SUMMARY

The object of this work was to synthesise selenium analogues of biologically-active sulphur compounds in the hope that they would show similar activity. Part I deals with the preparation and physical properties of the compounds synthesised and Part II with the spectrophotometric determination of selenium in organic compounds.

A survey of the biological properties of selenium in relation to animals and man leads to a brief account of isosterism and bio-isosterism with particular reference to these relationships involving oxygen, sulphur and selenium. A more detailed account of the selenium analogues of biologically-active sulphur compounds shows the changing interest from the toxic properties to the possible utilisation of organo-selenium compounds stimulated by the suggestion that selenium may be a previously unknown essential trace element. A brief account of the biological properties of oxazolidines and thiazolidines concludes the introduction.

Initially, the synthesis of the selenium analogues of the barbiturates was attempted but was concluded when these compounds were reported. As an alternative, the synthesis of selenium analogues of hydantoins was attempted but was unsuccessful. A series of 5-mono-substituted-4-selenazolidones was prepared from α-halo acids and selenoureas, together with the hydrolysis products, the selenazolid-2,4-

diones. The cyclisation was shown to take place by the initial formation of an isoselenohydantoic acid which could be isolated and cyclised to the expected product. Disubstitution led to unstable products, and only 5,5-dimethylselenazolid-2,4-dione could be isolated in a state of purity. Acetylation was successful only in the case of 2-imino-4-selenazolidone which also formed sulphonamide derivatives, but all attempts to prepare 5-alkylidene derivatives were unsuccessful. 5-Alkylidene derivatives were prepared from 2-acetylamino-4-selenazolone and selenazolid-2,4-dione. Methylation of each series took place in the 3-position and the products, together with the 2-dimethylamino compounds were used as models in the investigation of tautomerism. 2-Alkylidenehydrazones of the selenazolid-2,4-diones were also prepared.

Se-Benzylselenouronium chloride, picrate and p-toluenesulphonate were isolated but the attempted preparation of the formate, acetate and benzoate was unsuccessful. Se-Methylselenouronium iodide and picrate were also isolated.

The attempted titration of the 2-imino-4-selenazolidones and the 2-amino-4-selenazolones showed that these compounds were too weakly basic to allow the calculation of pK_a values but the 3-methyl derivative isolated readily titrated as also did the selenazolid-2,4-diones.

The ultraviolet absorption spectra of the 2-imino- and 2-amino-compounds were measured in water and at different pH values. Comparison of the ultraviolet and infrared absorption spectra showed that the 2-imino form predominated in the 2-imino-4-selenazolidones. The tautomerism of the ring system in related compounds is discussed.

In Part II the various methods used for determination of selenium in organic compounds are surveyed and a spectrophotometric method described, in which the organo-selenium compound is oxidised to selenious acid which gives a golden-brown sol on reduction with ascorbic acid using chlorpromazine hydrochloride as stabiliser. This reaction could be used for the microdetermination of selenium by measuring the extinction of the golden-brown solution at 420 m/u. Chlorpromazine hydrochloride was compared with other known stabilisers but only cetomacrogol appeared to be as efficient

The incomplete biological testing of representative selenazolidine derivatives shows that these are unlikely to prove of any medicinal value.

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SYNTHESES AND STUDIES IN ORGANO-SELENIUM COMPOUNDS

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INTRODUCTION

Selenium was discovered in association with sulphur in the sludge from a sulphuric acid plant by Berzelius and Gahn in 1817[1]. Nowadays, it is regarded as an important element and is widely used in the electronic, glass, rubber and ceramic industries[2], but despite this it is still doubtful whether it will ever command a wide use in medicine on account of its toxicity. This toxicity to animals was first noted by Japha as early as 1842, and interest in this property since that time[1,3,4,5,6] has been such that even today selenium is regarded as a toxic element. In view of this emphasis on the toxic properties of selenium, it is perhaps not surprising that there has been a certain reluctance to seek medicinal uses for its derivatives. The recent work of Schwarz and Folts[7] has, however, given a new impetus to the investigation of selenium in biological systems. Some use is also being made of its toxic properties in the production of selenium-containing insecticidal sprays[8], and of its fungitoxic properties in the treatment of dandruff and similar skin ailments[9].

BIOLOGICAL ACTIONS OF SELENIUM.

Although selenium has received much attention because of its toxicity[1,3,4,5,6], this is by no means general to all biological systems. Thus it was noted by Trelease and Trelease[10] that some insects are so resistant to selenium that the larvae thrive on food sources which would be fatal to most animals. It is also known that selenium is essential for the optimum growth of certain plants[11] and that selenium as selenite is an essential factor for the production of formic dehydrogenase in some coliform organisms[12]. More recently, Schwarz and Folts[7], after showing that selenium is an integral part of Factor 3, which is necessary for the prevention of chronic dietary liver necrosis in the rat, suggested that selenium may be a hitherto unknown essential trace element.

The intensive study of this rare and, at that time, little known element was begun some thirty years ago when it was shown to be the cause of a serious and often fatal disease of livestock[13]. It is absorbed from the soil by plants[14] in sufficient quantities to make them highly toxic to animals, and the research assumed added importance when the possibility of human injury, due to the consumption of contaminated grains, vegetables, eggs and dairy products

was recognised. Selenium is the only mineral element known to be present in healthy food plants and forage crops in sufficient quantity to make them lethal to animals[15], a fact which would seem to suggest that plants are more resistant than animals.

Selenium in Plants[1,4]

Cameron[14] in 1880 was the first to show that selenium was absorbed by plants and cereals grown on seleniferous soils normally have a selenium content of less than 100 parts per million (p.p.m.), the most common range being 5-10 p.p.m.[1] Various symptoms of injury are apparent in plants if a sufficiently high proportion of selenium is present, although small quantities stimulate plant growth[16]. These symptoms include stunting of growth, changes in foliage colour, withering and drying of leaves and premature death of the plant[4]. Different chemical forms of selenium have different toxicities[4] and absorption may be prevented by the application to the soil of other substances such as sulphate, but this seems of doubtful value[1,6].

Different plant species have differing tolerance to selenium[17] and some plants always contain amounts of

selenium which would be toxic to most other plants, when collected on seleniferous soils, the highest content reported being 15,000 p.p.m.[4] Such plants, belonging to the genera Astragalus, Stanleya, Conopsis and Xylorrhiza, seem to require selenium for optimum growth[11] and their presence indicates the occurrence of selenium in the soil. These "indicator plants"[4] have been invaluable to geologists and agricultural chemists in mapping seleniferous areas. No satisfactory explanation has been offered for the ability of indicator plants to absorb such large quantities of selenium under conditions where ordinary plants absorb only traces. One explanation which has been advanced is that indicator plants having a very high tolerance for selenate and selenite, possess a detoxication mechanism leading to the elaboration of soluble organic selenium compounds[17]. These, when returned to the soil, are readily available to all types of plants[13]. Thus it is well known that organic selenium as, for example, in an aqueous extract of Astragalus, is more readily absorbed than selenite[18], being also less toxic and stored to a greater extent. Selenium is found mainly in the plant tops[4] usually as inorganic selenium (selenate), while trace amounts of elementary selenium have been found in roots.

While it has been suggested that selenium replaces

sulphur in proteins and other organic compounds[4,5,14], this is not definitely known, although cereal selenium has been shown to be organic in nature[19], while the inorganic material is almost exclusively selenate[4]. Most of this selenium is water soluble even when present in the relatively insoluble gluten of wheat grain.

A crystalline amino acid complex containing sulphur and selenium has been isolated from Astragalus pectinatus by Horn and Jones[20]. This material analysed as a complex

$$HOOC.CH(NH_2)CH_2XCH_2CH_2CH(NH_2)COOH$$

I

of two parts of selenocystathionine (I, X=Se) and one part cystathionine (I, X=S). Heath and Eppson[21] stated that all the selenium in A. bisulcatus was in organic form and recently, Trelease, Di Somma and Jacobs[22] isolated a selenoaminoacid from the same plant by ion-exchange and filter paper column techniques. The indications were that this amino acid was Se-methylseleno-cysteine (II, X=Se),

$$CH_3.X.CH_2CH(NH_2).COOH$$

II

still not completely separated from S-methylcysteine (II, X=S), which was also shown to be present. Franke and

Painter[23] studied sulphur and selenium absorption in cereal plants and concluded that there was little relationship between sulphur and selenium.

Toxicity of Selenium to Animals.

Moxon[1] noted Japha's recognition of the toxic action of selenium. Later investigations have shown that toxicity varies with different chemical forms of selenium[4] and in general, elementary selenium is non-toxic due to its insolubility[6]. Comparison of a number of organic selenium compounds has shown them to be less toxic than inorganic selenium while selenium in food plants is more toxic than inorgenic selenium[6,24]. Probably the greatest potential danger to human beings comes from inorganic selenium compounds which are present in plants[4], as selenate and the more toxic selenite, and so are potential food contaminants.

Among organic selenium compounds, aliphatic derivatives are less toxic than aromatic compounds[25]. Stereochemical features among closely related compounds can be important, as in the case of selenocystine (III) where the

$$HOOC(NH_2)CHCH_2SeSeCH_2CH(NH_2)COOH$$

III

L-isomer is much more toxic than the D-compound[26].

While different chemical forms of selenium vary in their toxic effects, different species also show varying susceptibility to any one form of selenium[1,6], while adult animals, generally, are less susceptible than young ones[27].

Toxicity in Forage and Pasture Animals.

In 1295, Marco Polo described a disease of pack animals in a region of Western China in which a poisonous plant grew. Similar effects were later described by Madison in 1860 relating to the Great Plains of North America and again in 1912 by Stein relating to a similar region of Western China to that traversed by Marco Polo[4].

This disease came to be known as "alkali disease"[4] because of the supposed relationship to alkali or mineral salt in water or soil. Various other suggested causes[6] were also shown to have no connection with "alkali disease" and it was only when the problem became of such economic importance and several agricultural experimental stations in the United States undertook research into the problem in the early 1930's, that it became evident that selenium was the cause of the disease[13]. This followed from the work of Franke[6] who showed that the source of the poison was animal feeding stuffs and that of Robinson[28], who

established the presence of selenium in them. Further evidence in support of these findings was obtained when it was shown that similar symptoms to those of alkali disease could be produced by feeding an artificial seleniferous diet[6].

The disease is of two forms, acute and chronic. Using the classification of Trelease and Beath[4], the disease can be subdivided into a) acute and b) chronic disease, which is again subdivided into "blind staggers" and "alkali disease". The acute form, in which death due to respiratory failure often occurs within a few hours, is normally the result of a single ingestion of highly seleniferous forage plants. No treatment is known since death usually occurs before diagnosis. The two types of chronic disease would seem to suggest that the type of selenium compound ingested has an effect on the symptoms and pathology. Thus "blind staggers" is due to repeated ingestion of native range weeds containing selenium in water soluble forms, the characteristic symptoms being that the animals tend to wander about with a stumbling, blundering gait. The chief symptoms of the second type of chronic disease, still known as "alkali disease" or "bob-tailed disease", are loss of the longer body hair of the mane and

tail and deformation and sloughing of the hoofs, the animals becoming quite lame. This form of the disease is caused by fodder crops grown on seleniferous soils, containing protein-bound selenium insoluble in water. Bromobenzene[29] and arsenic[1] alleviate the symptoms, but the most satisfactory treatment is to discontinue feeding the infected fodder and grain.

Toxicity of Selenium to Poultry.[1,4,6]

Concentrations of selenium which are too low to cause noticeable symptoms in farm animals, interfere with the hatchability of chicken and turkey eggs. The embryos developed but were malformed and unable to break the shell. The deformaties took the form of misshapen or missing beaks, eyes missing, feet and wings malformed as well as abnormal down which gave a greased appearance. That selenium was responsible was shown by injecting sodium selenite (0.1 p.p.m.) into the air cell of normal fertile eggs which produced typical deformed embryos.

Effect of Selenium on Insects.

Trelease and Trelease[10] showed that some insects are so resistant to selenium that they thrive on food sources

which would be fatal to most animals. Gnadinger[8], however, in a search for an effective insecticidal spray against red spider species, found that selenium in a solution of potassium ammonium sulphide was a very effective and specific insecticidal agent. Experiments quoted by Moxon[1], showed that aphids were killed by selenium in wheat plants at a concentration which caused no injury to the plant, while cotton plants could be made toxic to cotton stainer and pink bollworm by applying sodium selenate to the soil. "The application of selenium to soils, however, is not recommended since the concentrations needed will produce vegetation toxic to man and animals."[1]

Toxicity of Selenium in Laboratory Animals.

A marked individual variation in susceptibility to selenium toxicity in rats, cats, rabbits and dogs was noted by Anderson and Moxon[30], while rats were found to be more resistant than rabbits and cats[31]. Repeated small doses of selenium, as selenite, were found to be cumulative, but no evidence of tolerance could be found[31].

The symptoms of naturally toxic rations containing selenium and normal rations containing added inorganic selenium were shown to be virtually identical[4,6]. Sub-acute

toxicity produced by feeding 15-25 p.p.m. inorganic selenium[1] is characterised by a marked decrease in food intake, decreased growth, progressive and marked anaemia, and definite pathological changes, especially in the liver, which shows necrotic lesions. This last is interesting in view of the recent findings of Schwarz and Foltz[7], that selenium is an integral part of essential Factor 3 in preventing liver necrosis in rats. The net result of sub-acute toxicity is that the animals are jaundiced and emaciated. Chronic symptoms were produced by feeding 5-15 p.p.m. selenium.

A characteristic feature of selenium poisoning is the garlic breath which is noted shortly after injection of sublethal doses of selenium[32,33]. The mechanism of action is unknown but it has been suggested[6] that it may be due to differential action of selenium compounds on enzymes concerned with metabolic processes. The distribution of selenium compounds in the animal body provides a useful background against which to commence studies of this sort, and this is discussed in the following section. Papers recently published by Heinrich and MacCann describe the cardiovascular[34] and respiratory[35] effects of sodium selenite on dogs.

Distribution of Selenium in the Animal Body.

One of the specific properties of selenium is its ability to be bound in trace amounts to a variety of proteins throughout the animal organism[36]. Dudley[37] found that in acute selenium poisoning in cattle, selenium was distributed throughout the whole organism in widely varying proportions, the highest concentrations being in the liver, blood, kidney, spleen and brain. Similarly, Smith, Westfall and Stohlman[38] found that the highest concentration of selenium in cats was in the liver and kidney. In chronic selenium poisoning of the blind staggers type, the distribution in cattle was similar to that in acute poisoning, although the blood concentration was greater[4]. With alkali disease, on the other hand, the concentration of selenium was found to be lower than in blind staggers in all tissues, but higher in the hoof[4]. The highest concentration of selenium, however, was found in rats in the liver, muscle, gastrointestinal tract and blood[39].

Retention of organic selenium was found to be greater than that of inorganic, the ratio being as much as 100 in some tissues[38]. In chronic poisoning, appreciable amounts were fixed in all tissues and organs with the exception of body fat, the most marked difference between inorganic and

organic selenium being found in brain, muscle, skin and bone, tissues in which inorganic selenium was retained to only a minor degree. These differences in fixation, storage and retention suggested the possibility of different chemical interaction during transport of selenium in the body. Different types of bonding of selenium were considered, and Smith, Westfall and Stohlman[38] concluded from their results that tissue selenium, especially in organic poisoning, was mainly in some firm protein combination. They suggested that inorganic selenium may also be built up into a protein-selenium complex, since selenium was present in the protein fractions in all cases in concentrations of 30-94 per cent of the total concentration of the tissues under examination. Non-protein selenium predominated only in animals acutely poisoned with massive doses of inorganic selenium.

Selenium in Blood of Experimental Animals.

A protein-like selenium compound was reported in the red blood corpuscles of the horse by Dudley[37], who also reported that 98 per cent of the selenium in the circulating blood in acute inorganic selenium poisoning was non-protein bound. McConnell[39] showed that selenium first appeared

in the blood of rats in the plasma, but as the total blood concentration decreased, the concentration in the red blood corpuscles became greater than that in the plasma since elimination from the plasma was faster than deposition in the red blood cells. The circulating blood contained 2-3.9 per cent of the total administered dose of selenium[40].

The results obtained by McConnell and Cooper[40] indicated that selenium was present in blood proteins in some organoselenium complex and the possibility of chemical fixation against adsorption on the surface of the protein molecules was discussed. The fixation of selenium in red blood cells presumably did not take place in bone marrow since sulphur and selenium differ in their abilities to concentrate there[40]. McConnell[41] has since shown that the proteins of leukocytes have affinity for trace amounts of selenium, resulting in the definite fixation in the proteins, 80-90 per cent being protein bound. According to McConnell[36] the leukocyte proteins contain selenium analogues of sulphur amino acids. McConnell, Wabnitz and Roth[42] have shown that serum proteins in the dog have selenium bound to them in at least two different forms. The albumin immediately accepts the selenium in a loosely bound form, and in this form, selenium is transported throughout the body, gradually being released to form more stably bound complexes with other

proteins in blood and tissues. The loosely bound form is tentatively described as being an anion-protein combination. The form in which selenium is incorporated in mammalian proteins is not definitely known. Recent chromatographic and tracer experiments by McConnell and Wahnitz[43] have shown that dog liver protein hydrolysates have at least three different fractions containing selenium, in the cystine-selenocystine area, another in the methionine-selenomethionine area and a third in the leucine area. McConnell, Wahnitz and Roth[42] report similar unpublished results with dog plasma protein hydrolysates. The mechanism whereby these selenium analogues of sulphur amino acids are formed has not been clearly established but the possibility of production from inorganic selenium has been discussed[43]. This is feasible since it is known that inorganic selenium compounds are converted biologically to the selenium analogues of methionine[44,45]; cysteine and cystine[44,45,46].

Selenium in Liver of Experimental Animals.

Smith, Westfall and Stohlman[38] have shown that liver selenium in acute selenium poisoning is predominantly non-protein, but in sub-acute and chronic poisoning, the bulk of the selenium is protein-bound. Liver protein contained 30-94 per cent of the total liver selenium in inorganic

poisoning and 78-92 per cent in animals poisoned with organic selenium, three quarters of which was associated with the globulins[47]. McConnell[39] showed that the liver had a higher concentration of the ingested selenium (19 per cent) than any other organ, and that this was associated with methionine, cystine and leucine fractions of protein hydrolysates[43]. More recently McConnell, Roth and Dallam[48] investigated the subcellular structures (mitochondria, microsomes and nuclei) of rat liver cells and showed that these structures fix selenium in their proteins, suggesting that they play a part in selenium fixation.

Excretion of Selenium.

Selenium leaves the blood stream slowly to be absorbed by certain tissues, but is eliminated chiefly in the urine[38,29]. Two other excretory pathways, faeces[49] and breath[33], are also used in selenium elimination and McConnell[32] has stated that 3-10 per cent is exhaled within 24 hours. It has also been reported[38] that in acute poisoning, 43 per cent can be recovered from the urine within 48 hours. In chronic selenium poisoning, 50-80 per cent of the inorganic selenium daily dose, but only 40 per cent of the organic daily dose, is eliminated in cat's urine. This is due to increased retention of organic selenium rather than to a marked

difference in availability. Prompted by the fact that tissue bound sulphur is believed to be the source of sulphur for mercapturic acid synthesis[50] and by the fact that selenium is protein bound in animal tissues[38,40,41,47], apparently in the selenocystine-cystine fraction[43], McConnell Kreamer and Roth[51] recently showed the presence of selenium in the mercapturic acid fraction of dog urine.

Dietary Factors affecting Selenium Toxicity.

Feeding trials with rats have been used in an attempt to find dietary factors which would reduce or prevent selenium toxicity. Moxon[1] surveyed the work done in this field and of the materials added to diets, only protein, arsenic and bromobenzene were effective.

It is generally accepted that a high protein diet alleviates the symptoms[1,4,6] but reports on individual amino acids, however, are somewhat anomalous. Thus Moxon and Rhian[3] reported cystine as having no alleviating effect on selenium poisoning and in this they agreed with Schneider[27] and Smith and Stohlman[52]. The latter also discredited methionine which was reported to have no effect on the excretion of selenium[33]. Fels and Cheldelin[53] reported that methionine partially reversed the yeast growth inhibitory action of selenate but later reports by the same

authors stated that, while cysteine and glutathione were effective in reversing selenate inhibition of E.coli, methionine was ineffective[54]. In yeast[55], methionine was partially effective, its effect being enhanced by cysteine and thymine but not by glutathione, cystine or biotin. Have and Williams[45] found that methionine and cystine partially alleviated the toxicity of low concentrations of selenite in E.coli but were ineffective against higher concentrations.

Moxon[1] discussed the beneficial effects of arsenic on selenium toxicity and mentioned that inorganic arsenic, as arsenate or arsenite, was equally effective against selenium as selenite, the selenium analogue of cystine and seleniferous wheat. It has been suggested that arsenic counteracts the toxicity of selenium by combination with it in the gastrointestinal tract but deposition of selenium in the tissues was unaffected by the presence of arsenic.

Organic arsenicals have also been shown to be effective against selenium poisoning[56].

Bromobenzene, when fed to rats and dogs was shown[29] to be effective against selenium poisoning by increasing the urinary excretion, but Westfall and Smith[47] showed that bromobenzene was ineffective in rabbits.

Effect of Selenium on Enzyme Systems.

There is very little definite information on the mechanism of selenium toxicity but some information has been obtained on its effect on unicellular organisms and enzyme systems[1,3,6,57], and it was concluded that selenium was a general dehydrogenase inhibitor[3,6].

Selenium has been used to determine the specificity of certain dehydrogenases[58] while Bornheim and Klein[59] subdivided enzymes into three groups on the basis of their reaction with or to selenium. The work of Wright, quoted by Moxon and Rhian[3], in which organic and inorganic selenium was shown to inhibit the activity of urease but not arginase in rat liver slices, indicated the relationship of selenium and those enzymes which are dependent on the action of sulfhydryl groups[6]. An enzymatic reaction between selenate and adenosine triphosphate (A.T.P.) was reported by Wilson and Bandurski[60] and it was suggested that this reaction may contribute to the understanding of selenate reduction in plants and selenium toxicity in animals.

Schultze[61] surveyed the recent work on Vitamin E, Factor 3 and selenium and concluded that more work is necessary before the rôle of tocopherols, selenium compounds and antioxidants in relation to enzyme systems can

be understood. Selenium compounds are effective against chronic dietary liver necrosis and this is discussed in the next section. Respiratory decline was noted in chronic liver necrosis, and Schwarz, Mertz and Simon[62] stated that for this to be apparent, simultaneous absence of selenium and Vitamin E was necessary. Olson and Dunning[63] noted decreased Coenzyme A activity in liver of rats with acute dietary necrosis, while traces of selenium increased the incorporation of radioactive sulphur into Coenzyme A of liver[64], besides decreasing the pyruvate oxidase activity. Schultze[61] concluded that the failure so far to ascribe a specific rôle to Vitamin E at the enzymatic level emphasised its rôle as a non-specific antioxidant, and that selenium compounds may well function in a similar manner. In support of this, protection against necrosis without feeding Vitamin E or selenium has been obtained[65] by the addition of antioxidants.

Factor 3.

In 1951 Schwarz[66] recognised a separate dietary agent, called "Factor 3", which prevents liver necrosis in the rat. This factor is present in brewers' yeast, in dried defatted pork kidney, in casein and in soybean meal

and has recently been shown to be an organo-selenium compound[7].

Schwarz[67] showed that necrotic liver degeneration can be produced in the rat by feeding a Vitamin E and Factor 3 - free diet based on 30 per cent Torula yeast as the sole source of protein. This deficiency had previously been shown to be responsive to cystine and Vitamin E. It had also been noted that while alkali-treated casein did not influence the development of liver necrosis, and had in fact been used in diets producing the deficiency, ordinary "Vitamin E - free", alcohol-extracted casein inhibited the damage.

Later Scott, Hill, Norris, Dobson and Nelson[68] showed that chicks fed on a similar Torula yeast diet, developed exudative diathesis. This defisiency disease, which caused loss in weight and eventually death, was already known to be produced in chicks only by certain types of Vitamin E - free diets. The liver, however, in contrast to the rat, appeared to be unaffected. These deficiency symptoms in chicks fed on a Torula yeast diet were prevented not only by tocopherol but also by brewers' yeast[68] which is known to be free of Vitamin E but an effective source of Factor 3[69].

When Factor 3 was shown to contain selenium[7], the element in inorganic form was tested at very small dose levels and was shown to be effective in protecting against dietary necrotic liver degeneration in the rat[7,70], multiple necrotic degeneration in the mouse[71], exudative diathesis[72,73,74] and muscle degeneration in the chick[75] and other symptoms formerly thought to be solely due to deficiency of Vitamin E. When the relative potencies of organic and inorganic selenium compounds were compared considerable chemical specificity was noted[70]. Against necrotic liver degeneration in the rat, selenium was shown to be five hundred times as effective as Vitamin E[7] and this agreed closely with its activity against exudative diathesis in the chick compared to Vitamin E[72].

The view was now taken that these deficiency symptoms were the manifestations of a multiple deficiency of Vitamin E and Factor 3[76]. The rôle of cystine, previously regarded as an essential factor, was stated to be due to trace contamination by selenium. Support for this conclusion came from the report that cystine protects against exudative diathesis but not encephalomalacia in chicks, a condition which is not affected by selenium[74]. On the other hand, Yang, Dialameh and Olson[66] concluded that their specimen of

cystine did not contain sufficient selenium to account for its action against necrotic liver degeneration. A preliminary suggestion[77] that molybdenum, osmium and cobalt might also be protective agents against necrotic liver degeneration in rats was disproved by Schwarz, Roginski and Foltz[78], but the possible effects of other dietary components is unknown[61].

While Factor 3 - active selenium protects against many deficiency symptoms, it does not, however, replace Vitamin E, as has been shown using the rat resorption gestation bioassay for Vitamin E[76]. This is also the case with muscular dystrophy in rabbits[79,80], while muscular dystrophy in chicks is only partially prevented by sodium selenite whereas Vitamin E protects completely[81].

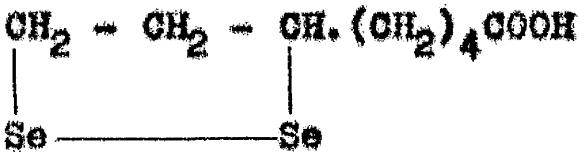
That a third factor was involved in the production of dietary liver necrosis in rats was shown by Schwarz[66] by eliminating the two previously accepted essential dietary components, Vitamin E and cystine, from the yeasts under examination. This was done by boiling twice with water and ethanol to leave an insoluble Vitamin E - free residue which consisted mainly of protein. After acid hydrolysis, neutralisation and concentration, a little cystine and considerable tyrosine precipitated. This cystine- and Vitamin E - free material was still active to the extent of about

5 per cent of the original yeast. Schwarz concluded that this third essential factor was water-soluble and stable to acid hydrolysis and was firmly bound to protein. He also showed that it was not identical with any of the common vitamins which were tested for activity[66]. Schwarz and Foltz[7] later showed that Factor 3 from acid hydrolysates of defatted kidney contained at least two chemically closely related substances, both of which were active. These they called α- and β-Factor 3. Factor 3 was water soluble, strongly anionic, stable to oxidation but sensitive to reducing agents. Dry ashing eliminated the activity entirely indicating its organic nature. The fractionation of α-Factor 3 lead to highly concentrated semi-crystalline preparations, some of which were found to develop a characteristic garlic odour on addition of alkali, an observation which led to the discovery of selenium as an integral part of α-Factor 3. In support of this, it was shown[7] that inorganic selenium is very effective against necrotic liver degeneration and that Factor 3 activity may be correlated with the selenium content. No detailed study of β-Factor 3 is recorded.

Patterson, Milstrey and Norstad[73] also attempted to isolate the factor from casein and kidney. They found that the active principle was insoluble in water and ethanol but

that it became soluble and diffusible after sulphuric acid and trypsin hydrolysis. It was also shown that the active factor could be adsorbed on to both anionic and cationic exchangers, suggesting that the factor in acid hydrolysed kidney was amphoteric. Ashing destroyed the activity but in presence of calcium oxide, the ash retained its activity, suggesting that it was due to an element which forms a volatile inorganic acid i.e. As, Se, Te. These three elements were then tested and selenium, as selenite, was shown to be active against exudative diathesis in chicks, while arsenic and tellurium were inactive.

The suggestion by Bergson[82] that 6-selenoctic acid



(IV)

(IV) might be related to, or even identical with Factor 3 was later shown to be incorrect[83].

Selenium and its Relationship to Public Health.

According to Trelease and Beath[4], the main interest and importance of the selenium problem is concerned with the continuous ingestion of small quantities of selenium

and the subsequent possibilities of functional and organic changes[84]. On the other hand, human cases of selenium poisoning are more likely to occur from the industrial use of selenium than from consumption of food in seleniferous areas[1,6].

Selenium is utilised in various industries[2] and selenium-bearing dusts, fumes, vapours and liquids may represent definite hazards due to toxicity by inhalation, via the alimentary canal or by skin absorption. Dudley[85] surveyed dust, vapour and gaseous sources of poisoning and described the symptoms, which need not be specific, as pallor, gastro-intestinal disturbances, garlic breath and perspiration, irritation to nose and throat, coating of the tongue and nervousness. Clinton[86] reported the effects of selenium fume exposure in a scrap smelting plant and describes the intensely irritating characters of the fumes, which, he suggested, are a safeguard against severe exposure. An unusual consequence of inhalation of hydrogen selenide was described[87], in which a chemist developed hyperglycaemia controllable only by increasingly large doses of insulin. The same effect could not be produced in animals, however, and it was suggested that this response may be effected only in "predisposed subjects".

Pringle[88] described dermatitis which occurred after

contact with selenium dioxide in various forms. Certain workers seemed to be immune, while sepsis was an important factor in the production of the dermatitis. Selenious acid, accidentally dropped on to a finger nail, caused perforation and extreme pain[89].

Human Poisoning from Contaminated Food.

Studies of rural families in highly seleniferous areas lead to the conclusion that, apart from gastro-intestinal and liver symptoms, both probably due to continual selenium ingestion, no other evidence of ill-health could be directly attributable to selenium[90,91].

Williams, Lakin and Byers[92] referred to an unpublished report by Roca, which described a disease of man and animals in a river valley in Mexico. This disease was caused by eating local vegetation and the symptoms in animals were identical with those of "alkali disease". Besides this, there were also cases of people losing hair and teeth, and being affected by a form of paralysis. Another report[93] also relating to Mexico, described nail trouble in man, similar to the hoof disorders of selenised animals.

When Gnadinger[8] described the investigations leading to the use of a selenium containing insecticidal spray, he

cautioned that the toxicity to the consumer was unknown and should be investigated before treating food crops. He reasoned, however, that such sprays would be less dangerous than arsenical sprays. Hoskins, Boyce and Lamiman[94] reported that selenium sprays adversely affected the selenium content of citrus fruits, although the proper use of such sprays offered no health hazard to man. Another report also suggested the cautious use of selenium sprays for food crops[95].

Selenium as a Dermatological Agent.

Matson[9] described the formulation and dermatological action of selenium sulphide. Initially, this preparation was used in effectively controlling various degrees of seborrheic dermatitis of the scalp. It is reported to be 85-95 per cent effective in the mild form known commonly as "dandruff", and has been suggested as the therapeutic standard in this field. Investigation showed, however, that its effectiveness was not limited to the scalp and that similar conditions of other glabrous skin areas were also controlled. Granulation of the eyelids, which frequently is associated with dandruff, was effectively controlled and justified the caution necessary since selenium sulphide is irritant to the eye. There has been some reluctance to prescribe this

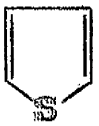
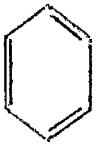
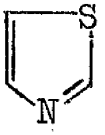
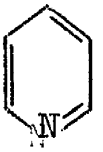
preparation on account of the toxic properties of selenium, but Watson[9] considered there was an ample safety margin for use under medical supervision. The chief reason for its safety is the insolubility, and suicide attempts by swallowing large quantities have all been unsuccessful.

Taboury[89] mentioned that the waters of Roche-Posay are recognised as a cure for certain skin ailments, a fact well known even in Roman times. The selenium content of these waters is 0.2 p.p.m., appreciably less than the toxic dose.

ISOSTERISM AND BIO-ISOSTERISM

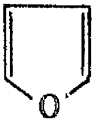
The earlier work in this field has been adequately reviewed by Friedman[96] and Schatz[97]. In 1919, Langmuir introduced the term "isosteric compounds" or "isosteres" relating to molecules in which the number and arrangement of electrons was the same. This concept was further extended in 1925, when Grimm introduced the term "pseudo-atom" instead of Langmuir's "isosteric comolecule", whilst Erlenmeyer in a series of papers from 1932 onwards, related the structures of isosteric organic compounds to their biological activity. This work gave great impetus to the modern concept of isosterism in organic chemistry, especially in relation to biological activity. According to Erlenmeyer, all elements in the same group of the periodic table are isosteric so long as they have the same number of electrons in their outermost shell. But, as early as 1933, this concept was further extended with the suggestion that -S- and -CH=CH- groupings in an aromatic nucleus are isosteric if only the "peripheral" electrons in the -C=C- pseudoatom are counted. This work was prompted by the noted similarity in physical properties between thiophene (V) and benzene (VI) and between thiazole (VII) and

pyridine (VIII)[98].

(V) (VI) (VII) (VIII)

Albert[99] also remarked on the volume of published work on the physical similarity between thiophene and benzene derivatives, although no close chemical similarity was claimed. Pyrrole (IX), furan (X) and thiophene (V) can,

(IX) (X)

however, be compared chemically and this is discussed later.

Both Metzner and Erlenmeyer used the term "pseudocycle" to show the steric relationship between the cyclic and open chain forms of physiologically active molecules. Metzner in 1946 showed that, in certain circumstances, the $-CH_2-CH_2-$ grouping could be replaced by -CO-O- without change in biological activity. Fieser used the term "isolog" usually meaning "isostere", although isologous

compounds need not always be isosteric. The same terminology was also used by Mautner and Clayton[100] referring to several series of oxygen, sulphur and selenium compounds, which had previously been described by Mautner[101] as "almost sterically identical". If compounds fit the broadest definition for isosteres and have the same type of biological activity, or are directly antagonistic, they are called "bio-isosteric" by Friedman[96]. Thus Seifrig showed that nitrous oxide (N_2O) and carbon dioxide (CO_2) were both reversibly anaesthetic to a slime mould. These two compounds had been termed isosteric by Langmuir, so fit Friedman's definition of bio-isosteres[96].

The biological equivalence of isosteric groups was first shown by the immunological work of Landsteiner who formed artificial antigens by coupling diazotised aromatic groups with proteins. Erlenmeyer, Berger and Leo and Tomcsik, Schwarzweiss, Triesler and Erlenmeyer also showed that isosteric atoms are serologically similar. This was criticised by Heidelberger[102] as requiring more and better supporting evidence, and also since arsanilic acid and stibonic acid are quoted as reacting in different ways in an antibody test. However, the conclusions of Erlenmeyer agreed with those of Pauling[103].

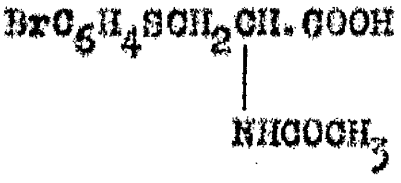
Since it was shown by Bell and Roblin that

sulphonamide antagonism to p-aminobenzoic acid is an anti-metabolite effect due to close similarity of structure, isosteric replacements in other essential nutrients have been investigated, often with successful results. Isosteric compounds may have the same type of activity, but frequently exhibit antagonistic effects, but, in either case, it is evidence that the same biological mechanism is involved and that the compounds are truly bio-isosteric[96]. Isosteres need not be bio-isosteres, but simple isosteric replacements often give compounds of interest and value, and the successful results already obtained through isosteric replacement show that this type of variation is useful in modelling new compounds. Isosterism, however, will not accomplish for molecules what the periodic table has accomplished for the elements, namely correlation of similar behaviour with similar electronic structure[97]. Molecular size and shape must also be considered in determining biological properties.

Bio-isosterism in the group oxygen, sulphur, selenium and tellurium is considered by Schatz[97], but selenium and tellurium derivatives are stated to be only of minor importance. Friedman[96] notes that sulphur is surprisingly less bio-isosteric with oxygen than might have been expected, probably due to polarity differences, while Mautner[104] in recent extensive work on selenium analogues of biologically-

active sulphur compounds, has repeatedly emphasised the isosteric similarity between sulphur and selenium. He draws attention to the fact that the radius of doubly bound sulphur (0.94 A^0) is close to that of doubly bound selenium (1.07 A^0), and that when sulphur and selenium analogues were compared, they crystallised in identical forms, which often differed from the oxygen analogues[101,105].

Considering now biological reactions, it was suggested[29] that the mechanism for the excretion of selenium from the body resembled that for sulphur. Thus dogs excrete sulphur compounds in the presence of bromobenzene as p-bromophenylmercapturic acid (XI), and, similarly,



(XI)

selenium compounds are excreted as "selenomercapturic acid"[51].

In a different context, Mautner and Günther[104] have shown that selenopantethine (XII, X=Se) can replace

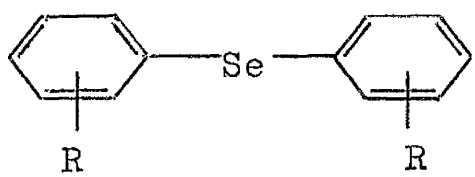
(XII)

pantethine (XII, X=S) completely on a mole for mole basis

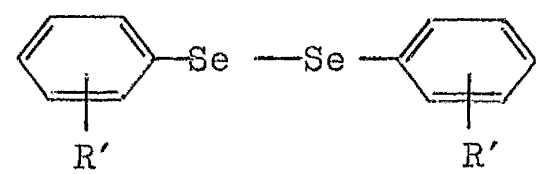
in Lactobacillus helveticus. It is stated that the selenium compound can replace its sulphur analogue in the biogenesis of "Coenzyme A", which thereafter performs a biological function. These are but two random examples which illustrate the fact that selenium can react in biological systems as would sulphur, and can in some cases replace it.

SELENIUM ANALOGUES OF BIOLOGICALLY ACTIVE SULPHUR COMPOUNDS

Interest in organoselenium compounds as possible therapeutic agents dates back only some twenty years. In 1940, Natti[106], stimulated by the antistreptococcal properties of the sulphonamides and sulphones, prepared several selenium compounds with a view to their possible use in the treatment of cancer and leprosy. These compounds were diphenyl selenide (XIII) or diphenyl diselenide (XIV) derivatives.



(XIII)



(XIV)

R = o- and p-NO_2-
o- and p-NH_2
o- and p-CH_3CONH-

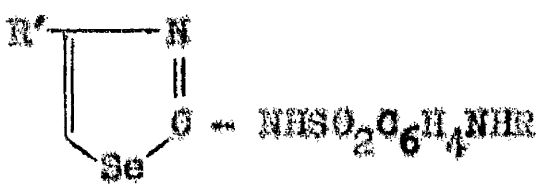
R′ = o- and p-NH_2-
o- and m-CH_3CONH-
p-OH-

Some of these compounds were tried out in the treatment of leprosy and streptococcal infections. No action was noticed against streptococci, while in leprosy, only the simplest derivatives showed favourable activity.

In 1940, Painter, Franke and Gortner[107] investigated

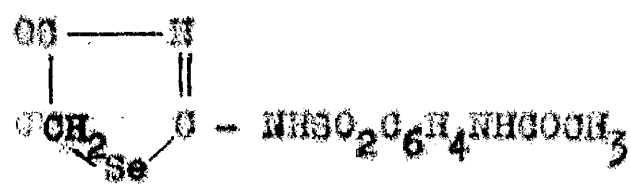
some organoselenium compounds with a view to studying selenium compounds in cereals. Their investigations were handicapped by the limited knowledge of the chemistry of these compounds and showed that organoselenium compounds were mainly stable in neutral organic solvents. They were less stable than sulphur compounds in air or in neutral solution and decomposed in alkaline media.

Roy and Guha[108] synthesised sulphanilamide derivatives containing selenazole (XV) and "selenohydantoin"(XVI) residues



R' = CH_3, C_6H_5.

(XV)



(XVI)

The selenazole derivatives (XV, R = $COCH_3$) were then deacetylated to give compounds of structure (XV, R = H). Although biological results of these compounds were promised, no report has been found.

Stekol[109] in a short note described the product obtained from the interaction of cysteine hydrochloride and sodium selenite as selenium tetracysteine, and Painter[110] in 1947 reported a new synthesis of dl-selenocystine (III) and derivatives of selenocysteine (XVII), as part of an

investigation of selenium compounds in plants. The isolation

$HOOCCH(NH_2)CH_2SeSeCH_2CH(NH_2).COOH$ (III)

$R.SeCH_2CH(NH_2).COOH$ (XVII)

R = C_6H_5, $C_6H_5CH_2$

of these plant products in a pure form was exceedingly difficult since it seemed probable that the selenium compounds occurred in conjunction with analogous sulphur compounds with closely related properties. Painter's approach was to synthesise the selenium analogues of sulphur-containing amino acids and compare them pharmacologically and chemically with the isolated plant products. This was reasonable since it was accepted by this time that selenium occurred as a constituent of amino acid material, and Horn and Jones[20] had recently isolated an amino acid material containing both sulphur and selenium from A. pectinatus. Painter[111] continued this work by synthesising the selenium analogues of dl-methionine (XVIII) and dl-homocystine (XIX) since cystine

$CH_3SeCH_2CH_2CH(NH_2)COOH$ (XVIII)

$HOOCCH(NH_2)CH_2CH_2SeSeCH_2CH_2CH(NH_2)COOH$ (XIX)

(or cysteine) and methionine carry nearly all the sulphur in cereal proteins. Homocystine, although never identified in plants, was of interest because of its known ability to

supply animals with their sulphur-containing amino acid requirements. In the same year, Klosterman and Painter[112] improved on the synthesis of the two selenium compounds. Later, Klug and Petersen[113] reported experiments which suggested that Stekol's selenium tetracysteine[109] and Painter's selenium dicysteine[111] were really mixtures of cystine and, probably, selenium dicysteine. This, however, conflicts with the work of Williams and Ravve[114], who had synthesised the selenium analogue of dl-cystine in the previous year by two different routes and obtained a compound which melted at 215°, as did the compounds obtained by Painter[111], and Fredga[115].

Weisberger, Suhrland and Seifter[116] reported in 1956 that selenium cysteine (XVII, R=H) was effective in low concentrations in decreasing the incorporation of radioactive L-cysteine in leukaemic leukocytes <u>in vitro</u>. Diets deficient in the sulphhydryl amino acid L-cysteine had been shown[117] to suppress the growth of malignant tumours in animals. It therefore appeared that substances which inhibited the cellular incorporation of L-cysteine could have an effect similar to a dietary deficiency of cysteine in suppressing tumour growth. It was not known whether selenocystine also competitively inhibited cystine incorporation in the intact animal, but it was shown that selenocystine decreased the incorporation of S^{35} L-cystine by rat Murphy lymphosarcoma

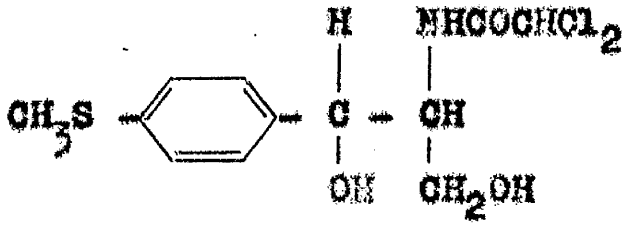
tumour cells both *in vitro* and *in vivo*, whilst benzyl-selenocysteine (XVII, R=$CH_2C_6H_5$) did not. Selenocystine also decreased the growth of the tumour in the intact animal.

Under clinical conditions, Weisberger and Suhrland[118] reported that selenocystine had a rapid and striking effect on leukocytes in both chronic and acute leukaemia. The effect was greater on immature than on mature leukocytes, and the action took place at the source with a decrease in spleen size, as well as by attack of the leukocytes in circulation. It was interesting to note that selenocystine was effective in patients with acute leukaemia resistant to cortisone, aminopterin or 6-mercaptopurine. A decrease in leukocyte count or in spleen size was also noted in chronic myeloid leukaemia resistant to irradiation, Fowler's solution, urethane and Myleran. One case is quoted where the patient became resensitive to 6-mercaptopurine after selenocystine treatment. The mechanism of action is unknown, but selenocystine seems to have a specific toxicity to immature leukocytes since no discernible changes in any organ could be attributable to selenium toxicity. Nausea and vomiting, which were often very severe, proved a serious disadvantage with selenocystine and difficulty was found in continuing treatment.

It has been shown by Cowie and Cohen[46] that

selenomethionine (XVIII) could completely replace methionine for normal growth of a methionine-requiring mutant of E.coli and that radioactive selenium from selenite was incorporated into proteins. Tuve and Williams[44,45] identified selenomethionine in protein hydrolysates of E.coli and suggested that selenocystine may also be produced by E.coli grown in the presence of sodium selenite. Thus since cysteine is an intermediate of methionine production in E.coli and since selenomethionine has been identified, it is believed, although so far not proven, that selenocystine may also be produced.

Supniewski, Misztal and Krupinska[119] described some sulphur (XX) and selenium (XXI) derivatives of chloromycetin



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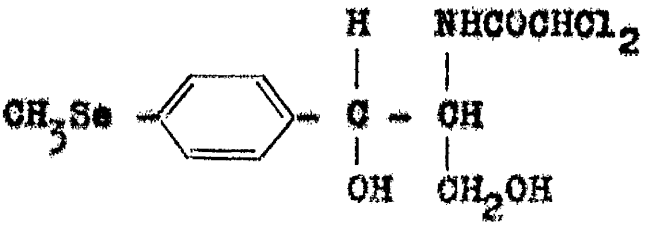
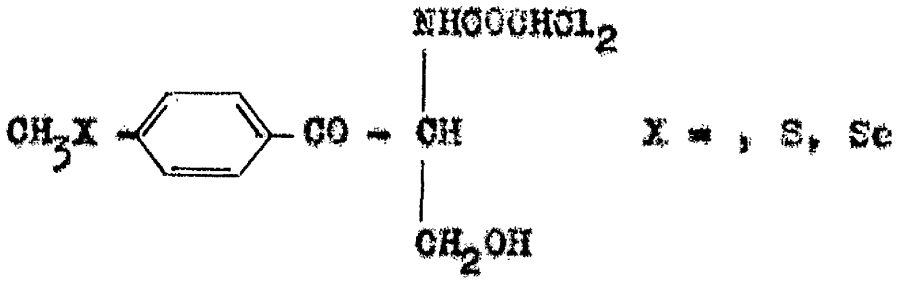


(XXI)

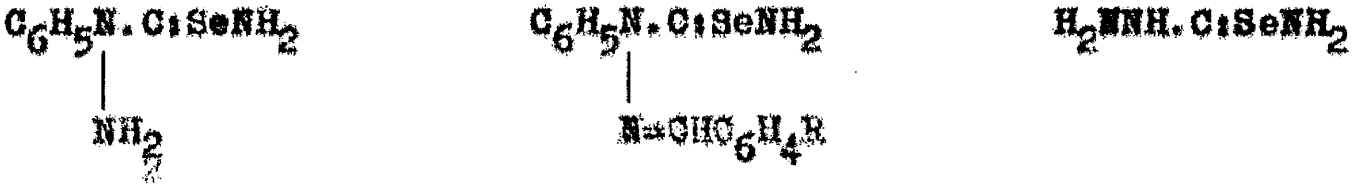
which were strongly antibacterial, with the selenium analogue about ten times more active than the sulphur analogue. Gram-positive bacteria were most sensitive and both the selenium and sulphur analogues were strongly active against acid-fast bacteria. The toxicity in mice of both series of compounds was comparable with that of chloromycetin. The

lethal doses were 500 mg. per kilogram and the toxic symptoms identical with those of chloromycetin, while the selenium compound, but not the sulphur compound, decreased respiratory movement, lowered arterial pressure and had a diuretic effect on the cat. According to the same authors, the ketone derivative of chloromycetin has strong antifungal activity, but this action was not noted in its sulphur and selenium analogues. No structure was given for the derivatives, but they are presumed to be as shown in structure (XXII).



(XXII)

In 1956, Mautner and Kumler[105] synthesised 2-phenyl-



selenosemicarbazide (XXIII) and a number of its derivatives

(XXIV), but failed to prepare selenosemicarbazide (XXV) itself.

The highest antitubercular activity was found in p-substituted benzaldehyde thiosemicarbazones whilst thiosemicarbazide itself had lower but still considerable in vitro activity. These compounds were also shown to inhibit fungal growth but replacement of sulphur by oxygen resulted in complete or partial loss of both antitubercular and antifungal activity of these compounds. It has been stated that thiosemicarbazones exert antimicrobial activity by forming copper chelates, so that the lower tendency of the oxygen analogues to form such chelates would explain their lower effectiveness compared with the corresponding sulphur compounds. As an extension of this, Mautner and Kumler[105] suggested that the selenium analogues might have greater chelating ability since selenium is related to sulphur in the same way as sulphur is to oxygen. The antifungal activity of 2-phenylselenosemicarbazide (XXIII), selenium-substituted benzaldehyde derivatives, phenylseleno-urea and their sulphur and oxygen analogues was tested by Mautner, Kumler, Okano and Pratt[120] against plant and animal pathogens and a saprophytic organism. It was found that the selenium compounds were ten to one thousand times more active on a molar basis than the sulphur compounds, while

the oxygen analogues showed negligible activity. The possibility of released selenium being the active agent was proved to be of little importance. It was concluded that selenium compounds are of sufficient activity to warrant further research.

Various carbonyl derivatives of selenosemicarbazide (XXVI) were prepared by Hüls and Renson[121] and selenosemi-

$RR'C{=}N.NH.C{:}Se.NH_2$ R and R' = CH_3, CH_3; C_2H_5, H; CH_3, C_2H_5; C_3H_7, H;

(XXVI)

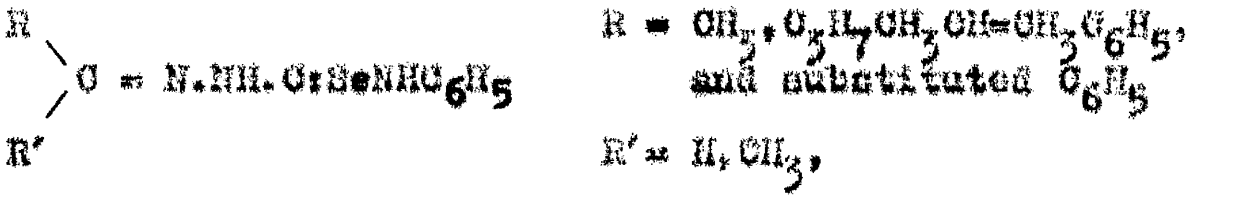
carbazide (XXV) was isolated in the preparation of its propionaldehyde derivative (XXVI, R = C_3H_7; R' = H). In a later report, Hüls and Renson[122] described the preparation of various aldehyde selenosemicarbazones by displacement from acetone selenosemicarbazone (XXVI, R,R' = CH_3). In the case of benzaldehyde selenosemicarbazone (XXVII), the

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!>\!C = N.NH.C{:}SeNH_2 \xrightarrow{C_6H_5CHO} C_6H_5CH = N.NH.C{:}SeNH_2$$

(XXVII)

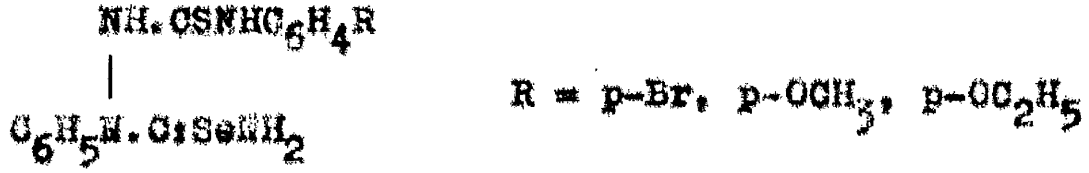
reaction was as shown but acetone could not be displaced by other ketones. 4-Phenylselenosemicarbazones (XXVIII) were also prepared from aldehydes and ketones, but no report of biological activity has so far been published by these

workers.



(XXVIII)

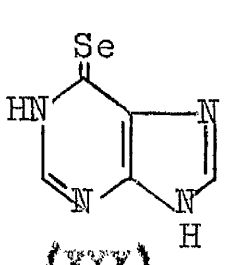
Bednarz[123] synthesised, and tested for biological activity, several derivatives of 2-phenylselenosemicarbazide of two types (XXIV, R = $N(CH_3)_2$) and (XXIX). These compounds had



(XXIX)

a weak effect on Staphylococci, B.subtilis and E.coli, a moderate effect on Mycobacterium B.C.G., M.phlei and M.smegmatis and a very strong effect on M.tuberculosis.

In the same year, Mautner[101] synthesised various selenopurine and selenopyrimidine derivatives. 6-Selenopurine (XXX), 6-methylselenopurine (XXXI), 2-selenouracil (XXXII), 2,4-diselenouracil (XXXIII) and 2-selenothymine (XXXIV) were synthesised by similar methods to those for the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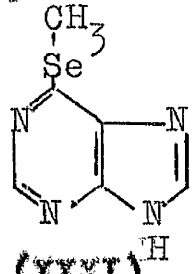
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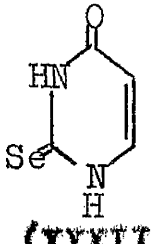
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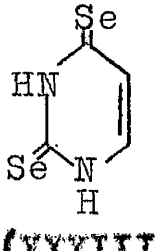
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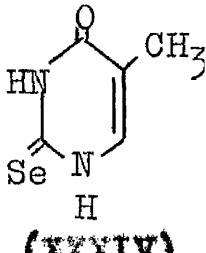
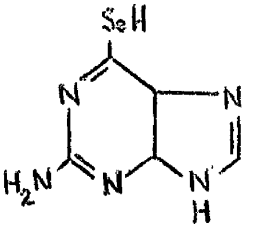

(XXXIV)

preparation of the thioanalogues by replacing thiourea with selenoureas. It had been noted that the most useful purine and pyrimidine bases were those in which the size of the new atom or group was closely similar to the group of atom replaced. The radius of double bonded selenium (1.07 A^{o}) is close to that of double bonded sulphur (0.94 A^{o}). Sterically, therefore, the compounds synthesised were almost identical with the sulphur analogues.

The actions of 6-selenopurine (XXX) and 6-mercaptopurine were compared against Ehrlich ascites tumour systems and Lactobacillus casei, as well as a wide range of micro-organisms[124]. 6-Selenopurine (XXX) inhibited a 6-mercaptopurine resistant strain of L.casei as efficiently as it did the wild strain. In contrast, mouse leukaemia L-1210, resistant to 6-mercaptopurine, showed full cross resistance to the selenium compound[125]. 6-Selenopurine (XXX) was inhibitory to a fairly wide range of micro-organisms, being more active than the corresponding mercapto compound. It was also shown that the sulphur and selenium compounds appeared to act by similar mechanisms. Another somewhat more detailed report[126] showed that 6-selenopurine had lower antitumour activity and greater host toxicity than equimolar quantities of 6-mercaptopurine with some tumour cells and equivalent activity with others. In both the

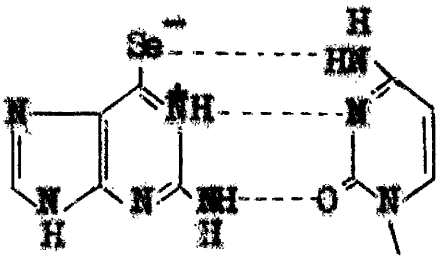
selenium and sulphur compounds, methylation decreased activity. Since 6-selenopurine is unstable, its effectiveness implies that its action is swift and selective. The possibility that the decomposition products may be the active species was not supported by the ineffectiveness of the equally unstable methyl compound (XXXI) and 2-selenouracil (XXXII).

Mautner and Jaffe[127] recently described the synthesis and preliminary biological testing of 6-selenoguanine (2-amino-6-selenopurine) (XXXV), after noting that 6-thio-



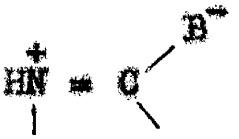
(XXXV)

guanine exhibited antimitotic activity and was incorporated into deoxyribonucleic acid (DNA). Although the mechanism of action of 6-thioguanine was unknown, it was suggested by Mautner and Jaffe[127] that charge separation might lead to unusually strong hydrogen bonding (XXXVI) with the amino group of cytosine facing thioguanine in the double helix of



(XXXVI)

DNA, and that this could be expected to interfere with the replication of DNA. Charge separation of the type shown (XXXVII) has been found to be greater in thiocarbamyl



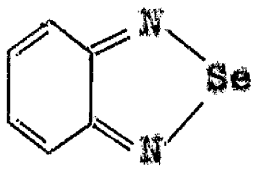
B = O, S, Se

(XXXVII)

compounds than in carbamyl, while replacement of sulphur by selenium gives even more marked polarisation[100,101].

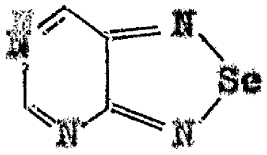
Since 6-selenopurine (XXX) showed antitumour activity in mice[126,128], despite its instability, 6-selenoguanine was similarly tested, having first been shown to be more stable than 6-selenopurine. Effective growth inhibition of L.casei could be obtained with 6-selenoguanine (XXXV) using one tenth the required concentration for thioguanine, but the selenium analogue was more toxic to mice than the thio compound in a single dose, although this position was reversed on repeated administration. In vivo testing in mice showed that the two compounds had comparable anti-tumour activity, while the selenium analogue seems to have an appreciably higher therapeutic index. It was also shown that, if a tumour was resistant to 6-mercaptopurine, this resistance extended to selenoguanine as well as to thio-guanine.

Sawicki and Carr[129] still engaged in the synthesis of possible purine antimetabolites for cancer chemotherapy studies, investigated the ultraviolet and visible spectra of benzo,2,1,3-selenadiazole derivatives (XXXVIII). In

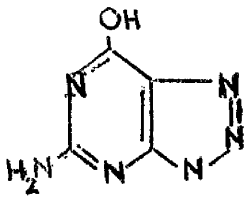


(XXXVIII)

the following year Carr, Sawicki and Ray[130] synthesised some 8-selenopurines (XXXIX) related to known purine



(XXXIX)

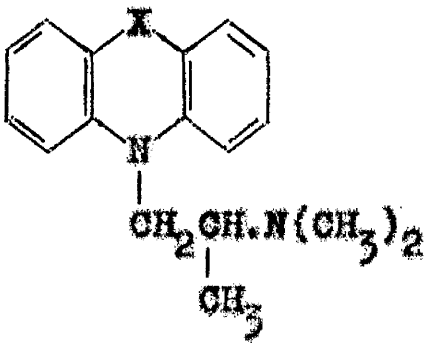


(XL)

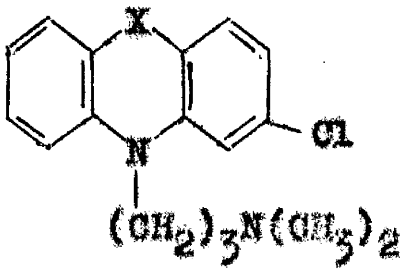
antimetabolites. Carcinostatic activity resulted[131] from the substitution of a nitrogen atom for the 8-carbon of guanine (XL). Selenium was introduced as a more radical change than nitrogen and while Mautner's purine derivatives (XXX - XXXIV)[101] had an exocyclic selenium atom, the compounds prepared by Carr, Sawicki and Ray[130] had a heterocyclic selenium atom. The possible mechanism of action was discussed. No report of the biological testing of these compounds has been found.

The possibility of organoselenium compounds having some chemotherapeutic value was strengthened by the report by Schwarz and Foltz[7] of the essential role of selenium in the animal body (see page 20).

Müller, Buu-Hoi and Ripa[132] compared the selenium analogues of promethazine (XLI, X=S) and chlorpromazine (XLII, X=S) and reported that both the selenium analogues (XLI, X=Se) and (XLII, X=Se) had antihistaminic activity similar to that of the respective sulphur compounds.

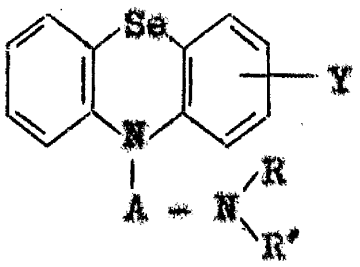


(XLI)



(XLII)

A number of phenoselenazine derivatives (XLIII), unsubstituted or substituted in the 10 position, have also



(XLIII)

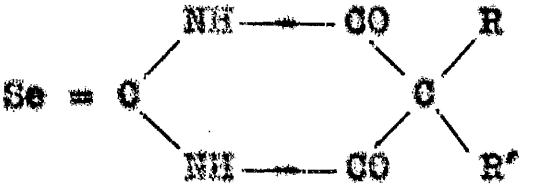
Y = hydrogen, halogen, trifluoromethyl, lower alkyl or lower alkoxyl group.

A = divalent, straight or branched alkylene chain of 2-6 carbon atoms.

R,R'= hydrogen, benzyl, lower alkyl or with the nitrogen attached to a monocyclic 5- or 6-membered heterocyclic ring.

been described[133]. The compounds are particularly useful as depressants of the central nervous system, i.e. as tranquillisers or mild sedatives. They also have fungicidal and antibacterial activity, as well as antihistaminic and antiemetic properties. The 10-substituted compounds, useful mainly as intermediates, also showed significant antifungal, vermifugal and antibacterial activity. This type of compound is stated to be both stable and of low toxicity.

Mautner and Clayton[100] prepared 2-selenobarbituric acid (XLIV, R,R'= H) and some 5-substituted derivatives (XLIV, R=H, R'= C_6H_5, $CH_2C_6H_5$, C_5H_{11}). Only one 5,5-disubstituted derivative, 5-phenyl-5-ethyl-2-selenobarbituric acid (XLIV, R = C_2H_5, R'= C_6H_5), was sufficiently stable to be



(XLIV)

isolated. These compounds were synthesised as part of an investigation of the relative lipid solubilities of oxygen, sulphur and selenium compounds. 6-Selenopurine (XXX) and 2-selenouracil (XXXII), previously prepared by Mautner[101], were similarly investigated. It was found that 6-seleno-

purine (XXX) had slightly greater lipid solubility than the thioanalogue while 2-selenouracil (XXXII) was less soluble at physiological pH than the thioanalogue. In the case of the barbiturates tested, the sulphur and selenium analogues had very similar lipid solubilities.

Mautner and Clayton[100] concluded from their results that the replacement of oxygen by sulphur in compounds of the type tested, was an effective method of increasing lipid solubility with only minor changes in the steric configuration of the molecule. Further replacement by selenium did not reduce the solubility in organic solvents, though substitution in this case involved replacement of an essentially non-metallic element by an essentially metallic element. It is therefore to be expected that lack of lipid solubility will not be a major problem in synthesising selenium analogues of physiologically active oxygen and sulphur compounds[100].

In 1960, Gunther and Mautner[104] briefly surveyed the work done in the previous decade, mainly by Mautner, in the preparation of selenium analogues of biologically active sulphur compounds. The work was extended to include a more detailed study of selenopantethine (XII, X=Se), which had already been shown to replace pantethine (XII, X=S)

completely on a mole for mole basis in L.helveticus. Thus it was shown that the selenium analogue can replace pantethine in the biogenesis of Coenzyme A which is then able to react normally. This is only possible, however, if the selenopantethine (XII, X=Se) is supplied preformed to the organism.

Pichat, Herbert and Thiers[134] reported the preparation of selenocystamine (XLV) selenohypotaurine (XLVI) and selenotaurine (XLVII) in a proposed comparison of the

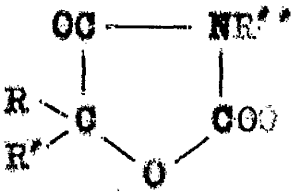
$(HCl.NH_2-CH_2-CH_2-Se)_2$ (XLV) $NH_2CH_2CH_2SeO_2H$ (XLVI) $NH_2CH_2CH_2SeO_3H$ (XLVII)

metabolism of organic sulphur and selenium compounds.

The trend of chemical literature dealing with organoselenium compounds in the past twenty years shows the changing interest in this element. Syntheses of the selenium analogues of biologically active oxygen and sulphur compounds has been stimulated by reports of such compounds having similar biological activity. Thus the emphasis has shifted from the toxic effects of selenium compounds to their possible utilisation in chemotherapy.

BIOLOGICAL ACTIVITY OF OXAZOLIDINES AND THIAZOLIDINES

3,5,5-Trimethyloxazolid-2,4-dione (XLVIII, R,R', R''= CH_3)[146] and 3,5-dimethyl-5-ethyloxyzolid-2,4-dione (XLVIII, R=C_2H_5, R', R''= CH_3) have found medicinal use

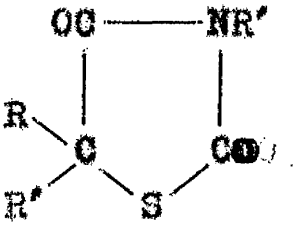


(XLVIII)

as a valuable pair of anticonvulsants[135]. 5,5-Dialkyl-oxazolid-2,4-diones (XLVIII, R, R'= alkyl, R''= H) however, do not show this activity but generally have sedative, hypnotic and narcotic properties[136] which are absent in the 3-alkyl derivatives[137]. More complex substitution in the 3-position has led to compounds with antifungal and anti-bacterial[138,139], analeptic[140,141], central nervous depressant[142] and tranquillising activity[143].

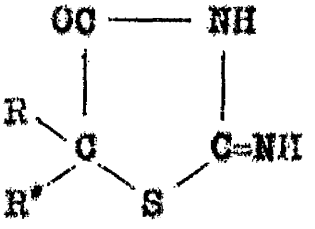
Replacement of the heterocyclic oxygen atom by sulphur or nitrogen in 3,5,5-trimethyloxazolid-2,4-dione (XLVIII, R,R', R''= CH_3) led to inactive compounds[144]. Doran and Shonle[145] noted that no pharmacological data was available for 5.5-dialkylthiazolid-2,4-diones (XLIX, R,R'= alkyl, R''= H) and found that these compounds had marked

anaesthetic and sedative action of short duration when



(XLIX)

given intravenously, but tremors and convulsions nullified their possible therapeutic usefulness. These findings were confirmed by Erlenmeyer[146], who did not refer to the side effects. Thiazolid-2,4-dione (XLIX, R,R', R''= H) has also been reported to have some antithyroid activity[147]. The intermediate 2-imino-4-thiazolidones (L, R,R'= alkyl)

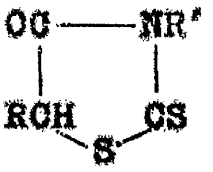


(L)

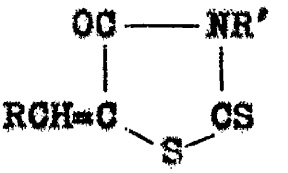
prepared by Doran and Shonle[145], showed a moderate degree of sedative activity when administered orally.

The rhodanines (LI), related to the thiazolid-2,4-diones (XLIX), are well known antifungal and antibacterial agents[148] and the thiazolid-2,4-diones (XLIX) have also shown these properties, since the N-C-S grouping common to both, has been related to the activity[149]. 5-Benzylidene-

rhodanine (LII, R = C_6H_5, R'= H) has shown hypertensive,

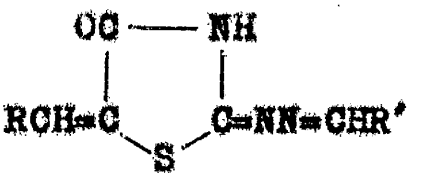


(LI)



(LII)

cardiac, intestinal and uterine effects[150]. A series of papers on thiazolid-2,4-dione derivatives (XLIX, R,R'= H, R''= aryl) showed that 3-substitution gave compounds which had antitubercular activity[151], also shown by thiazolid-2, 4-dione-2-hydrazone derivatives[152,153] (LIII, R = aryl, R'= aryl).



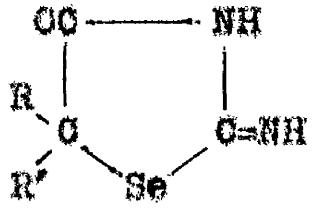
(LIII)

DISCUSSION OF EXPERIMENTAL WORK

Introduction.

The object of the present work was the synthesis of selenium analogues of biologically-active oxygen and sulphur heterocycles. Initially, the syntheeis of selenium analogues of the barbituric acids was attempted but was abandoned when these compounds were reported in 1959 by Mautner and Clayton[100].

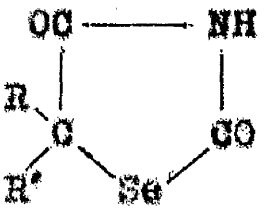
As an alternative, the synthesis of selenium analogues of the hydantoins was attempted but has so far proved unsuccessful. The synthesis of a homologous series of 2-imino-4-selenazolidones (LIV, R=H; R'= alkyl, C_6H_5) isomeric



(LIV)

with the selenohydantoins, was then undertaken. The first two members of this series had been synthesised by Hofmann[154] in 1889 and by Frerichs[155] in 1902, but since no reference has been found of biological activity, it seemed of interest to investigate the biological properties of these compounds and some of their derivatives especially the selenazolid-2, 4-diones (LV, R=H, R'= alkyl, C_6H_5), obtained from the 2-imino compounds by hydrolysis[154]. Since the 2-imino-4-

selenazolidones (LIV) are potentially capable of existing



(LV)

in different tautomeric forms, their physical examination by spectrophotometric and potentiometric methods was undertaken.

ATTEMPTED SYNTHESIS OF 2-SELENOBARBITURIC ACIDS

Preparation of Starting Materials.

Selenourea.

Selenourea has been prepared by various methods involving the reaction of hydrogen selenide and cyanamide in aqueous[156] or ethereal solution[157]. Cyanamide is not available commercially due to its instability[158], but is generally prepared from calcium cyanamide[156,159,160]. Since this also decomposes on storage, each new batch was analysed for cyanamide using the method of Wanussi[161]. After a series of trial experiments, the method of Werner[159,160] was adopted for the preparation of cyanamide. The final ether solution was evaporated to small bulk and hydrogen selenide[162] bubbled through slowly, in an atmosphere of nitrogen. Pale pink needles separated and the flow of gas was stopped after three hours. Since selenourea turns black on storage, it was prepared in batches of about 20-25g. as required.

2-Selenobarbituric acids.

2-Selenobarbituric acids.

The general methods available for the synthesis of barbiturates and thiobarbiturates include the condensation of urea or thiourea with a) malonic esters or their derivatives in presence of the alkoxides of sodium or

magnesium (Method A)[163], b) malonic acid or its derivatives in presence of acetic anhydride (Method B)[164], c) malonyl chloride alone[165], or in dry solvents[166] (Method C) or d) cyanoacetic esters (Method D)[167]. Replacement of urea or thiourea by selenourea in some of the above methods might have been expected to give 2-selenobarbiturates but in no case was it found possible to obtain the required compounds, despite varying the concentrations of the reactants and the reflux time.

Thus Method A[165] yielded a yellow solid which melted about 250°, but could not be purified. The ultraviolet absorption spectrum in 0.1N sodium hydroxide had λ_{max} at 285 mμ. Since barbituric acid itself has λ_{max} at 258 mμ, and since a bathochromic shift of about 30 mμ may be anticipated by replacement of oxygen by selenium[105], the value obtained was of the required order expected for a selenobarbiturate.

Method B[164] gave a similar yellow solid which melted at 243-5°. This material contained nitrogen and selenium and although the nitrogen analysis was satisfactory, the carbon and nitrogen analysis was not. In 0.1N sodium hydroxide solution, the ultraviolet absorption spectrum had λ_{max} at 285 mμ. Method C[165] gave a small quantity of yellow amorphous material melting about 245°, but attempted

crystallisation from various solvents caused decomposition. The ultraviolet spectrum showed λ_{max} at 280 mμ. Using dry solvents[166], sticky syrupy products were obtained on evaporation. Finally, method D[167] gave an amorphous yellow product melting about 180-190°. Once again this could not be purified. The ultraviolet absorption spectrum in alkaline solution showed λ_{max} at 259 mμ.

5,5-Diethyl-2-selenobarbituric acid.

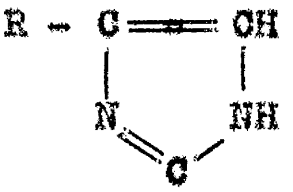
In the attempted synthesis of this product using method A[163], unpleasant, choking fumes were evolved on acidification. The yellow amorphous material obtained on acidification was a mixture of organic and inorganic material, and ether extraction gave, on evaporation, an unstable, yellow liquid which showed λ_{max} at 275 mμ in alkaline solution. Using method B[164], only impure semicrystalline solids were obtained.

At this stage of the work, a preliminary note by Mautner and Clayton[163] described the preparation of 2-selenobarbituric acids, hence this work was concluded. The method used by these workers[100] was essentially the same as method A[163], but the time of heating had been drastically reduced from seven hours to 45 minutes and the catalyst had been added dropwise to the refluxing mixture. It had been

hoped that the introduction of two ethyl groups into the 5-position would stabilise the molecule, but this produced the opposite effect. This agreed with the work of Mautner and Clayton[100], who could not isolate 5,5-diethyl-2-seleno-barbituric acid, due to its instability.

ATTEMPTED SYNTHESIS OF SELENOHYDANTOIN

A general route to hydantoins and thiohydantoins has been the condensation of amino acids with potassium cyanate or thiocyanate[169]. Since 2-selenomercaptan glyoxalines (LVI, R = aryl) had been prepared from potassium



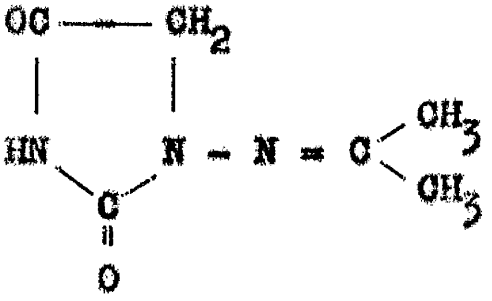
(LVI)

selenocyanate and the hydrohalides of substituted acetophenones[170], this route seemed promising. The attempted condensation of glycine, its ethyl ester or their hydrochlorides, with potassium selenocyanate in ethanol[171] gave only starting materials. No reaction occurred when acetic anhydride[172] or ethanolic sodium hydroxide[173] were used as condensing agents, or, in the absence of a solvent[174]. The same result was obtained using potassium selenocyanate and selenourea in the presence of barium hydroxide and ethanol.[175,176] 2-Selenouracil (XXXII) has been prepared from selenourea and ethylformyl acetate in water[101]. It seems possible therefore, that the lower homologous acid, glyoxylic acid, might condense with selenourea under the same conditions to give selenohydantoin. The attempted condensation in

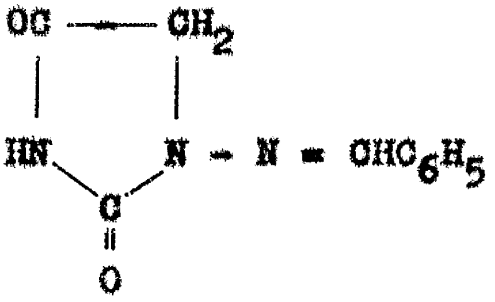
water gave only sticky, unstable products, while the same reagents in dry ethanol gave a yellow unstable solid, from which only selenourea could be isolated.

The attempted synthesis of diphenylselenohydantoin by the reaction of benzil and selenourea in alkaline solution[177] and from benzilic acid and selenourea in acetic anhydride[178] gave only starting material and unidentifiable products.

Jack[179] recently described the synthesis of 1-aminohydantoin derivatives (LVII) and (LVIII) from substituted semicarbazones and ethylchloroacetate under alkaline

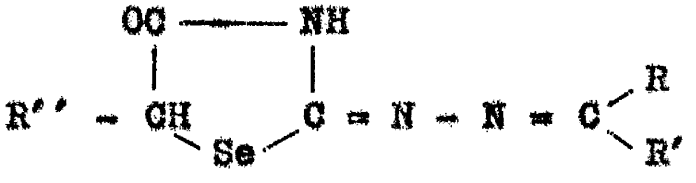


(LVII)



(LVIII)

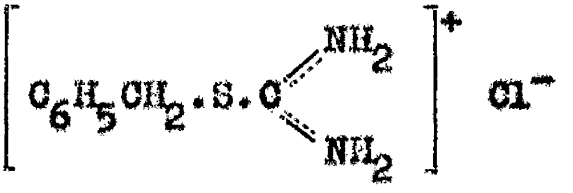
conditions. This method, however, produced the isomeric hydrazone (LIX, R,R' = CH_3, R'' = H) when acetone selenosemicarbazone was used.



(LIX)

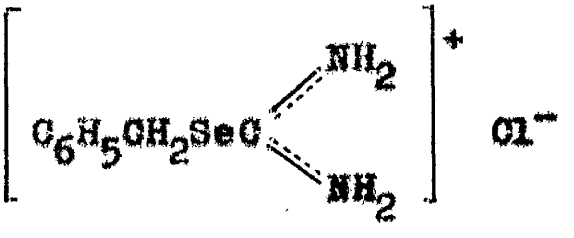
Se-ALKYLSELENOURONIUM SALTS

Thiourea unites with alkyl halides, sulphates, nitrates and thiocyanates to give S-alkylthiouronium salts[180]. S-Benzylthiouronium chloride[181] (LX), which occurs in two polymorphous forms, has been used to prepare salts of carboxylic[182] and sulphonic acids[183]. The ease of hydrolysis of some of these salts, and the small



(LX)

difference of melting point, led Dewey and Sperry[184] to prepare similar salts of p-chlorobenzylthiouronium chloride, which showed no evidence of polymorphism. The reaction of selenourea and benzylchloride in ethanol gave Se-benzyl-selenouronium chloride (LXI) in almost quantitative yield

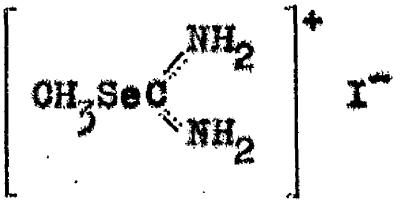


(LXI)

and no evidence of polymorphism was observed. Analysis of this compound for nitrogen and selenium was satisfactory, but chlorine analysis was reproducibly high, possibly due

to selenium interference. The sulphur analogue is readily hydrolysed to benzylmercaptan and dibenzyldisulphide[185]. That this is also true of the selenium compound was shown since, on attempted isolation of the base from the hydrohalide by the addition of alkali[181,185], the only crystallisable product was dibenzyldiselenide. This can be explained since hydrolysis would release benzylselenomercaptan, which is readily oxidised to dibenzyldiselenide[5].

A possible route to the selenohydantoins by condensing Se-benzylselenouronium chloride with chloracetic acid, and debenzylation with sodium in liquid ammonia[111] was envisaged, but the attempted cyclisation gave only uncrystallisable syrupy products, comparable with those obtained from the sulphur analogue (LX) and chloracetic acid[186]. Attempted condensation with chloracetyl chloride gave similar products and dibenzyl-diselenide. Taylor[180] found that methyl halides reacted more readily with thiourea than benzyl halides. On condensation of selenourea with methyl iodide, however, the yield of Se-methylselenouronium iodide (LXII) was less than that obtained from the comparable



(LXII)

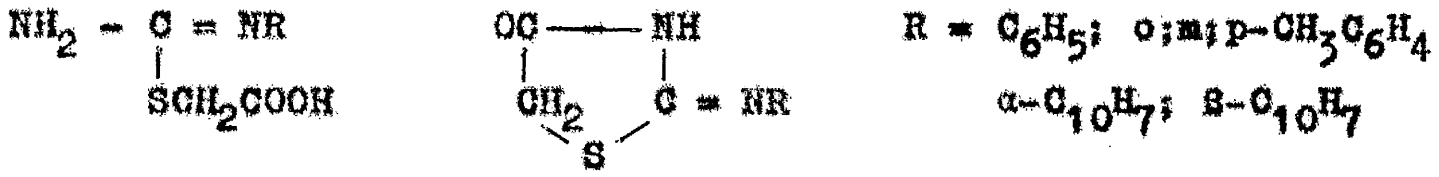
reaction with benzyl chloride. Once again the nitrogen and selenium analysis was satisfactory, but halogen was high.

The attempted titration of the Se-benzyl-(LXI) and Se-methyl-selenouronium (LXII) salts with potassium hydroxide was unsuccessful, and resulted in unpleasant-smelling breakdown products being released. The high pH of the aqueous solutions (Table I) suggested that these compounds were strongly basic, but no pK values could be obtained.

Se-benzylselenouronium formate, acetate and benzoate could not be formed, but the picrate and p-toluenesulphonate were well-defined crystalline solids.

ISOSELENOHYDANTOIC ACID HYDROCHLORIDE AND ITS CYCLISATION

When thiourea reacted with chloracetic acid in water in the cold, formamidinethiolacetic acid (isothiohydantoic acid) (LXIII, R=H) resulted, while heating under the same conditions gave 2-imino-4-thiazolidone (L, R,R=H).[187]

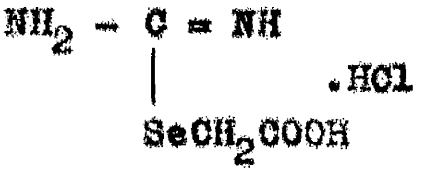


(LXIII) (LXIV)

In acetone, however, the hydrohalide of isothiohydantoic acid was isolated[187], which, when dissolved in water and left overnight, gave the free acid (LXIII, R=H)[188]. Heating for eight hours with glacial acetic acid gave 2-imino-4-thiazolidone (L, R,R'= H). Similar treatment of substituted thioureas gave the corresponding substituted products (LXIII) and (LXIV)[188].

The reaction of selenourea and chloracetic acid in water gave a crude pink solid which could not be purified by washing, but which cyclised on refluxing in dry ethanol for an hour to 2-imino-4-selenazolidone hydrochloride (LIV,R,R=H). In acetone, a much cleaner product was obtained, which on crystallisation from hot ethanol, again gave 2-imino-4-selenazolidone hydrochloride (LIV, R, R'= H). By washing

the crude product with acetone, isoselenohydantoic acid hydrochloride (LXV) could be isolated in a reasonably



(LXV)

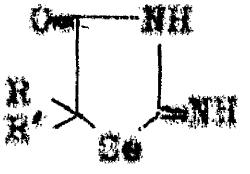
pure condition, although it was still not homogeneous and was slightly contaminated with selenium. The picrate, formed in methanolic solution, helped to confirm its identity. The hydrohalide (LXV) when refluxed for an hour with dry ethanol or glacial acetic acid, gave 2-imino-4-selenazolidone hydrochloride (LIV, R, R' = H). Similarly, heating with dilute hydrochloric acid or water gave selenazolid-2,4-dione (LV, R, R' = H), while sodium hydroxide gave no identifiable product.

The ultraviolet absorption spectrum of isoselenohydantoic acid hydrochloride (LXV) measured in water between 200 and 300 mμ, showed only end absorption, in contrast to the spectrum of 2-imino-4-selenazolidone hydrochloride (LIV, R, R' = H), see page 118. Calculation of the molecular weight of the picrate from ε at 355 mμ[189], gave reasonably good agreement with the theoretical value.

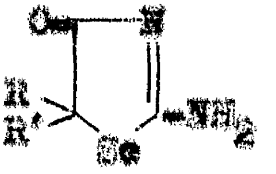
Thus it would appear that in the preparation of 2-imino-4-selenazolidone (LIV, R, R' = H), the initial condensation product is the open chain isoselenohydantoic acid (LXV) which then cyclises.

PREPARATION OF 2-IMINO-4-SELENAZOLIDONES

These compounds can be represented most appropriately by structures (LIV) or (LXVI).



(LIV)

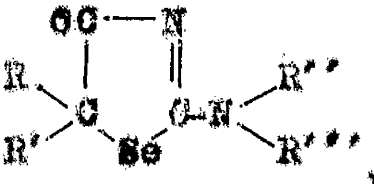


(LXVI)

R, R' = H, alkyl, aryl

2-Imino-4-selenazolidone (LIV, R,R' = H) was first prepared in 1889 by Hofmann[154], from chloracetic acid and selenourea. Frerichs[155] produced the same material, "selenohydantoin", by boiling N-phenyl-N'-selencyanacetylurea with water. He also prepared the 5-methyl derivative (LIV, R=H, R' = CH_3) from α-selenocyanpropionylurea by boiling with alcoholic ammonia. The parent selenazolidone (LIV, R,R' = H) was again prepared by Roy and Guha[103], who noted its instability, and preferred the hydrochloride for their subsequent preparation of the p-acetylaminobenzenesulphonamide derivative (XVI). This compound, however, was not deacetylated as were the other similar selenazole derivatives (XV), possibly due to

instability. Biological results were to be published elsewhere, but so far have not appeared. A number of 2-dialkylamino-4-selenazolone (LXVII) hydrohalides and



R = H
R' = CH_3; C_2H_5; H.
R'', R''' = CH_3; C_2H_5

(LXVII)

picrates were synthesised by Zingaro, Bennett and Hammar[190]. Again no biological testing was done.

The synthetic route used was that of Hofmann[154], in which selenoureas and α-halo acids were condensed in dry ethanol. This method, using thiourea, constitutes a general method for the preparation of the sulphur analogues[191]. The yields were not increased by longer heating[192], since decomposition also increased, giving a residue of grey selenium. The majority of the compounds prepared were isolated initially as the hydrohalide, which were crystallised from dry ethanol, or a mixture of ethanol and ether, as high-melting white solids. 5-Methyl-2-imino-4-selenazolidone hydrobromide (LIV, R=H, R' = CH_3) could only be crystallised by stirring the syrupy product under ether, while adding dry ethanol dropwise. These hydrohalides were reasonably stable in air and light but were best stored protected from both. Water rapidly caused decomposition,

easily recognised by the red colouration of the solution.

The 5-propyl-, 5-isopropyl- and 5-phenyl-derivatives (LIV, R=H, R' = C_3H_7, isoC_3H_7, C_6H_5) were obtained as the free base in somewhat lower yields. While the derivatives isolated as the hydrohalide readily formed picrates, all attempts to prepare picrates or hydrohalides from those isolated as the free base were unsuccessful. The conversion of the hydrohalides to the bases was best done using dilute ammonia, sodium carbonate or sodium acetate[192]. With excess sodium hydroxide, the precipitated base redissolved, showing amphoteric properties like the sulphur analogues which are also soluble both in acid and basic solutions. The bases, generally, were less stable than the salts.

When the synthesis of 5,5-dimethyl-2-imino-4-selenazolidone (LIV, R, R' = CH_3) was attempted, concentration of the reaction mixture yielded off-white crystals, which rapidly decomposed. Repetition in the absence of air and light was no more successful. Acid hydrolysis of the crude material gave 5,5-dimethylselenazolid-2,4-dione (LV, R, R' = CH_3). Attempted preparation of the picrate gave only a sticky yellow syrup. Similar difficulties were encountered in the attempted preparation of 5,5-diethyl-2-imino-4-selenazolidone (LIV, R, R' = C_2H_5) and although it was possible to isolate pale yellow needles, these decomposed under the mildest drying conditions. Acid hydrolysis of the crude product gave a deep red semicrystalline product which could not be purified. This instability is similar to that reported for the 5,5-dialkyl-

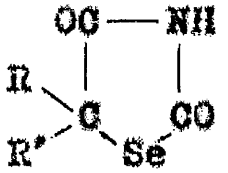
2-selenobarbituric acids (XLIV, R, R' = alkyl)[100]. The only 5,5-disubstituted derivative isolated was apparently 5,5-diphenyl-2-imino-4-selenazolidone (LIV, R, R' = C_6H_5), which has so far defied attempts at purification. Acid hydrolysis gave a brown, oily liquid which, on sublimation, gave white crystals which were too unstable for analysis.

2-Dimethylamino-4-selenazolone (LXVII, R, R' = H; R''; R''' = CH_3)[190] and 5,5-dimethyl-2-dimethylamino-4-selenazolone (LXVII, R, R; R''; R''' = CH_3) were synthesised using the method of Zingaro, Bennett and Hammar[190]. These were required for physical measurements relating to the tautomerism of 2-imino-4-selenazolidones. 5,5-Dimethyl-2-dimethylamino-4-selenazolone (LXVII, R, R; R''; R''' = CH_3) was recrystallised from dry ethanol and ether at 0°, in the absence of light.

Condensation of selenoureas and α-halo acids therefore provides a general method for the synthesis of 2-amino-4-selenazolones, and 2-imino-4-selenazolidones.

Preparation of Selenazolid-2,4-diones.

Chloracetic acid and selenourea heated together in water gave selenazolid-2,4-dione (LV, R, R' = H). The same



(LV)

product was obtained by hydrolysis of 2-imino-4-selenazolidone (LIV, R,R' = H). Since no higher homologues had been prepared, it seemed of interest to prepare other derivatives in view of the extensive work done on the oxygen and sulphur analogues[145,193]. Of the two methods investigated, acid-catalysed hydrolysis of the 2-imino-4-selenazolidones gave higher yields than the condensation of selenoureas and α-halo acids. The former method has been successfully applied to the synthesis of oxygen[194] and sulphur analogues[145]. The optimum time was two hours, after which the yields were good and could not be increased by more than a few per cent on longer heating.

The products were isolated by extraction of the acidic reaction mixture with ether and evaporation of the combined ether extracts to give pale yellow oils which solidified in vacuo. 5-Ethylselenazolid-2,4-dione (LV, R=H, R' = C_2H_5) crystallised on addition of a few drops of water. Purification was best done by sublimation or high vacuum distillation. Alternatively, selenazolid-2,4-dione (LV, R,R' = H) and its 5-ethyl derivative (LV, R=H, R' = C_2H_5) could be crystallised from water, but the other compounds were always tinged pink and were impure. On cooling, 5-phenylselenazolid-2,4-dione (LV, R=H, R' = C_6H_5) crystallised from the reaction mixture, in somewhat lower yields than the

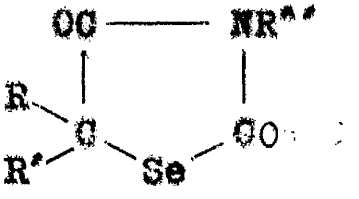
other derivatives. It was insoluble in ether. All these compounds were stable in air and light for several months, after which slight decomposition occurred.

Since 5,5-dimethyl-2-imino-4-selenazolidone (LIV, R,R'= CH_3) could not be isolated, 5,5-dimethylselenazolid-2,4-dione (LV, R,R'= CH_3) was prepared from selenoureа and α-bromo-isobutyric acid in water. Extraction and purification as before gave the required product, which was stable enough for analysis but after several days turned slightly pink. The preparation of 5,5-diethylselenazolid-2,4-dione (LV, R,R'= C_2H_5) by a similar method gave a deep red solid which could not be purified. The attempted preparation of 5,5-diphenyl-selenazolid-2,4-dione (LV, R,R'= C_6H_5) similarly gave a brown oil which gave highly unstable crystals on sublimation.

The selenazolid-2,4-diones (LV) are low melting solids, sparingly soluble in water giving acidic solutions and can be titrated with alkali. They are freely soluble in methanol, ethanol and ether.

METHYLATION OF SELENAZOLID-2,4-DIONES AND 2-IMINO-4-SELENAZOLIDONES

The possibility of O-methylation rather than N-methylation of oxazolid-2,4-diones (XLVIII, R,R′= alkyl, R′′= H) was discussed by Davis and Hook[194]. It was concluded that condensation of the potassium or sodium salts with methyl iodide in dry acetone or ethanol resulted in N-methylation. 3-Methylselenazolid-2,4-dione (LXVIII, R,R′= H, R′′= CH_3) and 3-methyl-5-ethylselenazolid-2,4-dione (LXVIII, R=H, R′= C_2H_5, R′′= CH_3) were prepared by



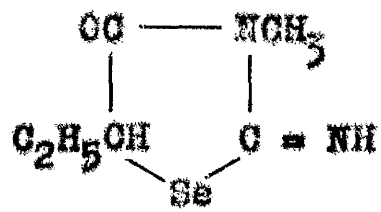
(LXVIII)

the action of methyl iodide on the potassium salts of the corresponding selenazolid-2,4-dione (LV, R=H, R′= H or C_2H_5), prepared in excellent yield in dry ethanol. These salts were pale yellow hygroscopic solids, which were therefore prepared *in situ* as required. The same products (LXVIII, R=H, R′= H or C_2H_5, R′′= CH_3) were also obtained by the action of ethereal diazomethane on the corresponding selenazolid-2,4-dione (LV, R=H, R′= H or C_2H_5). This was established by comparison of infrared and ultraviolet

absorption spectra which were identical.

That N-methylation has in fact taken place is supported by the identity of the products from methyl iodide and diazomethane, since diazomethane gave 3-methylthiazolid-2,4-dione (XLIV, R,R' = H, R'' = CH_3) with thiazolid-2,4-dione (XLIX, R,R',R'' = H)[195]. Thus methylation of thiazolid-2,4-diones and selenazolid-2,4-diones takes the same course.

5-Ethyl-2-imino-4-selenazolidone (LIV, R = H, R' = C_2H_5) reacted with methyl iodide in dry ethanol to give 3-methyl-5-ethyl-2-imino-4-selenazolidone (LXIX) as the



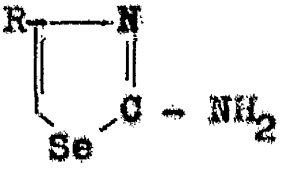
(LXIX)

hydriodide, from which a picrate could be prepared. The same reaction with 2-imino-4-selenazolidone (LIV,R,R' = H) gave a white crystalline compound which could not be purified for analysis and which decomposed on drying. The same difficulties were encountered with the picrate. This product and 3-methyl-5-ethyl-2-imino-4-selenazolidone hydriodide (LXIX) gave the corresponding 3-methylselenazolid-2,4-dione (LXVIII; R=H,R' = H or C_2H_5,R'' = CH_3) on acid hydrolysis. The

identity of these products was established by comparison of the ultraviolet and infrared absorption spectra, which were identical with those from the products obtained by action of diazomethane on the selenazolid-2,4-diones.

ACYL AND SULPHONYL DERIVATIVES OF 2-IMINO-4-SELENAZOLIDONE

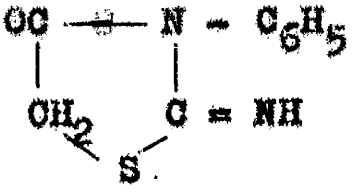
Acetylation of 2-aminoselenazoles (LXX) was reported by Backer[156], by the action of acetic anhydride on the free



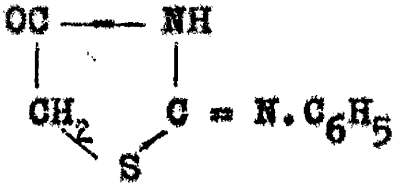
R = alkyl, aryl.
R' = H, alkyl

(LXX)

base. Wheeler and Johnson[196] isolated "phenylthiohydantoin" in two forms (LXXI) and (LXXII). The labile isomer (LXXI)

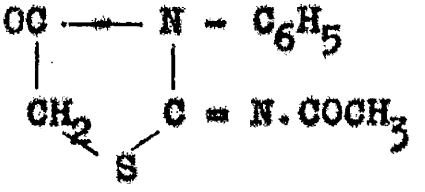


(LXXI)



(LXXII)

reacted with thiolacetic acid in benzene to give an acetyl derivative (LXXIII), while the stable isomer (LXXII), which

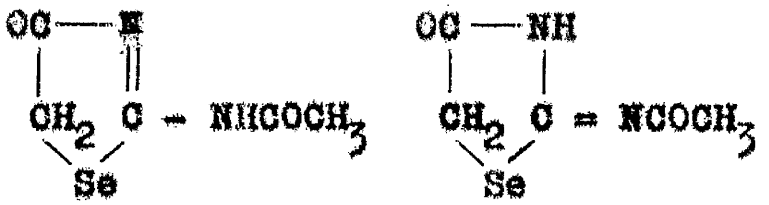


(LXXIII)

did not react with thiolacetic acid, gave a diacetyl derivative of uncertain structure with acetic anhydride.

2-Imino-4-selenazolidone (LIV) or its hydrochloride

gave an acetyl derivative with acetic anhydride and glacial acetic acid which was assumed to have structure (LXXIV, (a) or (b)) since the ultraviolet absorption spectrum (Table XII) suggested "isolation" of the acetyl chromophore from the ring system. Similar treatment of the 5-methyl- and 5-ethyl-

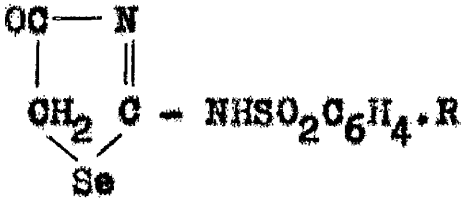


(a) (b)

(LXXIV)

derivatives (LIV, R=H, R' = CH_3 or C_2H_5) gave no acetylated product. Hydrolysis of the acetyl derivative (LXXIV) with dilute hydrochloric acid gave selenazolid-2,4-dione (LV, R,R' = H) and acetic acid.

Roy and Guha[108] prepared the p-acetylaminobenzene-sulphonamide derivative (XVI) but the deacetylated product was not isolated although similar selenazole derivatives (XV, R=H) were prepared. Using a similar method to that of Roy and Guha, the p-toluenesulphonamide- (LXXV, R = CH_3) and

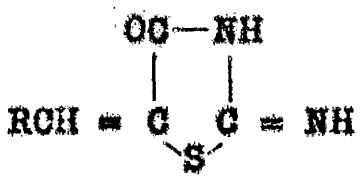


(LXXV)

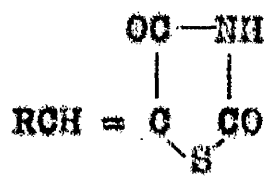
benzenesulphonamide derivatives (LXXV, R=H) of 2-imino-4-selenazolidone were prepared. The attempted isolation of benzoyl derivatives under neutral and alkaline conditions[197] gave gummy products, which could not be crystallised.

ALKYLIDENE DERIVATIVES OF SELENAZOLID-2,4-DIONE AND 2-IMINO-4-SELENAZOLIDONE

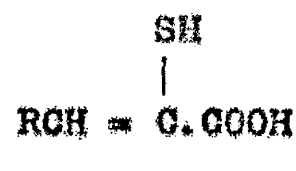
Open chain and cyclic compounds with a reactive methylene group react with aromatic aldehydes to give arylidene derivatives[198]. Thus 2-imino-4-thiazolidone (L, R,R' = H) and thiazolid-2,4-dione (XLIX, R,R';R'' = H) were condensed with aromatic aldehydes by Libermann, Himbert and Hengl[199] who hydrolysed the products (LXXVI) and (LXXVII)



(LXXVI)



(LXXVII)



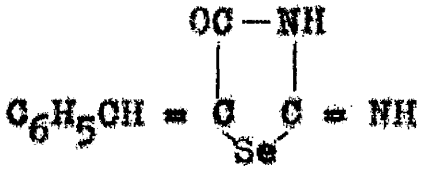
(LXXVIII)

R = aryl, heterocyclic

with alkali to produce substituted thiopyruvic acids (LXXVIII). The condensation was carried out in glacial acetic acid and sodium acetate[200]. Markeley and Reid[201] had earlier condensed 3-phenylthiazolid-2,4-dione (XLIX, R,R' = H, R'' = C_6H_5) with various aldehydes by the same method, while Ruhemann[202] used ethanol plus a few drops of piperidine as the condensing agent. Vladzimirskaya[203], in a recent exhaustive study, described the optimum conditions for the preparation of 5-benzylidene-2-imino-4-thiazolidone (LXXVI, R = C_6H_5) and 5-benzylidenethiazolid-

2,4-dione (LXXVII, R = C_6H_5). The former compound (LXXVI, R = C_6H_5) was prepared from thiourea, chloracetic acid and benzaldehyde in glacial acetic acid, while the latter (LXXVII, R = C_6H_5) was prepared from the same reagents in hydrochloric acid, neither method using sodium acetate.

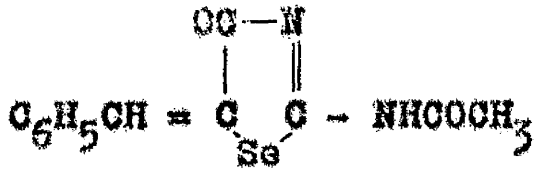
The attempted preparation of 5-benzylidene-2-imino-4-selenazolidone (LXXIX) using the method of Libermann,



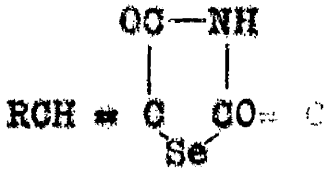
(LXXIX)

Himbert and Hengl[199] gave yellow crystalline products to which no structure could be assigned. 2-Imino-4-selenazolidone hydrochloride (LIV, R,R' = H) was obtained using the method of Vladzimirskaya[203], while no reaction took place in the presence of piperidine and ethanol[202].

Under the same conditions[199], 2-acetylamino-4-selenazolone (LXXIV) gave 5-benzylidene-2-acetylamino-4-selenazolone (LXXX), and selenazolid-2,4-dione (LV, R,R' = H)



(LXXX)



(LXXXI)

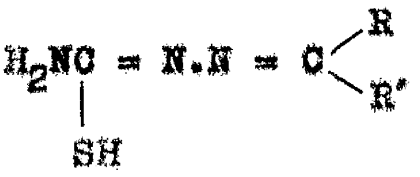
gave 5-benzylidene- and 5-salicylideneselenazolid-2,4-dione

(LXXXI, R = C_6H_5, C_6H_4OH).

Thus the methylene group of 2-imino-4-selenazolidone (LIV, R,R' = H) does not react normally under these conditions. That the acetyl derivative (LXXIV) and selenazolid-2,4-dione (LV, R,R' = H) do react may be explained since in both these compounds, a more electronegative group occupies the 2-position, which would activate the methylene group.

PREPARATION OF SELENAZOLID-2,4-DIONE-2-ALKYLIDENEHYDRAZONES

Wilson and Burns[204] and Stephen and Wilson[205] showed that thiosemicarbazones of aldehydes and ketones (LXXXII)



R = H, alkyl, aryl;

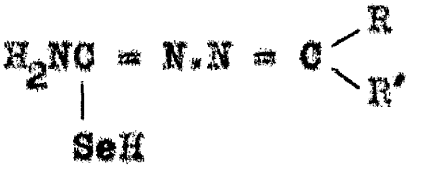
(LXXXII)

R' = alkyl, aryl, heterocyclic;
R'' = H, alkyl, aryl.

(LXXXIII)

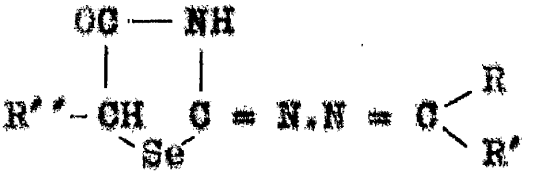
condensed with α-haloacids in the presence of sodium ethoxide, to give thiazole derivatives (LXXXIII). A similar reaction described by Jack[179], gave 1-substituted-amino-hydantoin derivatives (LVII) and (LVIII), from the corresponding semicarbazones. Chabrier and Cattelain[206] found that although aldehyde thiosemicarbazones readily condensed with chloracetic acid in ethanol, to give substituted hydrazones (LXXXIII, R'' = H), the reaction could not be used for ketone thiosemicarbazones. Addition of a large excess of sodium acetate however, allowed the reaction to be used for the condensation of both aldehyde and ketone thiosemicarbazones to give substituted hydrazones (LXXXIII, R'' = H), and a large number of these were prepared[207].

Acetone selenosemicarbazone (LXXXIV, R,R' = CH_3)[121] and benzaldehyde selenosemicarbazone (LXXXIV, R = H, R' = C_6H_5)[122] were condensed with α-haloacids using the



(LXXXIV)

method of Chabrier and Cattelain[207], to give selenazolid-2,4-dione-2-isopropylidenehydrazones (LIX, R,R' = CH_3;

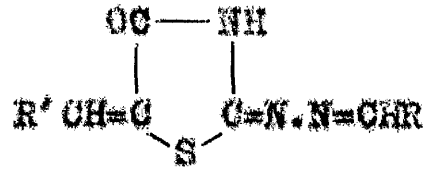


(LIX)

R'' = H, C_2H_5, C_6H_5) and selenazolid-2,4-dione-2-benzylidene-hydrazones (LIX, R = H, R' = C_6H_5; R'' = H, C_2H_5). Using Jack's method[179], acetone selenosemicarbazone and ethyl-chloroacetate gave selenazolid-2,4-dione-2-isopropylidene-hydrazone (LIX, R,R' = CH_3; R'' = H). Thus the condensation of acetone thiosemicarbazone (LXXXIII, R,R' = CH_3)[204] and acetone selenosemicarbazone (LXXXIV, R,R' = CH_3) with chloracetic acid under alkaline conditions gave 2-isopropylidene-hydrazone derivatives (LXXXIII, R,R' = CH_3; R'' = H) and (LIX, R,R = CH_3; R'' = H), while acetone semicarbazone gave an isomeric hydantoin derivative (LVII)[179]. This similarity

between sulphur and selenium compounds agrees with the suggestion of Friedman[96] that sulphur and selenium compounds are more often isosteric than those of oxygen and sulphur. It has also been noted that some sulphur and selenium compounds give crystalline structures differing from the corresponding oxygen analogues[101,105].

Vladzimirskaya[203] described the preparation of 5-arylidene-thiazolid-2,4-dione-2-arylidenehydrazones (LXXXV) from thiosemicarbazones, chloracetic acid and aromatic



(LXXXV)

aldehydes by refluxing in glacial acetic acid in the presence of sodium acetate and acetic anhydride for an hour. Similar treatment of acetoneselenosemicarbazone and benzaldehyde with chloracetic acid gave selenazolid-2,4-dione-2-benzylidenehydrazone (LIX, R,R''= H, R'= C_6H_5), which gave no depression of melting point on admixture with the product from chloracetic acid and benzaldehyde selenosemicarbazone. Comparison of the infrared spectra showed the two compounds to be identical. This can be explained since replacement of the isopropylidene by the benzylidene grouping is easily accomplished[122], and may take place before ring closure, as

in the preparation of benzaldehyde selenosemicarbazone[122], or after cyclisation, as seen from the reaction of selenazolid-2,4-dione-2-isopropylidenehydrazone with benzaldehyde.

Hydrolysis of the selenazolid-2,4-dione-2-substituted hydrazones (LIX) gave the corresponding selenazolid-2,4-dione (LV)[204]. This reaction was useful in establishing the structure of the product from Jack's reaction[179], and also that from the reaction of selenazolid-2,4-dione-2-isopropylidenehydrazone and benzaldehyde, both of which gave selenazolid-2,4-dione (LV, R,R' = H), showing that the substituent was in the 2-position.

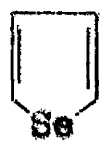
TAUTOMERISM OF SELENAZOLONES

The Fundamental Ring System.

Simple π-excessive heteroaromatics.

The selenazolones may be regarded as derived fundamentally from the five-membered heteroaromatic system selonazole (LXXXVI) which is the analogue of selenophene (LXXXVII). It is interesting therefore, to consider the

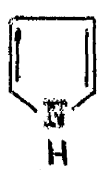
(LXXXVI)



(LXXXVII)

properties of selenazolone and its derivatives in the context of this relationship.

According to Albert[209], five-membered heteroaromatic substances such as pyrrole (IX), furan (X) and thiophen (V), in which the heteroatom is able to contribute a lone pair of electrons to the ring, so that the molecule is stabilised by the formation of an aromatic sextet of electrons, are



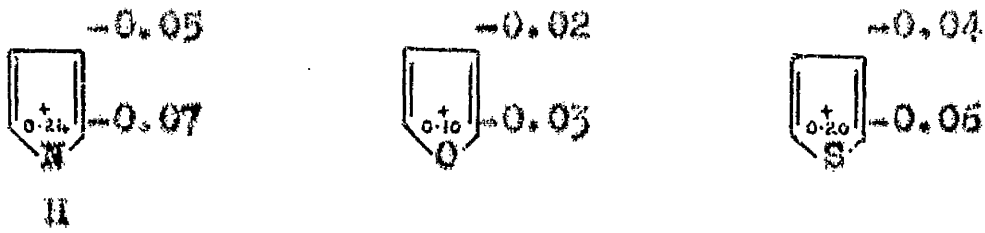
(IX)

(X)

(V)

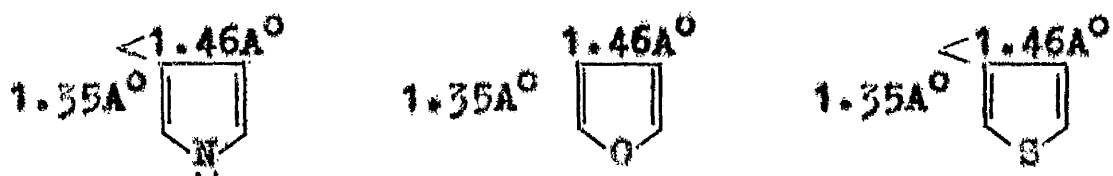
classed as π-excessive heteroaromatics. This description

arises from the redistribution of charge between the hetero-atom and the ring which, from valency-bond (resonance) calculations acquires a nett excess of charge[210]. In broad



II

terms, the classification as π -excessive is in keeping with the reactivity of these ring systems towards electrophilic reagents, although as explained by Albert[211], the actual nett charges calculated by this one method may not give a true picture of their reactivity. Thus thiophen undergoes electrophilic substitution less readily than furan, despite the apparently greater redistribution of charge shown by the former in these calculations. In the Friedel-Crafts reaction, for example, experiment has shown that reactivity decreases in the order pyrrole > furan > thiophen. Other, and perhaps more acceptable, criteria of aromaticity such as electron diffraction measurements of bond lengths[212], indicate that carbon-carbon double bonds are shorter and carbon-carbon single bonds longer than in benzene.

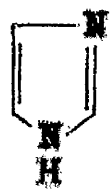


Percentage double bond character of C-heteroatom bond.

This evidence, together with calculations of the percentage double bond character of the carbon-heteroatom bond, suggests that aromaticity parallels decreasing electro-negativity of the heteroelement ($O > N > S$)[213]. Factors, such as the greater number of canonical forms which can participate in the resonance hybrid of thiophen, and their contribution to the gain in aromaticity of thiophen have also been discussed[213]. This arises from the position of sulphur in the second short period of the periodic table. Selenophen (LXXXVII) with a less electro-negative hetero-atom, would therefore be expected to exhibit even more pronounced aromatic character in the light of the foregoing evidence. Briscoe and Peel[214] described the properties of selenophen and stated that it resembles thiophen (V) in its remarkable stability and chemical inactivity. It has recently been shown[215] from the study of ultraviolet absorption spectra of α-substituted selenophens, that their behaviour was comparable with that of thiophens and inter-mediate between furans and benzenes. From calculations of transition energy differences, the same authors showed[216] that the selenophen nucleus was more highly aromatic in character than that of thiophen, and it was concluded that aromaticity decreases in the order selenophen $\geq$ thiophen $>$ furan.

Azalogues of Simple π -Excessive Heteroaromatics.

Replacement of any of the =CH- groups by nitrogen in the simple π -excessive heteroaromatics already discussed gives rise to azalogues of which only imidazole (LXXXVIII) oxazole (LXXXIX), thiazole (XC) and selenazole (LXXXVI)



(LXXXVIII)

(LXXXIX)

(XC)

(LXXXVI)

are relevant to this work. The doubly bound nitrogen which is electron attracting, counters the release of electrons to the ring by the first heteroatom so that although these substances are still π -excessive, they are less so than the parent compounds[217]. This opposing polarity of the new heteroatom enhances the weakly acidic character, as well as the basic properties of the parent rings. Water solubility is increased through the additional lone pair of electrons which is available for hydrogen bonding with water. In other respects, however, it is difficult to generalise about the influence of the second heteroatom. Pullman and Metzger[218] have derived charge distribution diagrams for thiazole which show a high electron density in the 5-position and thus electrophilic substitution takes place most readily there, especially if an electron-releasing group such as

amino, hydroxyl or alkoxyl occupies the 2-position[219]. Comparable data for the other substances is not available.

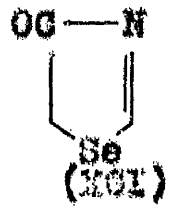
Imidazole and oxazole are markedly more stable to acid than pyrrole and furan. Thiophen is polymerised on prolonged treatment with concentrated sulphuric acid but thiazole is stable under similar conditions[220]. Selenophen resinified in the presence of sulphuric acid[214]. Selenazole could not be isolated by Metzger and Bailly[221], who attempted its synthesis by reducing diazotised 2-aminoselenazole with copper chloride. They concluded that although selenazole probably was formed, it decomposed spontaneously under normal conditions. It was also noted that previous attempts to prepare selenazole directly had been equally unsuccessful.

Keto-Enol Tautomerism in N-Heterocyclics.

The tautomerism of hydroxy derivatives of N-heterocyclics has long been recognised. The preparation of selenazolones from chloracetic acid has led to tacit acceptance that they should be formulated in the keto form. While this is undoubtedly correct, the evidence for it does not seem to have been reviewed and is therefore discussed.

Although the selenazole ring system (LXXXVI) and its analogues discussed above are essentially π -excessive in

character, the oxygen function in the selenazolones (XCI)



(XCI)

is adjacent to a doubly bound nitrogen, and so it is perhaps more correct to consider these derivatives as 2-hydroxy derivatives of a π-deficient heterocyclic[222]. 2-, 3- and 4-Hydroxypyridine, however, are all less π-deficient than pyridine itself and readily undergo electrophilic substitution[223], so that analogous substitution in the selenazole ring should enhance its π-excessive character. 2- and 3-Hydroxypyridine give identical red colours with ferric chloride, while 4-hydroxypyridine gives a yellow colour. In one reaction, however, these compounds differ from phenols. Methylation usually favours N-substitution rather than O-substitution[224].

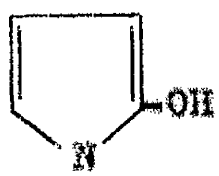
Such chemical evidence alone is insufficient for distinguishing between tautomers since every hydroxy derivative in this series can tautomerise, unlike the corresponding amino compounds[223]. Only physical properties can give the true picture existing at equilibrium. 2- and 4-Hydroxypyridines are weaker bases and acids than 3-hydroxy pyridine, the basic strengths of the former approximating much more closely to the N-methyl isomer than to the O-methyl isomer,

implying that the keto tautomer is favoured[224]. Spectroscopic evidence[225], based on comparison of the N-methyl and O-methyl derivatives with the parent substance, supports this conclusion. The 2-hydroxy derivatives of the other π-deficient heteroaromatics have similarly been shown to exist mainly in the amide form[224]. Albert[226] emphasises the importance of the aromatic nature of the 2- and 4-hydroxy compounds and concludes that any differences between these and their isomers are only a matter of degree. That the amide form predominates can be concluded if keto-enol tautomerism is considered in the selenazole ring in terms of hydroxyl substitution in a ring system which is essentially π-excessive in character.

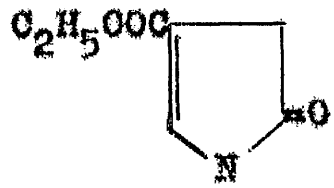
According to Katritsky and Lagowski[227], hydroxy-furans, -pyrroles and -thiophens exist largely or wholly in a non-aromatic tautomeric form, their reactions showing very little resemblance to phenols. In the carbonyl form, these compounds are the cyclic analogues of the very reactive vinylethers, -amines and -sulphides, accounting for the fact that monocyclic hydroxy-furans, -pyrroles and -thiophens are difficult to prepare, very unstable and little known, while no reference to hydroxyselenophens has been found.

2-Hydroxypyrrole (XCII) is unstable but the spectrum of 4-methoxy-2-hydroxypyrrole (XCIII) was shown to be

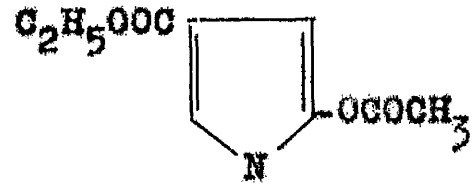
quite different from that of its O-acetyl derivative (XCIV),



(XCII)

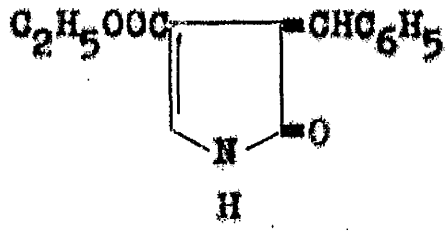


(XCIII)

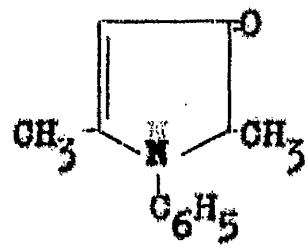


(XCIV)

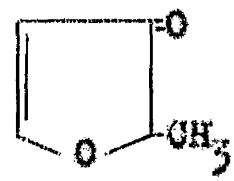
suggesting that structure (XCIII) predominated[228]. Chemical evidence, which is less reliable, supported the existence, but not the predominance of the amide form (XCIII) since condensation with benzaldehyde gave the 3-benzylidene derivative (XCV). However, no general conclusion may be derived from the study of a single substance. 2,5-Dimethyl-3-hydroxy-1 phenylpyrrole (XCVI) is insoluble in dilute



(XCV)



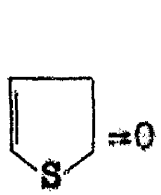
(XCVI)



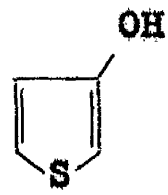
(XCVII)

alkali[229] and this again supports a keto structure, while 2-methyl-3-hydroxy furan (XCVII) yields only one third of a mole of methane with methyl magnesium iodide[230], suggesting that the keto form predominates. Although formally ketonic, these compounds are really amide in character and thus do not react with such agents as semicarbazides[226]. Hurd and Kreuz[231] studied the infrared spectra of 2- and 3-hydroxy-

thiophen. The evidence showed that the equilibrium favoured the keto form (XCVIII) in 2-hydroxythiophen while



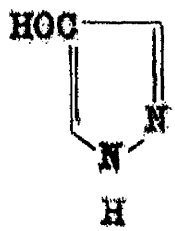
(XCVIII)



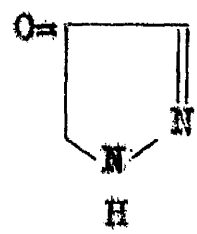
(XCIX)

the hydroxy form (XCIX) seemed to be favoured in 3-hydroxythiophen, which had a phenolic odour and formed an O-benzoate.

4-Hydroxypyrazoles behave like phenols (C) rather

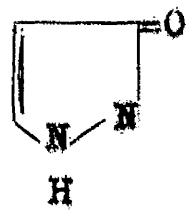


(C)

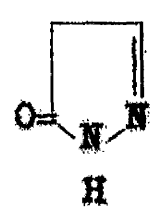


(CI)

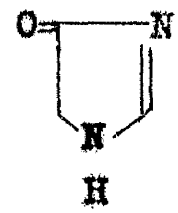
than pyrazolones (CI) although attempts to determine the relative amounts of keto and enol forms indicate an equilibrium[232]. In reaction they are amphoteric, forming salts with both acids and bases. 3- and 5-Hydroxypyrazoles (CII)



(CII)



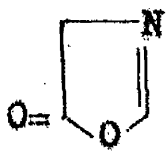
(CIII)



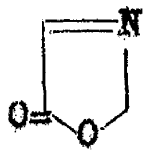
(CIV)

and (CIII), on the other hand, behave primarily as if they

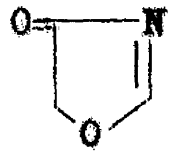
possessed the keto structure[233], as do the 4-imidazolones (CIV)[234]. There are five possible types of oxazolones



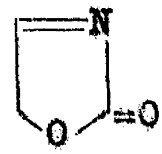
(CV)



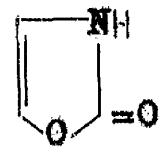
(CVI)



(CVII)



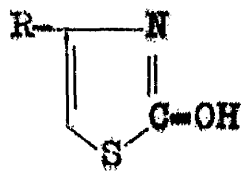
(CVIII)



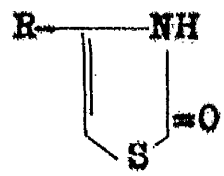
(CIX)

(CV - CIX)[235], of which only those of structure (CV) are numerous, while the existence of any of structure (CVII) is doubtful[236].

2-Hydroxythiazoles exhibit tautomerism similar to that of the 2-hydroxypyridines and may be represented by either the 2-hydroxythiazole (CX) or the 2-thiazolone (CXI) structure[237]. 2-Hydroxythiazole has not been isolated, but the ultraviolet absorption spectrum of 2-hydroxy-4-

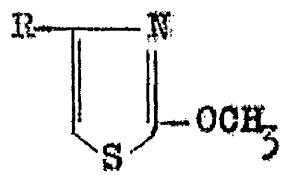


(CX)

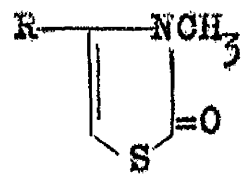


(CXI)

methylthiazole (CX, R = CH_3) indicates a tautomeric mixture, since the curve lies between the curves of the two corresponding methyl derivatives (CXII) and (CXIII). In chloroform



(CXII)



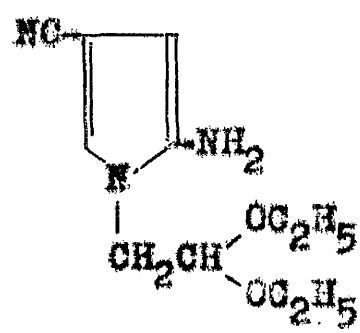
(CXIII)

solution, the infrared spectrum resembles that of the N-methyl derivative (CXIII), thus showing that in this solvent, the compound exists mainly in the keto form (CXI, R = CH_3)[195].

Tautomerism in Amino N-Heterocyclics.

As with the hydroxy derivatives, the introduction of an amino group on to furan, thiophen and pyrrole rings produces compounds which exist largely, if not entirely, in non-aromatic tautomeric form[227]. The reactions of such compounds thus show little resemblance to those of anilines. The free amino compounds are unstable, decomposing rapidly, and the potentially tautomeric compounds react both in amino and imino forms[238]. Physical evidence when available indicates the predominance of the amino form.

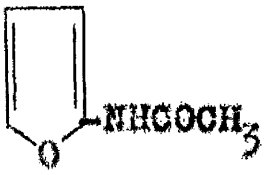
2- and 3-Aminopyrroles are unknown but Grob and Utzinger[228] examined 1-(2,2-diethoxyethyl)-2-amino-4-cyano-pyrrole (CXIV) in the infrared and concluded it to be in the



(CXIV)

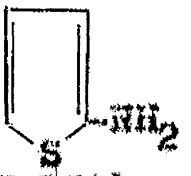
amino form. This is the only evidence so far of an amino-pyrrole and may be atypical. The simplest aminofuran is 3-amino-2-methylfuran (CXV) which is unstable in air,

(CXV)

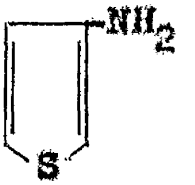


(CXVI)

although diazotisation and coupling with β-naphthol is possible. 2-Acetamidofuran (CXVI) has been prepared but decomposed on attempted hydrolysis. Nothing is known of the tautomeric equilibria of these compounds. 2- and 3-Aminothiophen (CXVII and CXVIII)[239], like hydroxythiophens

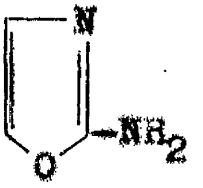


(CXVII)



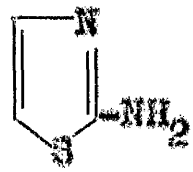
(CXVIII)

(XCVIII and XCIX), are highly unstable in air. 2-Amino-thiophen (CXVII) diazotises and couples normally, but nothing is known of the tautomeric composition of these substances at equilibrium in contrast to the amino derivatives of thiazole. Chemical evidence suggests that derivatives of 2-aminooxazole (CXIX) exist predominantly in

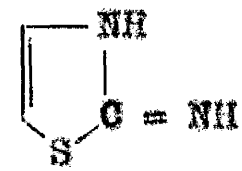


(CXIX)

the amino form[240]. In 2-aminothiazole (CXX), the amino to imino ratio in water at 20° is 20,000 to 1. The evidence produced for the predominance of the imino form (CXXI) is chiefly chemical and can be explained by resonance[241]. The two tautomeric forms (CXX) and (CXXI) do not however, have

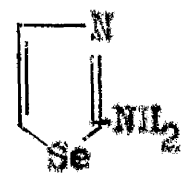


(CXX)



(CXXI)

separate existence and cannot be isolated, although derivatives of both forms are known. 2-Aminoselenazole (CXXII) can be diazotised but on attempted reduction with copper chloride the ring breaks down[221]. Derivatives of 2-aminoselenazole can be isolated[156,221,242].



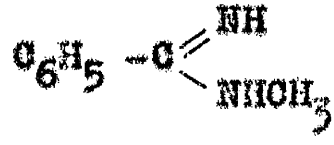
(CXXII)

Simultaneous Substitution of the Aromatic Ring Systems.

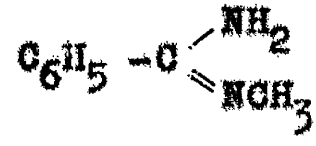
When another group which can participate in a tautomeric shift is introduced into an aminothiazole, many of the aromatic properties disappear and properties expected of a cyclic isothiourea or amidine predominate[243]. Thus the diaminothiazoles, hydroxyaminothiazoles, and mercapto-

aminothiazoles rarely exhibit aromatic properties. The same is true of the dihydroxythiazoles.

Wheland[244] discussed the tautomerism of amidines and noted that, although two non-equivalent structures for N-methylbenzamidine can be written (CXXIII) and (CXXIV),

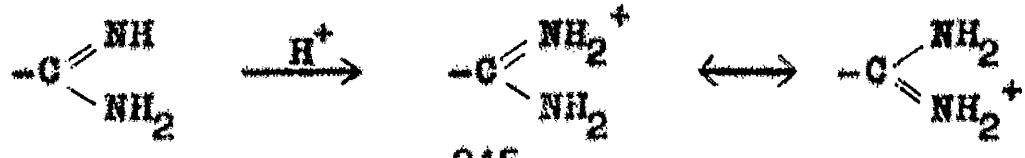


(CXXIII)



(CXXIV)

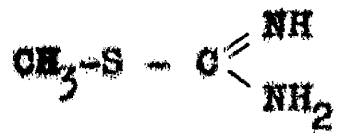
in fact only one substance exists. Angyal and Angyal[241] describe the potentially tautomeric heterocyclic amines as cyclic amidines or vinylogues of them. As in all amidines, cation formation will occur by addition of a proton to the



doubly bound nitrogen atom[245], i.e. to the ring nitrogen in amino forms and to the imino group in the imino forms, resulting in stabilisation by resonance. The potentially tautomeric amines are therefore stronger bases than their isomers and the stronger the amidine character of the amines, the greater is the basic strength[246]. The imino compounds derived from aminopyridines and -quinolines show a basic strength typical of amidines and 1,4-dihydro-2-imino-3-methylthiazole (CXXV) has a basic strength comparable to

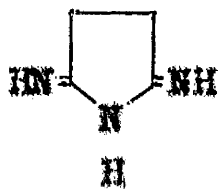
S-methylisothiourea (CXXVI). The imino forms of other

(CXXV)

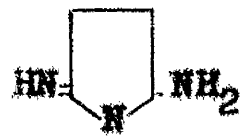


(CXXVI)

heterocyclic amines would presumably show the same non-aromatic basic strength and since all the heterocyclic amines so far studied have considerably lower pK_a values, they probably exist in the amino form. Angyal and Angyal[241] conclude that the amino form is the more stable and therefore predominates in the N-heterocyclic amines. In a study of heterocyclic amines and imines, in particular succinimidine, Elvidge and Linstead[247], however, concluded that the di-imino form (CXXVII) was more stable, and that structure (CXXVIII) makes no appreciable contribution.



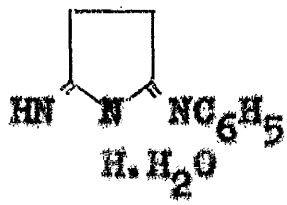
(CXXVII)



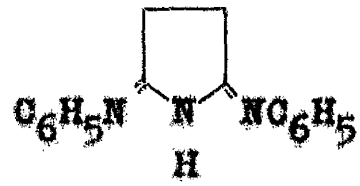
(CXXVIII)

This is based on the argument that it is expected that the amino form (CXXVIII) will absorb above 300 mμ, and so the absence of such absorption suggested this conclusion, although it was stated that the structures of the monophenyl-imine (CXXIX) and 2,5-diphenyliminopyrrolidine (CXXX) were

less certain and that the structural form (CXXVIII) may contribute.



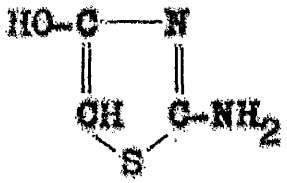
(CXXIX)



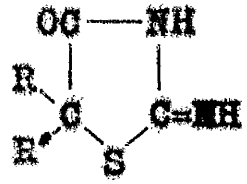
(CXXX)

Hydroxy aminothiazoles can exist in a variety of tautomeric forms which permit their classification as thiazoles, thiazolines, thiazolidines and in many cases derivatives of each form are known. The classification and assignment of structure are rather arbitrary[191]. Generally however, many compounds of this class exhibit few properties of the aromatic thiazole system. Frequently, the amino group cannot be diazotised and can be replaced by acid hydrolysis. This last reaction has been quoted as evidence for the predominance of the imino structure, but Angyal and Angyal[241] suggest that a more likely explanation is that the electron density is low at the ring carbon to which the amino group is attached, so that presumably, nucleophilic replacement would be facilitated. Alkaline hydrolysis on the other hand, cleaves the ring system of hydroxy aminothiazoles indicating their cyclic amidine structure[191].

2-Amino-4-hydroxythiazole (CXXXI) was among the first



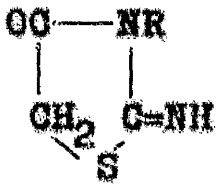
(CXXXI)

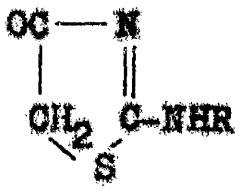


(L)

(CXXXII)

thiazole substances known. It is commonly represented as 2-imino-4-thiazolidone (L, R,R'= H), although 2-amino-4-thiazolone (CXXXII) is equally appropriate[191]. Condensation of thiourea and chloracetic acid (or its esters)[192] and its higher homologues gives a general method of preparation. The parent compound (L, R,R'= H) is a weak acid, dissolving in aqueous alkali. The 3-substituted-2-imino compound (CXXXIII) which is labile, also dissolves in alkali but the





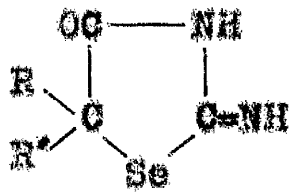
R = alkyl

(CXXXIII) (CXXXIV)

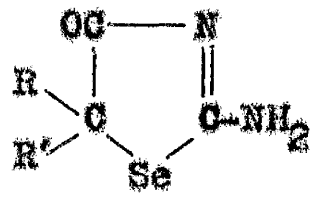
ring opens. Alkylation of the stable 2-amino isomer (CXXXII) usually gives a mixture of the 2-alkylamino- (CXXXIV) and 3-alkyl-2-imino-compounds (CXXXIII)[243]. Acid treatment of compound (L, R,R'= H) hydrolyses the 2-imino group to give thiazolid-2,4-dione (XLIX, R,R'= H), while on alkaline hydrolysis, the ring opens.

Structure of 2-Imino-4-selenazolidones.

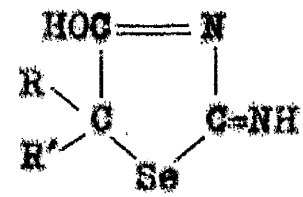
Structures (LIV, LXV, CXXXV, CXXXVI) show possible tautomeric forms of these compounds. The structure of



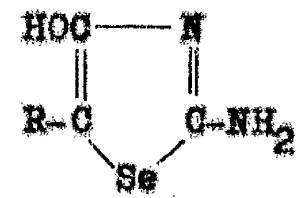
(LIV)



(LXVI)



(CXXXV)

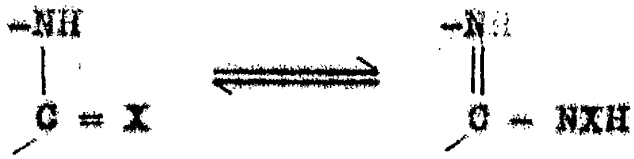


(CXXXVI)

5,5-dimethyl-2-dimethylamino-4-selenazolone (LXVII, R,R';R'';R'''= CH_3) is unambiguous. 2-Dimethylamino-4-selenazolone (LXVII, R,R'=H, R'';R'''= CH_3) and compound (LXVII, R,R';R'';R'''= CH_3) have identical ultraviolet absorption spectra (Table IV), hence tautomerism involving a selenazole ring system such as structure (CXXXVI) may be excluded among 2-amino-4-selenazolones showing a similar absorption spectrum. None of the compounds studied (Table X) showed a hydroxyl band at 3400 $cm.^{-1}$ in the infrared, but a strong band in the 1740 $cm.^{-1}$ region may be attributed to the 4-carbonyl group[249]. Further, if structure (CXXXVI) did exist, it could be expected to have the properties of an aromatic selenazole, the 2-amino group forming a diazonium salt[221]. The attempted preparation of such a salt was unsuccessful.

The Δ^3-selenazoline structure (CXXXV) can be excluded from the presence of the 4-carbonyl band in the

infrared, together with various reports which favour the keto rather than the enol form in comparable heterocyclic systems[224,250]. Marshall and Walker[251] in a study of potentially tautomeric pyrimidines, studied the protropic system shown. When X = O, this system is analogous to the



where X = O, S, NH

Δ^3-selenazoline structure (CXXXV). They report studies by Arndt which led to the conclusion that, when X = O, open chain amides never exhibit this tautomerism and that a potential amide group incorporated into a heterocyclic ring system rarely inhibits this tautomerism. They do, however, point out that these studies were carried out in ether and not in aqueous solution. Katritsky and Jones[250] in a review of protonation of amides, state that various studies indicate that amides exist predominantly in the keto form. Assuming, therefore, that this conclusion is valid, the Δ^3-selenazoline structure (CXXXV) is unlikely, and so can be eliminated from consideration as being, at most, of minor importance. The same argument could be applied to the previously eliminated structure (CXXXVI). Thus the two forms most likely to occur can be represented by structure (LIV) and (LXVI) in which the Δ^2-selenazoline ring involves

the predominance of either the exocyclic-imino or -amino group.

Chemical evidence alone is not valid for determining molecular structure in such circumstances[223], hence physical methods such as ultraviolet and infrared spectra and dissociation constants, where obtainable, were used to assist in assigning structures to the 2-imino-4-selenazolidones prepared.

Preparation and Structure of Model Compounds.

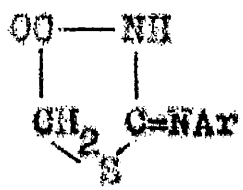
For comparison, compounds were prepared in which the protropic system of the Δ^2-selenazoline had either the 2-amino-(LXVII, R, R' = H, R'', R''' = CH_3) or 2-imino-structure (LXIX). In the preparation of these compounds, methyl substitution was used since it is assumed that this has little or no effect on absorption characteristics[241,250] and ionisation constants[224,241], and has been used to assign structure to other heterocyclic amino-imino systems. The salts were used in preference to the parent compounds since they are more stable and more soluble in water, ultraviolet absorption spectra in water being the most useful in such investigations[225]. Comparison of the ultraviolet absorption spectra of the selenazolidones (LIV) and their hydrohalides (Table III) showed no variation in λ_{max} and only minor

variations in intensity, so it would appear unlikely that the exocyclic group in the two position had changed[252].

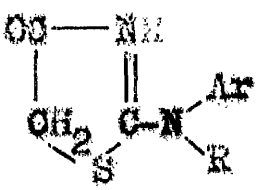
5,5-Dimethyl-2-dimethylamino-4-selenazolone (LXVII, R,R';R''= CH_3) has an unambiguous structure and is useful as a basis in the argument used. 2-Dimethylamino-4-selenazolone (LXVII, R,R'= H,R'; R''= CH_3)[190] is undoubtedly in the 2-amino form, while 3-methyl-2-imino-4-selenazolidone (LXIX) has the 2-imino structure. However, the structure of compound (LXIX) is not unambiguous, and for this reason, is discussed below.

Structure of 3-Methyl-2-imino-4-selenazolidones.

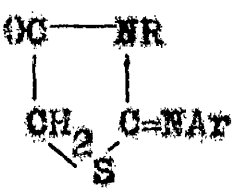
2-Arylimino-4-thiazolidones (CXXXVII) gave mixtures of 2- and 3-alkyl isomers (CXXXVIII) and (CXXXIX) on



(CXXXVII)



(CXXXVIII)



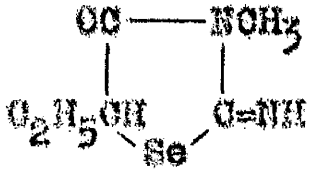
(CXXXIX)

Ar = aryl

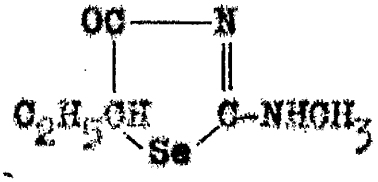
R = alkyl

alkylation[248], thus the same possibility could apply to 2-imino-4-selenazolidones (LIV). When 5-ethyl-2-imino-4-selenazolidone (LIV, R=H, R'= C_2H_5) was refluxed in ethanol with methyl iodide, the product gave a satisfactory elementary analysis, the possible structures being (LXIX), (CXL) or

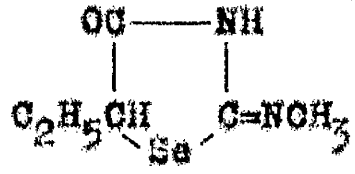
(CXLI). Similar treatment of 2-imino-4-selenazolidone (LIV, R,R' = H) gave a crystalline compound which could not



(LXIX)



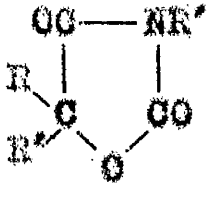
(CXL)



(CXLI)

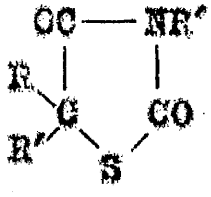
be purified. Since the ultraviolet and infrared experiments discussed later showed (Tables III and X) that 5-substitution did not affect the tautomeric equilibrium, the use of the 5-ethyl derivative seemed valid.

Acid hydrolysis of both methylated products gave oils which had identical ultraviolet and infrared spectra with the methylated products obtained from the potassium salts of the corresponding selenazolid-2,4-diones (LV, R = H, R' = H, C_2H_5) and methyl iodide. Identical products were also obtained by the action of ethereal diazomethane on the corresponding selenazolid-2,4-dione (LV, R = H, R' = H, C_2H_5). Oxazolid-2,4-diones (XLVII, R'' = H) gave 3-methyl



R, R' = H, alkyl

(XLVIII)

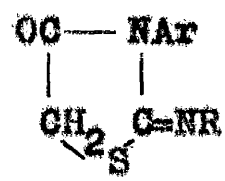


(XLIX)

derivatives (XLVIII, R'' = CH_3) on treatment of the sodium or

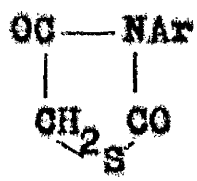
potassium salts with methyl iodide in dry ethanol[194], as did similar sulphur analogues[253]. Thiazolid-2,4-dione (XLIX, R,R';R''= H) gave the 3-methyl derivative (XLIX, R,R' = H, R''= CH_3) with diazomethane[195], so that by analogy, 3-methylation of the selenazolid-2,4-diones (LV, R = H, R'= H, C_2H_5) seems certain. Thiazolid-2,4-dione is reported[243] to have almost identical ultraviolet absorption characteristics with those of the 3-methyl derivative. Similarly 2-thiazolone (CXI, R = H) and its N-methyl derivative (CXIII, R = H) absorb at the same wavelength (240 m/u) while the O-methyl compound (CXII, R = H) absorbs at 237 m/u[195]. Selenazolid-2,4-dione (LV, R,R'= H) showed λ_{max} at 239 m/u, while the 3-methyl derivative (LCVIII, R,R'= H, R''= CH_3) showed λ_{max} at 243 m/u. Similarly the 5-ethyl derivatives showed λ_{max} at 236 and 240 m/u respectively.

In a study of alkyl derivatives of 2-arylimino-4-thiazolidones (CXXXVII), Dains and Eberly[248] refer to work done by Beckurts and Frerichs[254], in which ethylation of such thiazolidones was reported to give 2-ethylimino-3-aryl-4-thiazolidones (CXLII), since, on acid hydrolysis 3-aryl-thiazolid-2,4-dione (CXLIII) was obtained. An earlier paper



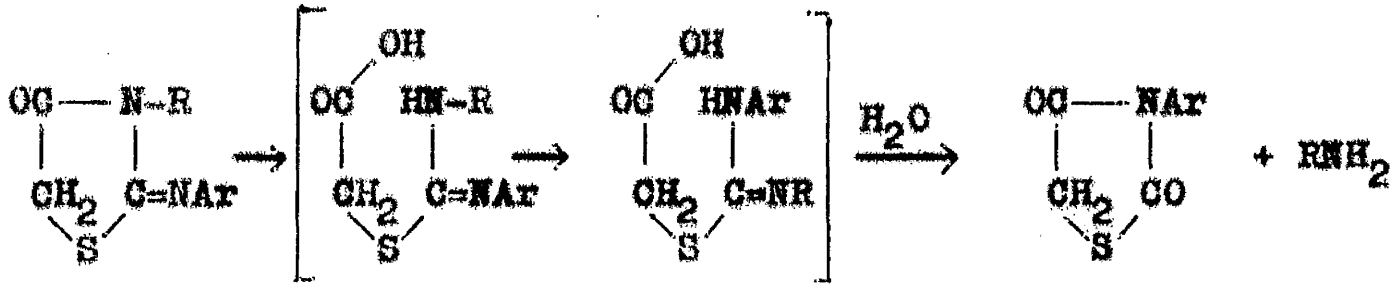
Ar = C_6H_5
R = C_2H_5

(CXLII)



(CXLIII)

by Wheeler and Johnson[196] showed that the 3-aryl form (CXLII, R = H) was labile, rearranging to the more stable 2-arylimino structure (CXXXIX, R = H) which gave on acid hydrolysis, both 3-arylthiazolid-2,4-dione (CXLIII) and thiazolid-2,4-dione (XLIX, R,R';R'' = H). Alkylation of 2-arylimino-4-thiazolidone (CXXXIX, R = H) was finally shown by Daine and Eberly[248] to give a mixture of isomeric products, 2-aryl-2-alkylamino-4-thiazolone (CXXXVIII) and 3-alkyl-2-arylimino-4-thiazolidone (CXXXIX), which gave on hydrolysis, thiazolid-2,4-dione (XLIX, R,R';R'' = H) and 3-alkylthiazolid-2,4-dione (XLIX, R,R' = H, R'' = alkyl) respectively. The assumption was made by Wheeler and Johnson[196] that these two hydrolysis products resulted from an intermediate arylthiohydantoic acid, the ring reclosing after loss of aniline or ammonia. Since the rearrangement probably takes place as shown below, the intermediate product would therefore be an arylisothiohydantoic acid (CXLIV) and

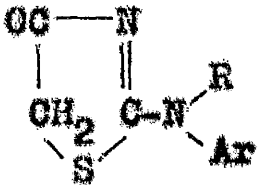


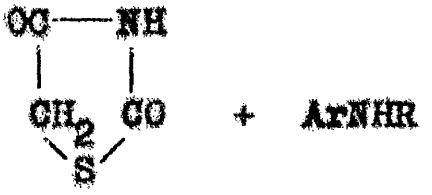
(CXLIV)

where R = alkyl; Ar = aryl

the elimination would be of a primary amine. Similarly the

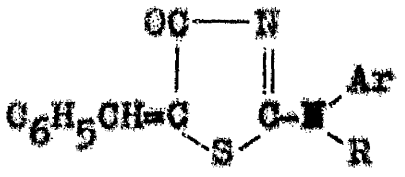
product from 2-aryl-2-alkylamino-4-thiazolone (CXXXVIII) is obtained by elimination of a substituted aniline.



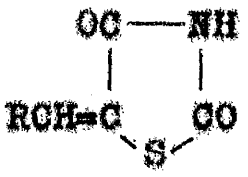


where R = alkyl; Ar = aryl.

Substitution with a 5-benzylidene group stabilised the ring[248] and this compound (CXLV) gave on acid hydrolysis, 5-benzyli-

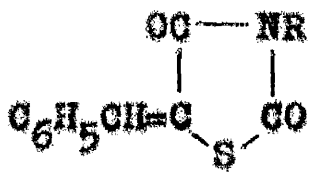


(CXLV)

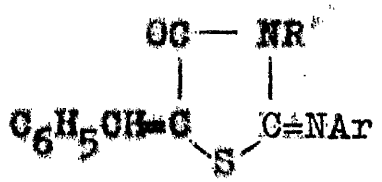


(LXXVII)

dene-thiazolid-2,4-dione (LXXVII, R = C_6H_5). Davis and Dains[255] described the preparation of alkyl derivatives of 2-arylimino-4-thiazolidone (CXXXVII) by condensing the sodium salts in alcoholic solution with alkyl halide. Hydrolysis with alcoholic hydrochloric acid usually resulted in ring opening, but again the 5-benzylidene derivatives were stable and gave on hydrolysis 5-benzylidene-thiazolid-2,4-dione (LXXVII, R = C_6H_5) and 5-benzylidene-3-alkylthiazolid-2,4-dione (CXLVI, R = alkyl) showing that two isomeric products



(CXLVI)

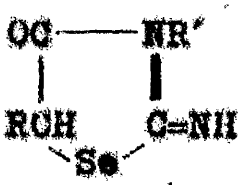


(CXLVII)

(CXLV) and (CXLVII) were produced by alkylation. It might therefore be expected that methylation of 2-imino-4-selenazolidones (LIV) would yield a mixture of the 2- and 3-alkyl derivatives (CXLVIII) and (CXLIX). However, the methylated

R = H or alkyl;
R′= CH_3

(CXLVIII)

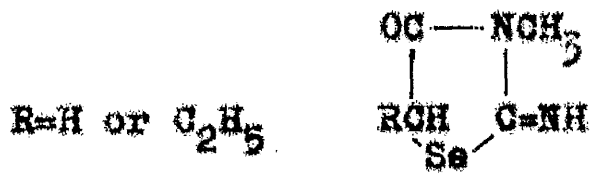


(CXLIX)

product of 5-ethyl-2-imino-4-selenazolidone and the picrate, had well defined melting points, a fact which does not suggest the production of a mixture. Again, hydrolysis of 2-dimethylamino-4-selenazolone (LXVII, R,R′ = H, R′;R′′′= CH_3) gave only selenazolid-2,4-dione (LV, R,R′= H). Since no 3-methylselenazolid-2,4-dione (LXVIII, R,R′= H, R′′= CH_3) was produced, it seems certain that no rearrangement has taken place. Thus, it would seem unlikely that if the 2-alkylamino compounds (CXLVIII) were produced in the methylation of 2-imino-4-selenazolidones (LIV), they would rearrange on hydrolysis to give the 3-methylselenazolid-2,4-diones (LXVIII, R = H, R′= H, C_2H_5, R′′= CH_3) which were obtained.

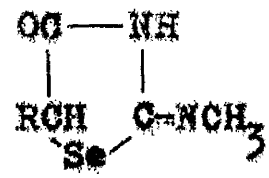
Moreover, the ultraviolet and infrared spectra of the methylated products (Tables IV and X) differ markedly from the model compounds undoubtedly in the 2-amino form (LXVII, R,R′= H or CH_3; R′;R′′′= CH_3), hence structures (CL) or (LXIX)

most adequately represent the methylated products. Amidines



R=H or C_2H_5

(LXIX)



(CL)

alkylate on the doubly bound nitrogen[256] and since by analogy[191] with the 2-imino-4-thiazolidones (L), the 2-imino-4-selenazolidones (LIV) may be regarded as cyclic amidines, it may be argued that alkylation leads to structure (CL). In this case, however, acid hydrolysis which gives compounds (LXVIII, R''= CH_3), would necessitate migration of the methyl group to the 3-position, which as previously discussed, seems unlikely.

Titration with potassium hydroxide of the hydrohalides of the compounds under examination (Table I), showed that only the methylated products could be titrated to give a pK_a value, the 2-imino-4-selenazolidones (LIV) and the model compounds representing the 2-amino form, being too weakly basic. It has been shown that the imino forms of comparable heterocyclic amines have basic strengths similar to those of amidines or isoureas[241], which are strongly basic, so that it might be expected that the 2-imino-4-selenazolidones (LIV) would be strongly basic. These compounds, however, like their sulphur analogues[191], are in fact weakly acidic (Table I),

despite the fact that they form stable hydrohalides. The titration of the methylated product may be ascribed to the base strengthening effect of an N-methyl substituent[246], well known in heterocyclic systems such as amino-pyridines, -quinolines and -thiazoles[257]. Thus the difference in basic strength of the 2-imino-4-selenazolidones (LIV) and the methylated form may be explained since, although both are formally cyclic amidines, the 2-imino-4-selenazolidones (LIV) cannot be titrated since they are such weak bases. This can be attributed to the equilibrium existing between the imino and amino forms since the 2-amino compounds (LXVII), are too weakly basic to be titrated. However, the methylated derivative, in which methylation fixes the molecule in the 2-imino form, can be titrated since, as mentioned above, in such heterocyclic systems, the imino form is more strongly basic than the amino.

Thus it seems reasonable to conclude in the light of the above discussion that structure (LXIX) is correct for the methylated products of 2-imino-4-selenazolidones, and so they can be used as model compounds of the 2-imino form in the following comparisons of physical properties.

pKa Determinations.

The hydrohalides of the compounds under examination

were titrated with potassium hydroxide as described. The titration curves obtained from 2-imino-4-selenazolidone (LIV, R,R'= H) and 2-dimethylamino-4-selenazolone (LXVII, R,R'= H, R'',R'''= CH_3) closely followed that of the free halo acid (see Appendix A, figure 5) and no pKa determination was possible. This indicates that these compounds are very weak bases. From the titration curve of 5-ethyl-3-methyl-2-imino-4-selenazolidone (LXIX), it was possible to calculate the pKa value (see Table I). This can be explained since the 2-imino form might be expected to be more strongly basic than the 2-amino form, as discussed in the previous section. The reason for the difference in basic strengths of the 2-imino form and the 2-imino-4-selenazolidones (LIV) was also discussed. Thus it may be concluded from the titration experiments, that the 2-imino-4-selenazolidones (LIV) as expected, are an equilibrium mixture of the 2-amino (LXVI) and 2-imino (LIV) forms. The attempted titration of Se-benzylselenouronium chloride (LXI) and Se-methylselenouronium iodide (LXII) as examples of isoselenoureas, was unsuccessful. However, from the pH in water (Table I) these compounds would appear to be the salts of strong bases.

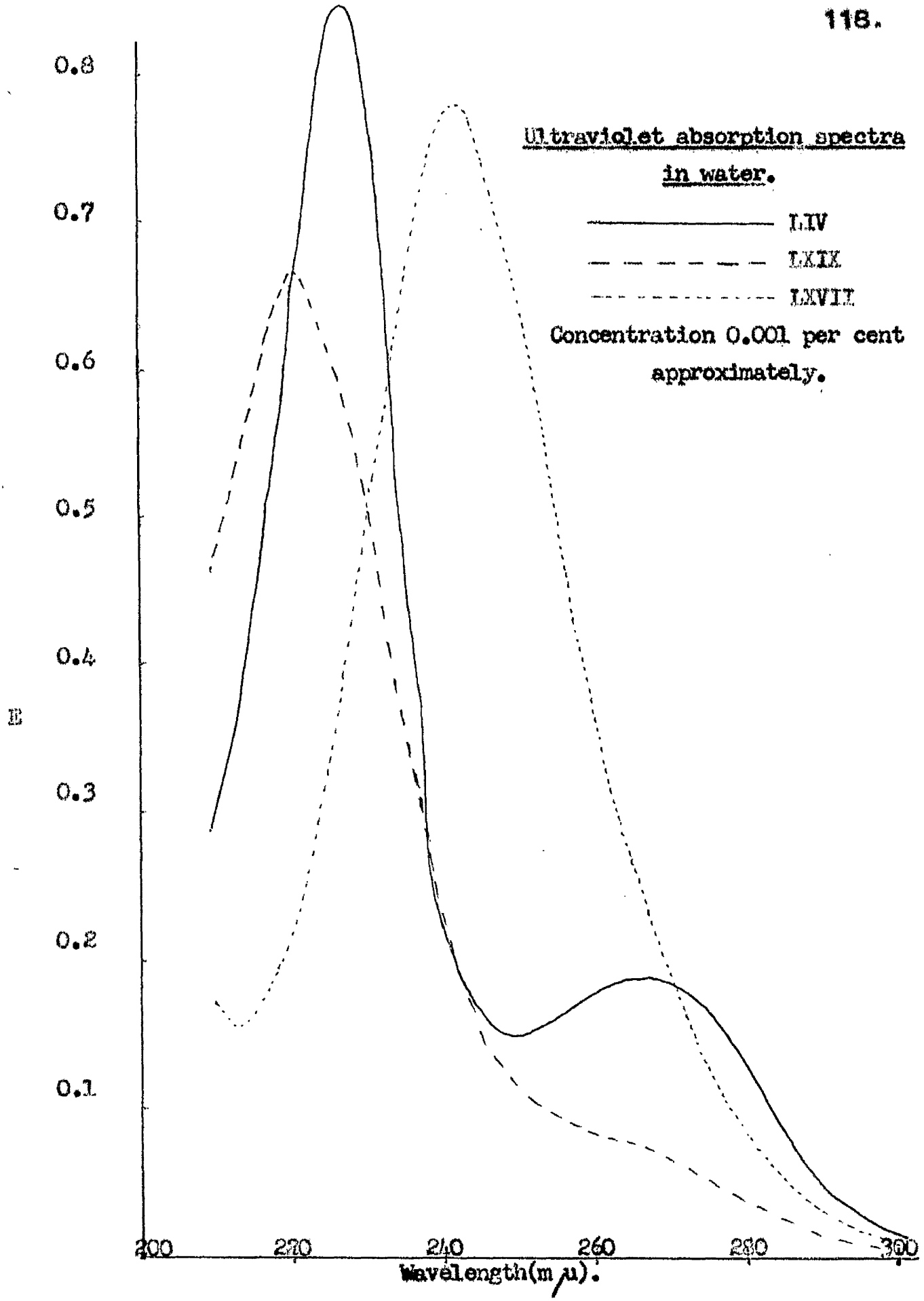

Ultraviolet absorption spectra in water.
LIV
LXIX
LXVII
Concentration 0.001 per cent approximately.
E
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
200
220
240
260
280
300
Wavelength(mμ).

Figure 1.

Ultraviolet Absorption Spectra.

The ultraviolet absorption spectra of several 5-substituted-2-imino-4-selenazolidones (LIV, R=H, R'= H, CH_3, C_2H_5, C_6H_5) were measured in water to determine the effect of 5-substitution on λ_{max} and extinction. The results are recorded in Table III, and from these it can be seen that 5-substitution has little or no effect on λ_{max}, while ε values are relatively slightly affected. Similarly 2-imino-4-selenazolidone (LIV, R,R'= H) and its hydrochloride have practically identical ultraviolet absorption characteristics, so the salts may be used in tautomeric comparisons. The ultraviolet absorption spectra of the model compounds previously mentioned were also measured (Table IV). Thus it was seen that 2-dimethylamino-4-selenazolone (LXVII, R,R'= H, R'';R'''= CH_3) and 5,5-dimethyl-2-dimethylamino-4-selenazolone (LXVII, R,R;R'';R'''= CH_3) had practically identical spectral characteristics, which differed markedly from those of 5-ethyl-3-methyl-2-imino-4-selenazolidone (LXIX, R = C_2H_5). The spectra of the 2-imino-4-selenazolidones (LIV) were more closely similar to that of the latter compound (see figure 1, page 118) which would suggest that the 2-imino structure (LIV) predominates in the 2-imino-4-selenazolidones.

Effect of Change of pH on the Ultraviolet Absorption Spectra.

When a drop of hydrochloric acid was added to approximately 5 ml. of the test solutions in water the ultraviolet absorption spectra of the compounds examined changed. This change was most marked in the case of 2-dimethylamino-4-selenazolone (LXVII, R,R' = H, R'',R''' = CH_3) and 5,5-dimethyl-2-dimethylamino-4-selenazolone (LXVII, R,R',R'',R''' = CH_3), where the spectra changed from showing a single peak at 245 and 246 mμ respectively, to having characteristics very similar to those of 2-imino-4-selenazolidone (LIV, R,R' = H), as can be seen from Table VI. Similar addition of acid to the test solutions of 2-imino-4-selenazolidone (LIV, R,R' = H) and 5-ethyl-3-methyl-2-imino-4-selenazolidone (LXIX, R = C_2H_5) showed an increase in intensity of the peak in the 270 mμ region, or the appearance of a definite peak in the same region if the spectrum in water showed only a shoulder (Table VI). If the hydrochloric acid added was approximately neutralised with sodium hydroxide, it was seen (Table VI) that the ultraviolet absorption spectra assumed their original characteristics, showing that the change occurring on addition of acid was reversible by addition of alkali.

Addition of a drop of 20 per cent sodium hydroxide

solution to 5 ml. of the test solutions had no effect (see Table VII.) on the ultraviolet absorption spectra of 2-dimethylamino-4-selenazolone (LXVII, R,R'= H, R';R'''= CH_3) and 5,5-dimethyl-2-dimethylamino-4-selenazolone (LXVII, R, R';R';R'''= CH_3). The peak absorption at 269 mμ in the spectrum of 2-imino-4-selenazolidone (LIV, R,R'= H) and the shoulder at 240-270 mμ in the spectrum of 5-ethyl-3-methyl-2-imino-4-selenazolidone (LXIX, R = C_2H_5) were both eliminated on addition of alkali, while the peak absorption at 228 and 227 mμ respectively was virtually unchanged. Further addition of acid in these cases showed only a partial reversal and, even considering concentration differences, this would seem to indicate some decomposition in alkaline solution (Table VII).

The above experiments (Tables VI and VII) show the effect of strongly acidic and alkaline conditions on the ultraviolet absorption spectra. The effects of intermediate pH values were shown by measuring the spectra in buffer solutions to cover the pH range at intervals of approximately one pH unit (Table IX).

A test concentration of approximately 0.0005 per cent was sufficient for 2-imino-4-selenazolidone (LIV, R,R'= H) and 2-dimethylamino-4-selenazolone (LXVII, R,R'= H, R';R''' = CH_3). Using a similar concentration of 5-ethyl-3-methyl-

2-imino-4-selenazolidone (LXIX, R = C_2H_5), variations in the extinction of the peak at 270 mμ with change in pH were not great enough. A test concentration of 0.005 per cent was therefore used, and although this concentration was too great to allow measurement of the peak absorption in the 220 mμ region, this was not considered a serious disadvantage since it was shown in the preliminary experiments (Tables VI and VII) that this peak was effectively constant over a wide pH range.

The extinction curves were then measured as described and are recorded in figures 2-4 (see Appendix C) and Table IX. From these results it can be seen that the ultraviolet absorption spectrum of 2-imino-4-selenazolidone (LIV, R, R' = H), shown in figure 2, is practically unchanged over the pH range 0-10, although at the lower pH values, λ_{max} at 229 mμ undergoes a hypsochromic shift to 211 mμ at pH 0, while λ_{max} at 270 mμ undergoes a similar but less pronounced shift to 265 mμ. Highly alkaline conditions, on the other hand, changed the spectrum appreciably, the peak at 269 mμ being eliminated. The changes in the absorption spectrum of 2-dimethylamino-4-selenazolone (LXVII, R, R' = H, R'', R''' = CH_3) with change of pH are shown in figure 3, the most notable feature being the similarity of the spectrum at low pH values with that of 2-imino-4-selenazolidone (LIV, R, R' = H)

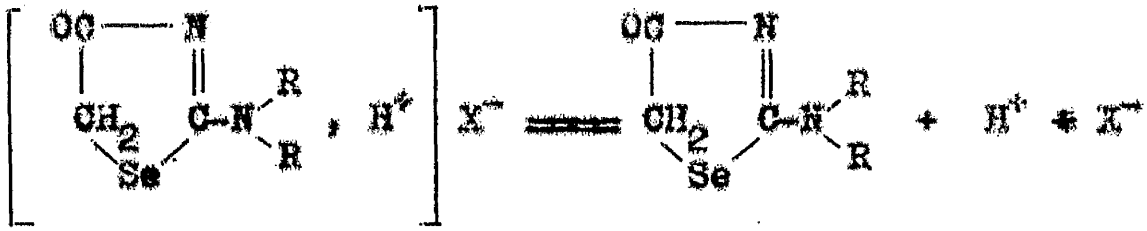
in water.

The variations with change in pH of the partial absorption spectrum of 5-ethyl-3-methyl-2-imino-4-selenazolidone (LXIX, R = C_2H_5) shown in figure 4, are much more gradual. Increase in pH decreases the intensity of λ_{max} until at pH 5 only a broad shoulder is seen. This suggests that the change taking place occurs between pH 4 and 5 and since there is still evidence of λ_{max} in the shoulder at pH 5, it seems likely that the critical pH is very slightly less than 5. This agrees well with the pKa value of 4.9 obtained by titration (Table I).

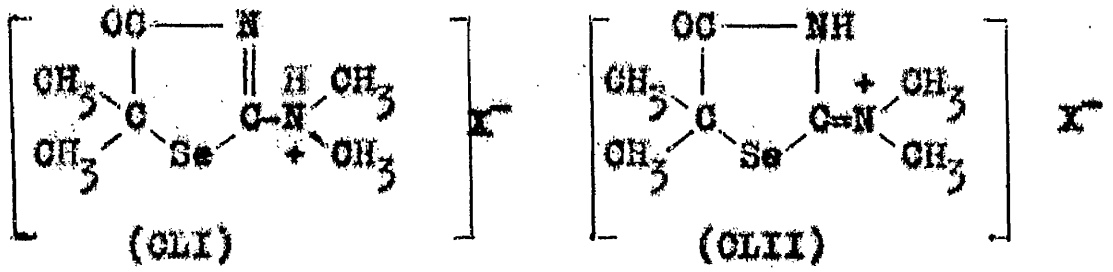
From these results it may be concluded that the change in the respective molecules takes place about pH 1 for the 2-amino form (LXVII) and about pH 5 for the 2-imino form (LXIX). If these pH values signify the ionisation constants, the results would agree with the observed facts that while the 2-imino form (LXIX) is a weak base, pKa *ca.* 5, it is still much stronger than the 2-amino form (LXVII), pKa *ca.* 1.

Thus the 2-amino form (LXVII) titrates as an exceedingly weak base (see Appendix A, figure 5), while the 2-imino form (LXIX) is sufficiently strongly basic to allow the calculation of a pKa value from titration (Table I).

Since the 2-amino form (LXVII) as the hydrohalide, titrates as if free halogen acid alone were present, the equilibrium shown must lie well to the right. At low pH, however, the

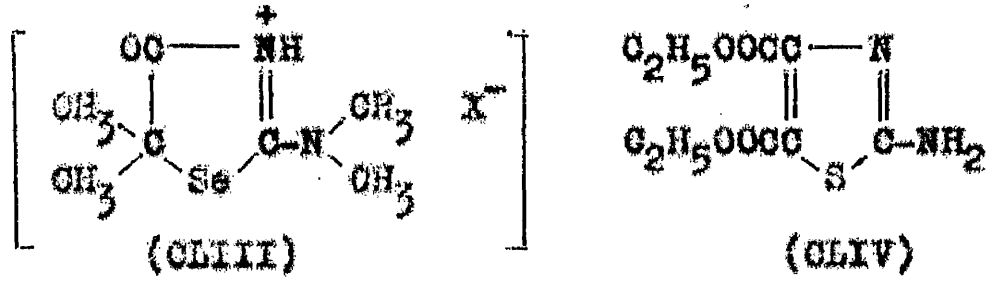


position may be reversed, with consequent alteration in the chromophoric system. At this stage, it is a matter of conjecture as to the actual form acquired by the cation. Thus, structure (CLI) would account for the hypsochromic



(CLI) (CLII)

shift of the 243 mμ band because of the conversion of the $-N(CH_3)_2$ auxochrome to $-\overset{+}{N}H(CH_3)_2$. Structure (CLII) has the advantage of being possible in the cation derived from the imino compound (LXIX) and would account for the similarity of the spectra at low pH (Table IX). On this evidence, another possible structure (CLIII) would appear to be unlikely. A similar hypsochromic shift was reported[252] for



(CLIII) (CLIV)

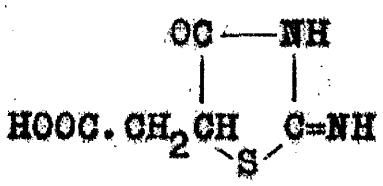
2-amino-4,5-dicarbethoxythiazole hydrochloride (CLIV) which had practically identical ultraviolet absorption characteristics with the free base. It was suggested that the first proton goes to the ring nitrogen and therefore does not effect the chromophoric system. In more strongly acid conditions, the hypsochromic shift may be due to dihydrochloride formation, preventing contributions from resonance structures.

Ionisation Constants from Ultraviolet Absorption Spectra.

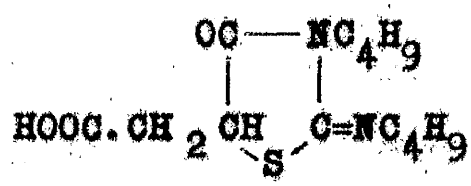
Ionisation constants have been determined using ultraviolet spectra[224]. Albert and Phillips[224] determined pK values of pyridine compounds by a titration method and also utilising the changes in spectra with change in pH. The wavelengths chosen for comparison were those where there was a marked difference between the extinction coefficients of the neutral molecule and the protonated form. Marshall and Walker[251] had previously criticised earlier work, since the pH values for comparison were chosen without knowledge or reference to the pK values of the substances studied. The result was that absorption measurements had been made on mixtures of ions and neutral molecules and changes in shape of the extinction curves with pH variations have been attributed to enolisation, whereas ionisation has really been the

explanation.

From the foregoing experiments on effect of pH on the ultraviolet absorption spectra (Table IX), it seems obvious that the peak absorption in the 270 mμ region is involved in the change taking place. In the case of 5-ethyl-3-methyl-2-imino-4-selenazolidone (LXIX, R = C_2H_5), the limiting pH value, i.e. where the peak at 270 mμ is just eliminated, is approximately pH 5. Since the calculated pKa value for this compound is 4.9 (Table I), it may be argued that the limiting pH value corresponds to the ionisation constant. While this may be true, no corroboration by comparison with the other compounds studied was possible due to their extremely weak basic character. That enolisation was the cause of the extinction variations could be concluded by comparison with the work of Arakelian, Dunn, Grieshammer and Coleman[258]. They found that in the ultraviolet absorption spectrum of 2-imino-4-thiazolidone-5-acetic acid (CLV), which showed λ_{max} at 220 and 251 mμ,



(CLV)



(CLVI)

the peak at 251 mμ was eliminated by 2- and 3-alkylation producing structure (CLVI). It was concluded that the peak at 251 mμ was probably due to tautomerism of

structure (CLV). However, in the 2-imino-4-selenazolidones (LIV), it would appear that pH is the dominant factor and that the spectral changes with pH are due to ionisation or dissociation.

Infrared Absorption Spectra.

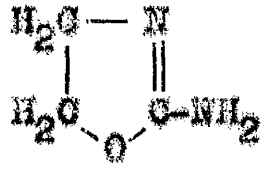
The bands occurring in the 1550-1750 cm.$^{-1}$ region are most important[259] in a study of amino-imino relationships and only the bands found in this region are listed in Table X. The complete spectra of the compounds used in structural elucidation are shown in Appendix B. The amide I and II bands are located in the same range as those of the C=NH group and as stated by Bellamy[260], the bands ascribable to -C=N- bands in cyclic systems are indistinguishable from those of C=NH terminal groups. However, by comparison of the spectra of model compounds of the 2-amino and 2-imino forms, it has been possible to draw some conclusion concerning the structure of the selenium compounds.

From a comparison of the spectra of 5-substituted-2-imino-4-selenazolidones (LIV) it can be seen (Table X) that in the 1550-1750 cm.$^{-1}$ region, 5-substitution does not affect the spectrum, and thus, as expected has no effect on the Δ^2-tautomeric equilibrium. Thus 2-imino-4-selenazolidone (LIV, R,R' = H) has three well-defined bands at

1730, 1680, and 1590 cm.$^{-1}$, which agree well with those of 5-ethyl-3-methyl-2-imino-4-selenazolidone (LXIX, R = C_2H_5) at 1750, 1650 and 1570 cm.$^{-1}$ 5,5-Dimethyl-2-dimethylamino-4-selenazolone (LXVII, R,R;R';R'' = CH_3) and 2-dimethylamino-4-selenazolone (LXVII, R,R' = H, R';R'' = CH_3) both show very well defined bands in the 1740 and 1630 cm.$^{-1}$ regions. An extremely weak band at 1560 cm.$^{-1}$ was recorded in the spectrum of compound (LXVII, R,R;R';R'' = CH_3), but was neglected as unimportant, in view of the fact that there was no trace of it in the spectrum of compound (LXVII, R,R' = H, R';R'' = CH_3) which has identical structure in the 2-position under discussion.

In a study of oxazolid-2,4-diones (XLVIII, R'' = H) a band in the 1754-1730 cm.$^{-1}$ region was assigned to the 4-CO group[249], and as expected, was present in the spectra of all the compounds examined. The band in the 1650 cm.$^{-1}$ region corresponds to the amide I band as explained by Bellamy[261], and was again present in all these compounds.

In a study of 2-amino-oxazoline (CLVII), the band



(CLVII)

in the 1650-1700 cm.$^{-1}$ region was ascribed to the isourea

grouping (O-C(=N)-N), while that ca. 1610 cm.$^{-1}$ signified only the 2-amino form[259]. The spectra of compounds with the 2-amino structure (LXVII) show strong bands at 1630 and 1640 cm.$^{-1}$ respectively, and these may be ascribed to the 2-amino form. The spectra of the other compounds, listed in Table X, show a band at slightly higher wavelength (1650-1680 cm.$^{-1}$) and this may correspond to the isourea band (1650-1700 cm.$^{-1}$) mentioned above.

The presence of the band at 1570-1590 cm.$^{-1}$ is regarded as highly suggestive in view of its presence in the spectra of those compounds in which the 2-imino group is suspected to be present (ultraviolet evidence) and its absence in the spectra of the 2-amino compounds. Thus, while 2-imino-4-selenazolidones (LIV) and 5-ethyl-3-methyl-2-imino-4-selenazolidone (LXIX, R = C_2H_5) show strong bands in three regions, 1730-1750, 1650-1680 and 1570-1590 cm.$^{-1}$, the latter is absent in the spectra of those compounds undoubtedly in the amino form (LXVII). Thus the predominance of the 2-imino form (LIV) is confirmed.

EXPERIMENTAL

Melting points are uncorrected.

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ATTEMPTED PREPARATION OF 2-SELENOBARBITURIC ACIDS

Preparation of Starting Material.

Cyanamide

Cyanamide was liberated from commercial calcium cyanamide using glacial acetic acid[159,160], and extracted into ether. The ethereal solution was evaporated to small bulk and the cyanamide content determined[161].

Selenourea

The cyanamide solution obtained above was made alkaline by the careful addition of concentrated ammonia. A stream of nitrogen was passed through for 15 minutes before passing in hydrogen selenide[162] simultaneously, until pale pink crystals separated. Each 100g. of calcium cyanamide yielded approximately 25g. of selenourea, m.p.205-10° (decomp.). Literature gives m.p.213°[155], 200°[157], 217°[221].

2-Selenobarbituric acid.

Method A.[163]

The attempted condensation of selenourea and diethylmalonate in the presence of sodium ethoxide for seven hours at 110° yielded on acidification and evaporation, traces of selenourea, some inorganic material and a pale yellow amorphous solid, m.p. *ca.* 250°. Light absorption in 0.1N sodium

hydroxide, λ_{max} 285 mμ. This could not be crystallised from the commoner solvents. The reaction was repeated varying the concentrations of the reactants, the reflux time and using sodium ethoxide, sodium methoxide and magnesium methylate as base catalysts but was unsuccessful.

5,5-Diethyl-2-selenobarbituric acid.

Replacement of diethylmalonate by diethyldiethylmalonate in the above experiments failed to give the required product, but gave instead a viscous, unpleasant smelling liquid. Light absorption in alkaline solution, λ_{max} 275 mμ.

Method B;[164]

The reaction of selenourea and malonic acid in the presence of glacial acetic acid and acetic anhydride gave on concentration, a deep yellow amorphous solid, m.p.190-5° (decomp.). Attempted recrystallisation from water gave an amorphous yellow material, m.p.243-5°(decomp.), containing nitrogen and selenium. Light absorption in 0.1 N sodium hydroxide, λ_{max} 285 mμ.

Found C, 35.94; H, 3.4; N, 14.85%

$C_4H_4N_2O_2Se$ requires C, 25.15; H, 2.11; N, 14.66%

The reaction was repeated varying the concentrations of the reactants and the reflux time but was unsuccessful.

5,5-Diethyl-2-selenobarbituric acid.

Selenourea and diethylmalonic acid[262] were treated as in Method B, but only an impure product was obtained.

Method C:[165]

Selenourea and malonyl chloride[263] were heated together to give a yellow-brown resinous mass. Attempted crystallisation from the usual solvents gave impure semi-crystalline products, m.p.245-50°(decomp.). Light absorption in 0.1N sodium hydroxide, λ_{max} 280 mμ. On repeating the experiment using dry chloroform[166], dry pyridine or dry dioxan as solvent, uncrystallisable syrups were obtained.

Method D:[167]

Selenourea was reacted with ethylcyanacetate in the presence of magnesium methylate. Acidification gave an opalescent solution which deposited a yellow-brown solid on standing overnight. Attempted crystallisation from ethanol gave a pale yellow, amorphous, hygroscopic material, m.p. 180-90°(decomp.). Light absorption in 0.1N sodium hydroxide, λ_{max} 259 mμ.

ATTEMPTED PREPARATION OF SELENOHYDANTOINS

Selenohydantoin.

Method A:[171]

Glycine ester hydrochloride and potassium selenocyanate[264] were refluxed in dry ethanol for an hour. Acidification and concentration yielded only starting material or their breakdown products. The experiment was repeated using glycine, glycine ester and glycine hydrochloride in dry ethanol and acetone as solvent but was unsuccessful.

Method B:[174]

Glycine ester hydrochloride and potassium selenocyanate were heated together at 140-50° for two hours. A solution of the crude product on acidification gave starting material. Using glycine or glycine hydrochloride also failed to give the required product.

Method C:[172]

Glycine and potassium selenocyanate were heated for thirty minutes in acetic anhydride and poured into cold water to produce a dark brown solid. Attempted crystallisation before or after treatment with dilute hydrochloric acid gave only unstable syrupy products. Using glycine

ester hydrochloride also failed to give the required product.

Method D:[173]

Glycine and potassium selenocyanate were refluxed for two hours in dry ethanol containing sodium hydroxide. Concentration and acidification gave starting material, selenium, and potassium and sodium chlorides.

Method E:[175,176]

Glycine and selenourea were refluxed for six hours in dry ethanol in the presence of barium hydroxide. Treatment with solid carbon dioxide and concentration gave only starting material.

Method F:

Glyoxylic acid (1.43g.) and selenourea (2.38g.) were heated in water (10ml.) for an hour. Concentration gave a deep-red-orange crystalline material, heavily contaminated with selenium. Recrystallisation from ethanol gave only traces of selenourea. Repetition using dry ethanol as solvent gave off-white needles on concentration. Crystallisation from dry ethanol (charcoal) gave selenourea.

Diphenylselenohydantoin.

Method A:[177]

Benzil and selenourea were refluxed for two hours with

potassium hydroxide in dry ethanol. Concentration, dilution with water and acidification gave a yellow amorphous precipitate. No purification was possible due to instability. The filtrate on concentration gave selenourea and potassium chloride.

Method B:[178]

Benzilic acid and selenourea were ground together in a mortar and refluxed in acetic anhydride for two hours. Addition of water and concentration gave a viscous, unpleasant smelling red mass which produced no identifiable product on attempted crystallisation.

SE-ALKYLSELENOURONIUM SALTS

Se-Benzylselenouronium chloride.

Selenourea (0.88g.) and benzyl chloride (1.0g.) were refluxed for thirty minutes in dry ethanol, using a calcium chloride guard tube. The yellow solution was filtered clear of selenium and concentrated. On cooling, a white solid (1.42g., 92%) separated. Recrystallisation from ethanol (charcoal) gave Se-benzylselenouronium chloride, as white lustrous plates, m.p.194-6°.

Found: N,11.2; Se,31.4; Cl,15.3;15.5%

$C_8H_{11}N_2SeCl$ requires N,11.23; Se,31.63; Cl,14.14%

Picrate: The picrate, rods, m.p.173-4°, was prepared using sodium picrate in water.

Found: N,14.65; Se,16.3%

$C_{14}H_{13}N_5O_7Se.2H_2O$ requires N,14.64; Se,16.41%

Attempted preparation of Se-benzylselenouronium formate, acetate and benzoate.

Addition of sodium formate in aqueous ethanol to Se-benzylselenouronium chloride in hot water gave only starting material on cooling.

Repetition using sodium acetate and sodium benzoate gave the same result.

Se-Benzylselenouronium p-toluenesulphonate.

p-Toluenesulphonic acid was neutralised with sodium hydroxide solution and added to an aqueous solution of Se-benzylselenouronium chloride. Cooling gave a white crystalline solid. Recrystallisation from ethanol (charcoal) gave Se-benzylselenouronium p-toluenesulphonate, as fine white needles, m.p.172-3°.

Found: N, 7.1; Se, 21.2%

$C_{15}H_{18}N_2O_3SSe$ requires N, 7.39; Se, 20.82%

Attempted Isolation of Se-Benzylisoselenourea.

Chemical method:[185]

Treatment of the hydrochloride in water with sodium hydroxide solution gave a pale yellow, nitrogen-free material, m.p.79-80°. Recrystallisation from ethanol (charcoal) or benzene-petroleum ether (b.p.60-80°) gave dibenzyldiselenide, as pale yellow needles, m.p.89-91°. (Literature[265] gives 93°.)

Found: Se, 45.9%. Molecular weight(Rast),341.

Calc. for $C_{14}H_{14}Se_2$ Se, 46.44%.Molecular weight, 340.

The same product was obtained using sodium acetate and heating for thirty minutes.

Electrolytic method:[185]

Attempted electrolysis of the hydrochloride in water gave a minute amount of an impure solid, m.p.80-2°.

Attempted Cyclisation of Se-Benzylselenouronium Chloride.

With chloracetic acid:

Se-benzylselenouronium chloride and chloracetic acid were refluxed in dry ethanol for an hour. Concentration gave starting material and a sticky syrupy product which could not be crystallised.

With chloroacetylchloride.

Se-benzylselenouronium chloride and chloracetyl chloride were refluxed in dry chloroform. Concentration gave a yellow syrupy product. Recrystallisation from dry ethanol gave dibenzyldiselenide, m.p.89-91°. Mixed m.p. with authentic sample[265], 90°. Using dry benzene as solvent or chloroform in the presence of calcium carbonate, gave an identical product, m.p.89-90°.

Se-Methylselenouronium Iodide.

Selenourea (1.95g.) and methyl iodide (5ml.) were refluxed for an hour in dry ethanol (10ml.). The solution was filtered and concentrated and ether added slowly with shaking till crystallisation began. On standing, pale

yellow coarse needles (3.3g., 79%) separated. Recrystallisation from ethanol ether mixture gave Se-methylselenouronium iodide, as coarse pale yellow needles, m.p.113-5°.

Found: N, 10.7; Se, 30.2; I, 50.3%

$C_2H_7N_2SeI$ requires N, 10.58; Se, 29.8; I, 47.89%

Picrate.

The hydriodide was dissolved in water and aqueous sodium picrate added to give immediate separation of a yellow amorphous solid. Recrystallisation from ethanol gave the picrate, as fine rods, m.p. 218-20°.

Found: N, 18.8; Se, 21.3 %

$C_8H_9N_5O_7Se$ requires N,19.14; Se, 21.57%

ISOSELENOHYDANTOIC ACID

Isoselenohydantoic acid hydrochloride.

Selenourea (2g.) and chloracetic acid (4g.) were suspended in acetone (50ml.) and left in a stoppered flask for 48 hours when a white semicrystalline solid separated, together with traces of grey selenium. The solid residue (3.8g.), m.p.130-5°(decomp.) was collected and well washed with acetone. Recrystallisation from hot dry ethanol (charcoal) gave 2-imino-4-selenazolidone hydrochloride, m.p.218-20°(decomp.).

Selenourea (1.2g.) and chloracetic acid (2.3g.) were treated as above and the product separated without disturbing the grey selenium. Thorough washing with acetone and drying in vacuo gave isoselenohydantoic acid hydrochloride, m.p. 168-70°(decomp.).

Found: N,13.3,13.15; Se,41.0,38.7; Cl,16.05; 16.1%
$C_3H_4N_2O_2SeCl$ requires N, 12.88; Se, 36.29; Cl, 16.30%

The same reaction was carried out in water but the product was deep pink in colour and could not be purified by washing. Heating in dry ethanol gave 2-imino-4-selenazolidone hydrochloride, m.p.217-20°(decomp.).

Picrate: Clusters of needles from dry ethanol, m.p.158°

Found: N,16.7; Se,19.5% Molecular weight[189],415.
$C_9H_9N_5O_9Se$ requires N,17.07; Se,19.23% Molecular weight,410.

Cyclisation of Isoselenohydantoic Acid Hydrochloride.

The hydrohalide was refluxed for an hour with dry ethanol. On cooling white crystals separated which on recrystallisation from dry ethanol (charcoal) gave 2-imino-4-selenazolidone hydrochloride, m.p.219-21°(decomp.). Heating with glacial acetic acid for an hour also effected cyclisation.

PREPARATION OF 2-IMINO-4-SELENAZOLIDONES

Preparation of Starting Materials.

Phenylchloracetic acid.

Phenyl chloracetic acid, m.p.58-60° was prepared by the method described by Walden[266].

Diphenylchloracetic acid.

Diphenylchloracetic acid, m.p.118-9°, was prepared by the method of Bistrzycki and Herbst[267].

Diethylbromacetic acid.

Diethylbromacetic acid, b.p.115-6°, (12 mm.), was prepared by the general method for α-bromo acids[268].

2-Imino-4-selenazolidone hydrochloride.

α-Chloracetic acid (3.84g.) and selenourea (5.0g.) were heated under reflux in dry ethanol (20ml.) for thirty minutes. The dark grey residue (0.2g.) was filtered from the hot solution which yielded on cooling an off-white crystalline product (4.1g., 61%). Recrystallisation from hot, dry ethanol (charcoal) gave 2-imino-4-selenazolidone hydrochloride as white needles, m.p.220-4°(decomp.). Light absorption in water, λ_{max} 227 and 269mμ, ε = 17,600 and 3,900 respectively.

Found: N,14.3 ; Se,39.4 ; Cl,17.6 %

$C_3H_5N_2OSeCl$ requires N,14.05; Se,39.58; Cl,17.77%

2-Imino-4-selenazolidone.

The free base (1.4g., 20%) as fine white needles, m.p. 189-90°(decomp.) was obtained from the filtrate by carefully neutralising with dilute ammonia and recrystallising from water. (Literature[154] gives m.p.190°(decomp.). Light absorption in water, λ_{max} 227 and 267 mμ, ε = 18,600 and 4,100 respectively.

Picrate. Fine needles, m.p.182-4° from dry methanol.

Found: N,17.6 ; Se,20.2 %.Molecular weight,[189] 394

$C_9H_7N_5O_8Se$ requires N,17.85; Se,20.13% Molecular weight, 392.

5-Methyl-2-imino-4-selenazolidone hydrobromide.

α-Bromopropionic acid (1.51g.) and selenourea (1.30g.) were refluxed in dry ethanol (10ml.) for thirty minutes. The hot solution was filtered to remove the insoluble material, the clear yellow solution concentrated to give a yellow syrup. Ether (2-3ml.) was added, followed by dry ethanol dropwise, with stirring, until crystallisation began. On standing small white crystals (1.45g., 57%) separated. Recrystallisation from dry ethanol (charcoal) gave 5-methyl-2-imino-4-selenazolidone hydrobromide m.p.178-180°(decomp.). Light absorption in water, λ_{max} 227 and 269 mμ, ε = 22,000

and 4,600 respectively.

Found: C,19.3 ;H,2.99;N,10.97;Se,30.81;Br,30.81%

$C_4H_7N_2OSeBr$ requires C,18.62;H,2.73;N,10.86;Se,30.61;Br,30.97%

5-Methyl-2-imino-4-selenazolidone.

The hydrobromide was dissolved in a minimum amount of cold water and concentrated ammonia added dropwise with shaking, till white needles separated. Recrystallisation from water gave 5-methyl-2-imino-4-selenazolidone m.p.175-180°. Drying over phosphorus pentoxide in vacuo at 100° for several hours raised the m.p. to 179-180°(decomp.). (Literature[155] gives m.p.179°). Light absorption in water, λ_{max} 228 and 270 mμ, ε = 17,300 and 3,500 respectively.

Found: N, 15.83%

Calc. for $C_4H_6N_2OSe$ N, 15.82%

Picrate. The picrate was prepared from the hydrobromide in water and aqueous sodium picrate. Recrystallisation from aqueous ethanol (charcoal) gave the picrate as fine needles, m.p.192-4°.

Found: N,17.16;Se,19.17% Molecular weight[189]407

$C_{10}H_9N_5O_8Se$ requires N,17.25;Se,19.44% Molecular weight, 406.

5-Ethyl-2-imino-4-selenazolidone hydrobromide.

α-Bromo-n-butyric acid (1.67g.) and selenourea (1.23g.)

were refluxed in dry ethanol (10ml.) for 30 minutes. Filtration and cooling gave fine white needles (2.3g., 85%). Recrystallisation from dry ethanol gave 5-ethyl-2-imino-4-selenazolidone hydrobromide, as white needles, m.p.220-4° (decomp.). Light absorption in water, λ_{max} 228 and 270 mμ ε = 18,600 and 3,700 respectively.

Found: C,22.30; H,3.33; N,10.20; Se,28.95; Br,29.55%

$C_5H_9N_2OSeBr$ requires C,22.08; H,3.33; N,10.30; Se,29.02; Br,29.38%

5-Ethyl-2-imino-4-selenazolidone.

The base was prepared from the hydrobromide as described under 5-methyl-2-imino-4-selenazolidone. Recrystallisation from aqueous ethanol gave 5-ethyl-2-imino-4-selenazolidone as fine white needles m.p.178-180°(decomp.). Light absorption in water, λ_{max} 228 and 269 mμ, ε = 17,950 and 3,700 respectively.

Found N, 14.96; Se, 40.90%

$C_5H_8N_2OSe$ requires N, 14.67; Se, 41.32%

Picrate: Needles, m.p.186-8° from dry methanol.

Found: N,16.47; Se,18.60% Molecular weight,422

$C_{11}H_{11}N_5O_8Se$ requires N,16.66; Se,18.79% Molecular weight,420

5-Propyl-2-imino-4-selenazolidone.

α-Bromo-n-valeric acid (1.2g.) and selenourea (0.82g.) were refluxed in dry ethanol (5ml.) for 30 minutes. The solvent was removed under reduced pressure and water (10ml.) added. Dilute ammonia was added dropwise till the pH was 8-9 when a pale pink microcrystalline material (1.08g., 80%) separated. Recrystallisation from ether gave 5-propyl-2-4-selenazolidone as very fine white needles, m.p.184-6° (decomp.).

Found: N, 13.88; Se, 38.31%

$C_6H_{10}N_2OSe$ requires N, 13.67; Se, 38.50%

5-Isopropyl-2-imino-4-selenazolidone

α-Bromo-isovaleric acid (1.63g.) and selenourea (1.04g.) were refluxed in dry ethanol (5ml.) for 30 minutes. The solvent was removed under reduced pressure and water (10-15ml.) added. Dilute ammonia was added dropwise till the pH was 8-9 when a pink amorphous solid (1.05g., 61%) separated. Recrystallisation from dry ethanol (charcoal) gave 5-isopropyl-2-imino-4-selenazolidone as shining white needles, m.p.211-3°(decomp.).

Found: N, 13.5, 13.7; Se, 38.37%

$C_6H_{10}N_2OSe$ requires N, 13.67; Se, 38.50%

5-Butyl-2-imino-4-selenazolidone hydrobromide.

α-Bromo-n-caproic acid (1.94g.) and selenourea (1.23g.) were refluxed in dry ethanol (10ml.) for 30 minutes. Concentration of the solution yielded, on cooling, an off-white crystalline solid (1.29g., 43%). Recrystallisation from dry ethanol (charcoal) gave 5-butyl-2-imino-4-selenazolidone hydrobromide as white needles, m.p.179-81°(decomp.).

Found: C,28.2; H,4.7; N,9.50; Se,25.98; Br,26.40%

$C_7H_{13}N_2OSeBr$ requires C,28.03; H,4.37; N,9.34; Se,26.32; Br,26.64%

5-Butyl-2-imino-4-selenazolidone.

The free base was obtained as a white amorphous solid (0.59g., 28%) from the filtrate by addition of water (10ml.) and dilute ammonia till the pH was 8-9. Recrystallisation from aqueous ethanol (charcoal) gave 5-butyl-2-imino-4-selenazolidone as a white microcrystalline solid, m.p.178-9°(decomp.).

Found N, 12.85; Se, 35.58%

$C_7H_{12}N_2OSe$ requires N, 12.78; Se, 36.04%

Picrate: The picrate was obtained from the base in dry methanol by addition of methanolic picric acid. Recrystallisation from aqueous methanol gave the picrate as canary yellow needles, m.p.126-30°(decomp.).

Found: N, 15.4; Se,17.2 % Molecular weight, 445.

$C_{13}H_{15}N_5O_8Se$ requires N,15.63; Se,17.62% Molecular weight, 448

5-Phenyl-2-imino-4-selenazolidone.

α-Chlorphenylacetic acid (1.73g.) and selenourea (1.3g.) were refluxed in dry ethanol (10ml.) for 30 minutes. The ethanol was removed under reduced pressure and water (10ml.) added. The pH was adjusted to 8-9 with dilute ammonia and gave a pale pink semicrystalline solid (1.46g., 61%). Recrystallisation from aqueous ethanol gave 5-phenyl-2-imino-4-selenazolidone as white cluster needles, m.p.200-4° (decomp.). Light absorption in water, λ_{max} 225 and 268, ε = 17,050 and 2,500 respectively.

Found:	N, 11.61;	Se, 32.61%
$C_9H_8N_2OSe$ requires	N, 11.71;	Se, 33.02%

Attempted synthesis of 5,5-dimethyl-2-imino-4-selenazolidone.

α-Bromo-isobutyric acid (1.7g.) and selenourea (1.26g.) were refluxed in dry ethanol (20ml.) for 30 minutes. Filtration of the solution and concentration under reduced pressure gave pale yellow needles which darkened rapidly. On collection, the crystals rapidly decomposed to a reddish-black mass. Repeating the experiment in an atmosphere of nitrogen and excluding light, gave similar products. The use of dry acetone, methanol or ether as solvent in the above reaction was equally unproductive, and all attempts to prepare a picrate were unsuccessful.

The crude material was hydrolysed by hydrochloric acid (1ml.) and water (20ml.) for two hours. Extraction with ether and evaporation gave a semicrystalline solid, m.p.75°. Sublimation gave 5,5-dimethylselenazolid-2,4-dione, as white cubic crystals, m.p.82-3°.

Found: N, 7.26; Se, 40.81%

$C_5H_7NO_2Se$ requires N, 7.29; Se, 41.11%

Attempted synthesis of 5,5-diethyl-2-imino-4-selenazolidone.

α-Bromodiethylacetic acid (2g.) and selenourea (1.3g.) were heated under reflux in dry ethanol (10ml.) for 30 minutes. Concentration and cooling gave pale yellow needles which decomposed rapidly to a deep orange-red solid on collection. Repetition in the absence of air and light and using acetone, methanol or ether as solvent, gave similar unstable products. Hydrolysis with dilute hydrochloric acid, extraction with ether and evaporation gave a deep-red crystalline material which could not be purified.

All attempts to prepare a picrate were unsuccessful.

Attempted synthesis of 5,5-diphenyl-2-imino-4-selenazolidone.

Diphenylchloracetic acid (2.51g.) and selenourea (1.33g.) were refluxed in dry ethanol for 30 minutes. On cooling, a greyish-white amorphous material (2.24g.) separated. This

could not be crystallised or purified by successive washing with ether and evaporation gave an unstable pink amorphous solid, which could not be purified.

2-Dimethylamino-4-selenazolone hydrochloride.

This was prepared from chloracetic acid and N,N-dimethylselenourea as described by Zingaro, Bennett and Hammar[190]. Recrystallisation from dry ethanol (charcoal) and ether gave pale yellow needles, m.p.227-9°(decomp.). (Literature[190] gives m.p.229-31°(decomp.)). Light absorption in water, λ_{max} 245 m u, ε = 17,400.

Hydrolysis of the hydrochloride with dilute hydrochloric acid gave selenazolid-2,4-dione m.p.146-7°. (Literature[154] gives m.p.147°).

5-Ethyl-2-dimethylamino-4-selenazolone hydrobromide.

This was prepared from α-bromo-n-butyric acid and N,N-dimethylselenourea as described[190]. Recrystallisation from dry ethanol (charcoal) and ether gave pale pink needles m.p.199-202°(decomp.). (Literature[190] gives m.p.201-3° decomp.). Light absorption in water, λ_{max} 245 mμ, ε = 17,200.

5,5-Dimethyl-2-dimethylamino-4-selenazolone hydrobromide.

α-Bromoisobutyric acid (2.31g.) and N,N-dimethyl-

selenourea (2.1g.) were refluxed in dry ethanol (25ml.) for an hour. The solution was cooled and ether added slowly with shaking until crystallisation began. On standing a pale yellow microcrystalline solid (1.95g., 47%) separated. Recrystallisation from dry ethanol (charcoal) and ether gave 5,5-dimethyl-2-dimethylamino-4-selenazolone hydrobromide as pale yellow platelets, m.p.230-2°(decomp.).

Found: N, 9.35; Se, 25.8 ; Br, 27.1 %

$C_7H_{13}N_2OSeBr$ requires N, 9.32; Se, 26.26; Br, 26.60%

Picrate: Needles, m.p.156-7° from water.

Found: N,15.4; Se,17.2% Molecular weight, 450

$C_{13}H_{15}N_5O_8Se$ requires N,15.63;Se,17.61% Molecular weight,448.

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Attempted preparation of 5,5-diethyl-2-dimethylamino-4-selenazolone hydrobromide.

Diethylbromacetic acid (1.66h.) and N,N-dimethylselenourea (1.28g.) were refluxed in dry ethanol (25ml.) for an hour. On cooling yellow needles separated. Recrystallisation from dry ethanol-ether gave yellow needles which rapidly decomposed and could not be purified. Attempted preparation of the picrate gave only sticky yellow material.

PREPARATION OF SELENAZOLID-2,4-DIONES

Selenazolid-2,4-dione.

Chloracetic acid (1.9g.) and selenourea (2.5g.) were heated in water (10ml.) for two hours. The solution was cooled and extracted with ether (5 x 10ml.). The combined ether extracts were evaporated to leave a pale yellow oil (1.9g., 61%), which crystallised on standing to a pale yellow solid, m.p.140°. Sublimation (97°, 0.1mm.) gave selenazolid-2,4-dione, as white cubes, m.p.146-7°. (Literature[154] gives m.p.147°).

The same product was obtained in 78 per cent yield on refluxing 2-imino-4-selenazolidone (1.5g.) in hydrochloric acid (2ml.) and water (25ml.) for two hours and isolating the product as described above.

5-Ethylselenazolid-2,4-dione.

α-Bromo-n-butyric acid (1.42g.) and selenourea (1.05g.) were heated in water (10ml.) for two hours. Extraction as before gave a yellow oil (1.6g., 71%). On addition of a few drops of water and shaking, white cluster needles separated. Recrystallisation from water (charcoal) gave 5-ethylselenazolid-2,4-dione, as white leaflets, m.p.69-71°

Found: C, 31.6 ; H, 4.1 ; N, 7.4 ; Se, 41.6 %

$C_5H_7NO_2Se$ requires C, 31.31; H, 3.67; N, 7.29; Se, 41.11%

5-Ethyl-2-imino-4-selenazolidone refluxed in hydrochloric acid and water gave the same product in 75 per cent yield.

5-Methylselenazolid-2,4-dione.

5-Methyl-2-imino-4-selenazolidone (0.4g.) treated as above gave a pale yellow oil (0.32g., 82%) which solidified to a pale yellow crystalline solid, m.p.72-3°, after standing for several days in vacuo. Microdistillation (80-85°, 0.1mm.) gave 5-methylselenazolid-2,4-dione, as white needles, m.p.74-5°.

Found: C, 26.6 ; H, 3.1 ; N, 7.9 ; Se, 43.9 %

$C_4H_5NO_2Se$ requires C, 26.99; H, 2.83; N, 7.87; Se, 44.35%

5-Propylselenazolid-2,4-dione.

5-Propylselenazolid-2,4-dione.

5-Propyl-2-imino-4-selenazolidone (0.813g.) was treated as above to give a yellow oil (0.54g., 67%) which solidified to a white crystalline solid, m.p.50°. Careful microdistillation (80-85°, 0.1mm.) gave 5-propylselenazolid-2,4-dione, as white, waxy platelets, m.p.55-6°.

Found: N, 6.7 ; Se, 38.1 %

$C_6H_9NO_2Se$ requires N, 6.80; Se, 38.31%

5-Isopropylselenazolid-2,4-dione.

5-Isopropyl-2-imino-4-selenazolidone (0.41g.) was treated as above to give off-white crystalline material (0.31g., 73%). Microdistillation (97°, 0.1mm.) gave 5-isopropylselenazolid-2,4-dione as white needles, m.p.73-4°

Found: N, 6.9 ; Se, 37.86%

$C_6H_9NO_2Se$ requires N, 6.80; Se, 38.31%

5-Butylselenazolid-2,4-dione.

5-Butyl-2-imino-4-selenazolidone (0.292g.) was treated as above to give an off-white solid (0.26g., 89%). Recrystallisation from aqueous ethanol (charcoal) gave 5-butylselenazolid-2,4-dione as shining white prisms, m.p.92-3°.

Found: N, 6.34; Se, 35.33%

$C_7H_{11}NO_2Se$ requires N, 6.36; Se, 35.88%

5-Phenylselenazolid-2,4-dione.

5-Phenyl-2-imino-4-selenazolidone (0.54g.) was refluxed for two hours in hydrochloric acid (1ml.) and water (20ml.). On cooling, off-white needles (0.33g., 60%) separated. Sublimation (105°; 0.1mm.) gave 5-phenylselenazolid-2,4-dione as fine white needles, m.p.160-2°, sinters at 156°.

Found: N, 5.90; Se, 32.6 %

$C_9H_7NO_2Se$ requires N, 5.81; Se, 32.74%

5,5-Dimethylselenazolid-2,4-dione.

α-Bromo-isobutyric acid (1.7g.) and selenourea (1.42g.) were refluxed with water (10ml.) for an hour. The grey selenium sludge was filtered off while hot and the solution allowed to cool. Extraction with ether (5 x 10ml.) and evaporation of the combined ether extracts gave white crystalline material (1.31g., 68%). Microdistillation (106-8°, 0.6mm.) gave 5,5-dimethylselenazolid-2,4-dione as white platelets, m.p.82.5 - 83.5°.

Found: N, 7.43; Se, 40.9 %

$C_5H_7NO_2Se$ requires N, 7.29; Se, 41.11%

Attempted synthesis of 5,5-diethylselenazolid-2,4-dione.

α-Bromodiethylacetic acid (2.1g.) and selenourea (1.4g.) were refluxed with water (15ml.) for an hour. Extraction with ether (5 x 10ml.) and evaporation of the combined ether extracts gave a deep red crystalline material which could not be purified.

Attempted synthesis of 5,5-diphenylselenazolid-2,4-dione.

Diphenylchloracetic acid (2.4g.) and selenourea (1.3g.) were refluxed with water (15ml.) for an hour. Extraction with ether and evaporation as above gave an unstable semi-crystalline material which could not be purified.

METHYLATION OF SELENAZOLID-2,4-DIONES AND 2-IMINO-4-SELENAZOLIDONES.

3-Methylselenazolid-2,4-dione.

Method A:

Selenazolid-2,4-dione (1.14g.), potassium hydroxide (0.44g.) and methyl iodide (5 ml.) were refluxed in dry ethanol (15 ml.) for two hours. The pale yellow solution was boiled to remove the excess methyl iodide and concentrated under reduced pressure. Water (10ml.) was added and the product extracted with ether (3 x 20ml.). Evaporation of the combined ether extracts gave a pale yellow oil (1.07g., 84%) which was dried overnight over sulphuric acid *in vacuo*. Microdistillation gave 3-methylselenazolid-2,4-dione as a pale yellow oil, b.p.64°(0.8mm.), with an alliaceous odour. On standing the oil solidified to a white waxy solid, m.p.33°.

Found: N, 7.6 ; Se, 44.1 %

$C_4H_5NO_2Se$ requires N, 7.67; Se, 44.35%

Method B:

Selenazolid-2,4-dione (1.6g.) was suspended in dry ether (10ml.) and ethereal diazomethane added in small portions until the solid material had completely dissolved and the evolution of gas had ceased. Evaporation of the

yellow ethereal solution gave a pale yellow oil (1.45g., 82%), b.p.65° (0.8mm.), which solidified to a white waxy solid, m.p.32-3°. Mixed m.p. with the authentic sample from method A, gave m.p.32°. The ultraviolet and infrared absorption spectra of the two products were identical.

3-Methyl-5-ethylselenazolid-2,4-dione.

5-Ethylselenazolid-2,4-dione (1.28g.) was treated as in method A, to give a pale yellow oil (1.05g., 74%). Distillation gave 3-methyl-5-ethylselenazolid-2,4-dione, as a pale yellow oil, b.p.82° (1mm.), with an alliaceous odour.

Found	N, 6.9; Se, 38.05%	$n_D^{21.5}$ 1.5439
$C_6H_9NO_2Se$	N, 6.80; Se, 38.31%	

Treatment of 5-ethylselenazolid-2,4-dione with diazomethane as in method B, gave an identical product in 76 per cent yield.

3-Methyl-5-ethyl-2-imino-4-selenazolidone hydriodide.

5-Ethyl-2-imino-4-selenazolidone (0.41g.) and methyl iodide (1ml.) were refluxed in dry ethanol (5 ml.) for an hour. The excess methyl iodide was boiled off and the yellow solution concentrated and cooled to give a white crystalline solid (0.44g., 62%) m.p. ca.210°(decomp.).

Recrystallisation from ethanol (charcoal) gave 3-methyl-5-ethyl-2-imino-4-selenazolidone hydriodide as white shining needles, m.p.223°(decompl).

Found: N, 8.4 ; Se, 23.3 ; I, 38.0 %

$C_6H_{11}N_2OSeI$ requires N, 8.42; Se, 23.71; I, 38.12%

Picrate. The picrate was obtained as fine canary yellow needles, m.p.192-4° from dry methanol.

Found: N,16.2 ; Se,18.4 % Molecular weight, 436 189

$C_{12}H_{13}N_5O_8Se$ requires N, 16.14; Se,18.19% Molecular weight, 434

Attempted isolation of the base from the hydriodide gave an unstable product which could not be purified for analysis.

Attempted preparation of 3-methyl-2-imino-4-selenazolidone hydriodide.

2-Imino-4-selenazolidone (1.03g.) and methyl iodide (5ml.) were refluxed in dry ethanol (20ml.) for nine hours. The excess methyl iodide was boiled off and the solution concentrated. Cooling gave a white crystalline solid (1.3g., 71%). Recrystallisation from ethanol (charcoal) gave white needles, m.p.198-200°(decomp.).

Found: N,8.5,10.2; Se,23.4,26.5; I,40.6,42.2%

$C_4H_7N_2OSeI$ requires N, 9.13; Se,25.72; I,41.34%

Picrate. Attempted preparation of the picrate gave needles which could not be purified.

Hydrolysis of 3-Methyl-2-imino-4-selenazolidone Derivatives.

Acid hydrolysis of 3-methyl-5-ethyl-2-imino-4-selenazolidone hydriodide gave 3-methyl-5-ethylselenazolid-2,4-dione, b.p.82°(1mm.). The infrared spectrum was identical with that of an authentic sample.

Similar treatment of the impure 3-methyl-2-imino-4-selenazolidone hydriodide gave 3-methylselenazolid-2,4-dione, m.p.32-3°. Again the infrared spectrum was identical with that of an authentic sample.

ACYL AND SULPHONYL DERIVATIVES OF 2-IMINO-4-SELENAZOLIDONE.

2-Acetylamino-4-selenazolone.

2-Imino-4-selenazolidone hydrochloride (0.81g.) was suspended in glacial acetic acid (5ml.) and acetic anhydride (2.5ml.) added dropwise with shaking. The mixture was heated on a boiling water bath for 15 minutes and more acetic anhydride (2.5ml.) added. The mixture was heated as before until all the solid material had dissolved (ca.1 hour). The orange coloured solution was concentrated and a yellow solid (0.73g., 88%) separated on cooling. Recrystallisation from aqueous ethanol (charcoal) gave 2-acetylamino-4-selenazolone as fine white needles m.p. (sealed tube) 236-40°(decomp.). Light absorption in water, λ_{max} 244 and 305 mμ, ε = 20,100 and 4,500 respectively.

Found: N,13.7 ; Se,38.3%

$C_5H_6N_2O_2Se$ requires N,13.67; Se,38.5%

Picrate. The picrate separated on mixing methanolic solutions. Recrystallisation from ethanol gave needles, m.p.203-4° (decomp.).

Found: N,16.4 ; Se,18.3% Molecular weight[189]430.

$C_{11}H_9N_5O_9Se$ requires N,16.14; Se,18.19% Molecular weight 434.

Treatment of 5-methyl- and 5-ethyl-2-imino-4-selen-

azolidone with glacial acetic acid and acetic anhydride as above gave only starting material.

Hydrolysis of 2-acetylamino-4-selenazolone by refluxing with 1:1 hydrochloric acid for two hours gave, on evaporation of ether extracts, a white crystalline solid. Microdistillation (97°, 0.1mm.) gave selenazolid-2,4-dione, m.p.146-7°. Mixed m.p. with authentic sample, 146°.

2-Toluenesulphonamido-4-selenazolone.

2-Imino-4-selenazolidone hydrochloride (0.64g.) and p-toluenesulphonylchloride (0.64g.) were heated in the presence of pyridine (5ml.) for two hours. The reaction mixture was cooled and water (20ml.) added together with concentrated ammonia (15ml.). Extraction with ether removed the pyridine and excess p-toluenesulphonylchloride. Acidification of the aqueous residue with glacial acetic acid gave a red-brown semi-crystalline precipitate (0.35g., 39%). Recrystallisation from ethanol (charcoal) gave 2-p-toluenesulphonamido-4-selenazolone, as glistening pale yellow rectangular plates, m.p.209-11°.

Found: N, 8.8 ; Se, 24.8 %

$C_{10}H_{10}N_2O_3SSe$ requires N, 8.84; Se, 24.90%

2-Benzenesulphonamido-4-selenazolone.

2-Imino-4-selenazolidone hydrochloride (0.32g.) and benzenesulphonylchloride (0.35g.) were heated for two hours in dry pyridine (5ml.). The solution was cooled and poured into cold water (10ml.) containing a few drops of concentrated hydrochloric acid. A deep red gum separated which on long standing gave deep-red needles, (0.27g., 46%). Recrystallisation from dry ethanol (charcoal) gave 2-benzenesulphonamido-4-selenazolone, as very pale yellow needles, m.p.168°.

Found: N, 9.0 ; Se, 26.4 %

$C_9H_8N_2O_3SSe$ requires N, 9.27; Se, 26.15%

ALKYLIDENE DERIVATIVES OF 2-IMINO-4-SELENAZOLIDONE AND SELENAZOLID-2,4-DIONE

Attempted preparation of 5-benzylidene-2-imino-4-selenazolidone.

a) 2-Imino-4-selenazolidone hydrochloride (0.5g.), benzaldehyde (0.4g.) and sodium acetate (0.55g.) were refluxed in glacial acetic acid (5ml.) for two hours at 160-170°. The solution was cooled and poured into cold water (25ml.) to give a yellow opalescent solution and a yellow green sludge. Extraction with ether (5 x 10ml.) and evaporation of the combined ether extracts gave a golden yellow liquid (0.20g.) which could not be crystallised. Evaporation of the extracted solution gave a yellow amorphous solid (0.7g.). This was thoroughly washed with water and the residue recrystallised from glacial acetic acid to give fine orange needles, m.p.237-9°(decomp.).

Found: N, 6.1 ; Se,17.4%

$C_{10}H_8N_2OSe$ requires N,11.16; Se,30.5%

The same product was obtained using 2-imino-4-selenazolidone.

The same reaction in glacial acetic acid and acetic anhydride gave 2-acetylamino-4-selenazolone, m.p.236-42°

(decomp.).

Found N, 13.8; Se, 38.4%

b) No reaction took place when 2-imino-4-selenazolidone and benzaldehyde were reacted in dry ethanol in the presence of piperidine.

c) Chloracetic acid (0.24g.), selenourea (0.31g.) and benzaldehyde (0.26g.) were refluxed for thirty minutes in glacial acetic acid (15ml.). The product which precipitated during the reaction was recrystallised from ethanol to give 2-imino-4-selenazolidone hydrochloride, m.p.218-20°(decomp.). Longer heating gave also selenazolid-2,4-dione, m.p. and mixed m.p. with authentic sample 146-7°.

5-Benzylidene-2-acetylamino-4-selenazolone.

2-Acetylamino-4-selenazolone (1.04g.), benzaldehyde (1.03g.), glacial acetic acid (3ml.) and acetic anhydride (0.5ml.) were refluxed in an oilbath at 150-60° for 45 minutes. The solution was cooled and water (10ml.) added dropwise to give a buff precipitate (0.54g., 37%). Recrystallisation from ethanol (charcoal) gave 5-benzylidene-2-acetylamino-4-selenazolone, as fine yellow needles, m.p. 270-85°(decomp.).

Found N, 9.55; Se, 27.1 %

$C_{12}H_{10}N_2O_2Se$ requires N, 9.56; Se, 26.93%

5-Benzylideneselenazolid-2,4-dione.

Selenazolid-2,4-dione (1.64g.), benzaldehyde (1.48g.), anhydrous sodium acetate (1.3g.) and acetic anhydride (10 drops) were refluxed in glacial acetic acid (4ml.) for three hours at 160-170°. The reaction mixture solidified on cooling and partially dissolved on addition of water. The insoluble solid (1.93g., 75%) on recrystallisation from dry ethanol (charcoal) gave 5-benzylideneselenazolid-2,4-dione, as pale straw-coloured needles, m.p.250-2°(decomp.).

Found: C, 47.5 ; H, 3.1 ; N, 5.6 ; Se, 30.75%

$C_{10}H_7NO_2Se$ requires C, 47.64; H, 2.80; N, 5.56; Se, 31.32%

5-Salicylideneselenazolid-2,4-dione.

Selenazolid-2,4-dione (1.3g.) and salicylaldehyde (1.41g.) were treated as above. The insoluble residue (1.3g., 61%) was recrystallised from dry ethanol or glacial acetic acid to give 5-salicylideneselenazolid-2,4-dione, as a yellow amorphous solid, m.p.214-6°(decomp.).

Found: N, 5.3 ; Se, 29.15%

$C_{10}H_7NO_3Se$ requires N, 5.23; Se, 29.45%

PREPARATION OF SELENAZOLID-2,4-DIONE-2-HYDRAZONES

Starting materials.

Acetone selenosemicarbazone was prepared using the method of Rolls and Renson[121] and used to prepare benzaldehyde selenosemicarbazone[122].

Selenazolid-2,4-dione-2-isopropylidenehydrazone.

Method A.[207]

Acetone selenosemicarbazone (0.82g.) chloroacetic acid (0.64g.) and anhydrous sodium acetate (0.64g.) were refluxed in 95 per cent ethanol (16ml.) for an hour. On cooling the reaction mixture solidified. Thorough washing with dry ethanol (4 x 10ml.) and hot water (4 x 10ml.) left pale cream semicrystalline solid (0.66g., 66%), m.p. ca.160°. Recrystallisation from aqueous ethanol (charcoal) gave selenazolid-2,4-dione-2-isopropylidenehydrazone, as white platelets, m.p.181-3°.

Found: Se, 35.95%

$C_6H_9N_3OSe$ requires Se, 36.20%

Method B.[179]

Clean sodium (0.23g.) was dissolved in dry ethanol (15ml.) and acetone selenosemicarbazone (1.78g.) added with

stirring. Ethylchloroacetate (0.62g.) was then added slowly and the mixture refluxed for ten minutes to give a pale yellow precipitate. Dry ethanol (15 ml.) containing clean sodium (0.115g.) was added, followed by ethylchloroacetate (0.31g.). The mixture was stirred and refluxed for ten minutes. The last series of additions was repeated and the mixture finally refluxed for thirty minutes. The ethanol was distilled off and hydrochloric acid (1.5ml.) in water (10ml.) added. Cooling in ice gave a red crystalline product (1.464g., 67%), m.p.168-73°. Recrystallisation from ethanol (charcoal) gave selenazolid-2,4-dione-5-isopropylidenehydrazone, as white lustrous needles, m.p.181-3°, mixed m.p. with previous product, 182°.

Found: Se, 36.0%

5-Ethylselenazolid-2,4-dione-2-isopropylidenehydrazone.

Acetone selenosemicarbazone (0.805g.), α-bromo-n-butyric acid (1.006g.) and anhydrous sodium acetate (0.66g.) were refluxed in 95 per cent ethanol (20ml.) for an hour. Cooling and removal of the solvent gave a white crystalline solid. Addition of water (15ml.), extraction with ether (4 x 15ml.) and evaporation gave a clear yellow liquid (0.76g., 69%). This was dissolved in dry ethanol (ca.5ml.) and left overnight at 0° to give 5-ethylselenazolid-2,4-dione-2-isopropylidenehydrazone, as fine flaky crystals, m.p.103-6°.

Found: C,38.3; H,5.56; N,16.84; Se,31.81%

$C_8H_{13}N_3OSe$ requires C,39.04; H,5.32; N,17.07; Se,32.07%

5-Phenylselenazolid-2,4-dione-2-isopropylidenehydrazone

Acetone selenosemicarbazone (1.23g.), α-chlorphenylacetic acid (1.21g.) and anhydrous sodium acetate (1.04g.) were refluxed in 95 per cent ethanol (20ml.) for an hour. The solvent was removed to leave a grey-white crystalline mass, to which water (10ml.) was added. The pH was adjusted to 8 by addition of concentrated ammonia and extraction with ether gave, on evaporation, white needles (0.78g., 39%), m.p.203-6°. Recrystallisation from ethanol (charcoal) gave 5-phenylselenazolid-2,4-dione-2-isopropylidenehydrazone, as very fine, faintly pink needles, m.p.208°.

Found: C,48.5 ; H,4.8 ; N,13.9 ; Se,26.79%

$C_{12}H_{13}N_3OSe$ requires C,48.99; H,4.45; N,14.29; Se,26.84%

Selenazolid-2,4-dione-2-benzylidenehydrazone.

Benzaldehyde selenosemicarbazone (0.26g.) and chloracetic acid (0.11g.) and anhydrous sodium acetate (0.2g.) were refluxed for an hour in 95 per cent ethanol (10ml.). The reaction mixture became opalescent after ten minutes. On cooling, the mixture solidified and partially dissolved on washing with hot water (20ml.). Filtration gave a creamy

white solid (0.23g., 75%). Recrystallisation from glacial acetic acid gave selenazolid-2,4-dione-2-benzylidenehydrazone, as very fine white needles, m.p.254-6°(decomp.).

Found: Se, 30.1 %

$C_{10}H_9N_3OSe$ requires Se, 29.67%.

5-Ethylselenazolid-2,4-dione-2-benzylidenehydrazone.

Benzaldehyde selenosemicarbazone (2.3g.), α-bromo-n-butyric acid (1.7g.) and anhydrous sodium acetate (1.0g.) were refluxed for an hour in 95 per cent ethanol (25ml.). Cooling gave a white semi-crystalline solid. Addition of water (25ml.) extraction with ether (4 x 15ml.) and evaporation of the combined ether extracts gave crude product (1.89g., 64%). Recrystallisation from ethanol (charcoal) gave 5-ethylselenazolid-2,4-dione-2-benzylidenehydrazone, as fine white needles, m.p.200-2°.

Found: Se, 26.9 %

$C_{12}H_{13}N_3OSe$ requires Se, 26.84%

Attempted synthesis of 5-Benzyleneselenazolid-2,4-dione-2-isopropylidenehydrazone.

Acetone selenosemicarbazone (0.9g.), chloracetic acid (0.71g.), anhydrous sodium acetate (0.7g.), benzaldehyde (1ml.), glacial acetic acid (10ml.) and acetic anhydride

(10 drops) were refluxed for an hour in an oilbath at 150-60° and gave selenazolid-2,4-dione-2-benzylidenehydrazone (1.17g., 89%) as pale yellow needles, m.p.250-2°(decomp.).

Found: Se, 29.2, 29.5%

The same product was obtained by treating selenazolid-2,4-dione-2-isopropylidenehydrazone with benzaldehyde in glacial acetic acid for an hour at 150-160°.

Hydrolysis of Selenazolid-2,4-dione-2-hydrazones.

Selenazolid-2,4-dione-2-isopropylidenehydrazone (0.33g.) was refluxed with water (10ml.) and hydrochloric acid (0.5ml.) for two hours. Extraction with ether (4 x 10ml.) and evaporation of the combined ether extracts gave selenazolid-2,4-dione, m.p.147°.

Similar treatment of selenazolid-2,4-dione-2-benzylidenehydrazone gave the same product.

PHYSICAL EXPERIMENTS

pK_a Determination.

Titration with potassium hydroxide.

The hydrohalide under examination (0.02mM.) was dissolved in purified water (4ml.) in a titration cell fitted with a magnetic stirrer. The cell was fitted with a water jacket through which a continuous stream of water at 25° was passed. A stream of nitrogen washed through water at 25° and then passed through soda lime, was bubbled through the test solution throughout the titration. Carbon dioxide - free potassium hydroxide (0.05N) was prepared from 0.05N potassium chloride by passing through an anion exchange column and filled directly into a micrometer burette as required, via a two-way tap. The titration was carried out by adding suitable volumes of potassium hydroxide solution from the micrometer burette to the test solution in the titration cell. The pH of this solution was measured initially and after each addition of potassium hydroxide, using a glass and calomel electrode system connected to a Pye Dynacap direct reading pH meter, previously standardised to pH 4.01 and 9.15 with standard buffer solutions.

A blank titration of halogen acid alone was carried

out and was used to correct the test titration volumes. The corrected volumes were then plotted against the pH values and the pKa values calculated where possible. The results obtained are recorded in Table I.

Table I.

Compound	Initial pH in water	pKa
2-imino-4-selenazolidone	5.4	-
2-imino-4-selenazolidone hydrochloride	2.1	-
5-ethyl-2-imino-4-selenazolidone	4.5	-
5-ethyl-2-imino-4-selenazolidone hydrobromide	2.5	-
5-isopropyl-2-imino-4-selenazolidone	5.9	-
5-phenyl-2-imino-4-selenazolidone	5.3	-
5-ethyl-3-methyl-2-imino-4-selenazolidone hydriodide	3.9	4.9
5,5-dimethyl-2-dimethylamino-4-selenazolone hydrobromide		-
2-dimethylamino-4-selenazolone hydrochloride	2.6	-
Se-methylselenouronium iodide	5.6	-
Se-benzylselenouronium chloride	6.8	-

The experiment was repeated on selenazolid-2,4-diones and the results are recorded in Table II.

Table II

Compound	Initial pH in water	pKa
selenazolid-2,4-dione	5.76	6.95
5-ethylselenazolid-2,4-dione	5.05	6.56

Ultraviolet Absorption Spectra.

The ultraviolet absorption spectra of several 2-imino-4-selenazolidones were measured in water on the Hilger Uvispek spectrophotometer using 1 cm. cells. The results are recorded in Table III.

Table III

Compound	Maximum		Minimum
	λ (mμ)	ε	λ (mμ)
2-imino-4-selenazolidone	227; 267	18,600; 4,100	249
2-imino-4-selenazolidone hydrochloride	227; 269	17,600; 3,900	250
5-methyl-2-imino-4-selenazolidone	228; 270	17,300; 3,500	249
5-methyl-2-imino-4-selenazolidone hydrobromide	227; 269	22,000; 4,600	210; 250
5-ethyl-2-imino-4-selenazolidone	228; 269	17,950; 3,700	250
5-ethyl-2-imino-4-selenazolidone hydrobromide	228; 270	18,600; 3,700	211; 250
5-phenyl-2-imino-4-selenazolidone	225; 269	17,050; 2,500	214; 252
2-acetylamino-4-selenazolidone	244; 305	20,100; 4,500	269

The ultraviolet absorption spectra of model compounds prepared for structural comparison were measured as before. The results are shown in Table IV.

Table IV.

Compound	Maximum		Minimum
	λ(mμ)	ε	λ (mμ)
2-dimethylamino-4-selenazolone hydrochloride	242	18,200	213
5-ethyl-2-dimethylamino-4-selenazolone hydrobromide	243	19,000	211
5,5-dimethyl-2-dimethylamino-4-selenazolone hydrobromide	243	18,600	220
5-ethyl-3-methyl-2-imino-4-selenazolidone hydriodide	227 260-70x	22,100; -	-

x denotes shoulder.

In all cases, a concentration of approximately 0.001 per cent was used.

The ultraviolet absorption spectra of selenazolid-2,4-diones were measured as before using spectroscopically pure ethanol. The results are shown in Table V.

Table V.

Compound	Maximum		Minimum
	λ(mμ)	ε	λ (mμ)
selenazolid-2,4-dione	239	2,300	217
5-methylselenazolid-2,4-dione	241	2,030	218
5-ethylselenazolid-2,4-dione	236	2,410	218
5-isopropylselenazolid-2,4-dione	241	2,500	218
5,5-dimethylselenazolid-2,4-dione	240	2,620	219
3-methylselenazolid-2,4-dione	243	1,750	218
5-ethyl-3-methylselenazolid-2,4-dione	240	3,300	221

Effect of Change of pH on Ultraviolet Absorption Spectra.

To 5ml. of the aqueous solutions used above was added hydrochloric acid (1 drop). Using 5ml. water containing hydrochloric acid (1 drop) as blank, the ultraviolet absorption spectra were measured on the Optica recording spectrophotometer. The spectra were then measured after further addition of 20 per cent sodium hydroxide (2 drops). The results are shown in Table VI.

Table VI.

Compound	Spectrum in water	+ hydrochloric acid	further addition of sodium hydroxide
	λ (mμ)	λ (mμ)	λ (mμ)
2-imino-4-selenazolidone hydrochloride	228;269	211;264	228
5-ethyl-3-methyl-2-imino-4-selenazolidone hydriodide	227;260-280^{x}	223;268	228;260-280^{x}
2-dimethylamino-4-selenazolone hydrochloride	245	224;245-270^{x}	246
5,5-dimethyl-2-dimethylamino-4-selenazolane hydrobromide	246	225;261	247

x denotes shoulder

The same procedure was carried out using sodium hydroxide initially. After measuring the ultraviolet absorption spectra, hydrochloric acid (1 drop) was added and the

spectra again measured. The results are recorded in Table VII.

Table VII

Compound	Spectrum in water	+ sodium hydroxide	further addition of hydrochloric acid
	λ (mμ)	λ (mμ)	λ (mμ)
2-imino-4-selenazolidone hydrochloride	228;269	228	228;260-285^{x}
5-ethyl-3-methyl-4-selen-azolidone hydriodide	227;260-280^{x}	228	228;260-285^{x}
2-dimethylamino-4-selen-azolone hydrochloride	245	245	224;245-275^{x}
5,5-dimethyl-2-dimethyl-amino-4-selenazolone hydrobromide.	246	246	227;245-280^{x}

x denotes shoulder.

Effect of various pH values on Ultraviolet Absorption Spectra.

Buffers of low absorption characteristics used by Albert and Phillips[224] included borate, phosphate and acetate while hydrogen-ion exponent solutions were used for lower pH values. Marshall and Walker[251] used similar buffer solutions but used various strengths of hydrochloric acid for low pH values and similar sodium hydroxide solutions for the higher pH range. Acetate buffer[269] was used initially for pH 1-5,

but it was found that no extinction values could be measured at 228 mμ due to the high extinction of acetate in this region, so N, 0.1N and 0.01N hydrochloric acid was used for pH 0, 1 and 2 respectively. Phosphate buffer[270] was used for pH 6-8 and borate buffer[271] for pH 9 and 10. N, 0.1N and 0.01N sodium hydroxide was used for pH 14, 13 and 12 respectively. The pH value of each buffer solution was checked on a Pye Dynacap direct reading pH meter before use, since the hydrochloric acid and sodium hydroxide solutions used in their preparation varied slightly from the required concentration. These measured pH values are recorded in Table VIII.

Table VIII.

Buffer	Acetate					Phosphate			Borate	
Theoretical pH	1.09	1.99	3.09	3.95	4.92	6.0	7.0	8.0	9.0	10.0
Measured pH	1.1	2.1	3.1	3.9	4.85	6.12	7.05	8.12	9.05	9.95

Stock aqueous solutions (approximately 0.001 per cent) of 2-imino-4-selenazolidone hydrochloride and 2-dimethylamino-4-selenazoline hydrochloride were prepared while a concentration of 0.01 per cent was used for 5-ethyl-3-methyl-2-imino-4-selenazolidone hydriodide. Buffer solution (5ml.) for each pH value was diluted to 10ml. with water and used as blank.

The test solutions (5ml.) were similarly diluted with buffer and the ultraviolet absorption curve measured as before. The resulting solutions would therefore be expected to have approximately the pH values shown in Table VIII, although dilution would have some effect. The results are recorded in Table IX.

Table IX

Compound		2-imino-4-selenazolidone hydrochloride	2-dimethylamino-4-selenazolone hydrochloride	5-ethyl-3-methyl-2-imino-4-selenazolidone hydriodide
Approximate pH	Medium	λ_{max} (mμ)	λ_{max} (mμ)	λ_{max} (mμ)
-	water	227;269	245	267
0	N hydrochloric acid	211;265	227;269	267
	acetate	265	- 265	265
1	0.1N hydrochloric acid	211;265	228;263	264
	acetate	265	245	269
2	0.01N hydrochloric acid	214;231x;265	245	270
3	acetate	269	247	270
4	acetate	270	246	269
5	acetate	270	245	265-285x
6	phosphate	229;270	245	NIL
7	phosphate	229;270	246	NIL
8	phosphate	229;270	246	-
9	borate	229;270	246	-
10	borate	229;270	245	-
12	0.01N sodium hydroxide	230	245	-
13	0.1 N sodium hydroxide	229	246	-
14	N sodium hydroxide	230	246	-

x denotes shoulder

Infrared Absorption Spectra.

These were measured in Nujol mull on a Perkin-Elmer Infracord spectrophotometer. The wavelengths in each infrared spectrum were checked by means of the polyetyrene band at 6.24 μ. The infrared absorption bands used for structural comparison are recorded in Table X.

Table X

Compound	Infrared absorption bands and strengths.
2-imino-4-selenazolidone hydrochloride	1730(m.); 1680(m.); 1590(m.) $cm.^{-1}$
5-methyl-2-imino-4-selenazolidone hydrobromide	1730(m.); 1650(m.); 1570(m.) $cm.^{-1}$
5-ethyl-2-imino-4-selenazolidone hydrobromide	1740(s.); 1650(s.); 1570(m.) $cm.^{-1}$
5-ethyl-3-methyl-2-imino-4-selenazolidone hydriodide	1750(s.); 1650(s.); 1570(s.) $cm.^{-1}$
2-dimethylamino-4-selenazolone hydrochloride	1730(s.); 1630(s.) $cm.^{-1}$
5,5-dimethyl-2-dimethylamino-4-selenazolone hydrobromide	1740(s.); 1640(s.) $cm.^{-1}$

s. = strong, m. = medium.

The infrared absorption spectra of selenazolid-2, 4-dione derivatives were recorded as above and the results are shown in Table XI.

Table XI.

Compound	Infrared absorption bands and strengths
selenazolid-2,4-dione	1740(s.); 1700(s.)$cm.^{-1}$
5-ethylselenazolid-2, 4-dione	1735(s.); 1700(s.)$cm.^{-1}$
3-methylselenazolid-2, 4-dione	1870(m) ; 1750(s.); 1730(s.); 1670(s.b.)$cm.^{-1}$
5-ethyl-3-methylselen-azolid-2,4-dione	1870(w.b.); 1745(s.); 1670(s.b.)$cm.^{-1}$
3,5,5-trimethylselen-azolid-2,4-dione	———

s. = strong, m. = medium, w. = weak, b. = broad

PART II

DETERMINATION OF SELENIUM

IN ORGANIC COMPOUNDS

INTRODUCTION

The choice of methods for elemental selenium analysis is very wide and includes volumetric[272-279], gravimetric[273,280-282], polarographic[283,284], catalytic[285-287], chromatographic[288], spectrographic[289], colorimetric[290-293] and fluorescent[294,295] techniques. Few of these are specifically designed for organo-selenium compounds, however, and this fact has been noted by several authors[273,279,281]. From an examination of the methods used for the determination of selenium in inorganic compounds, it would appear that at least some of these might be adapted for organo-selenium compounds, since a standard pattern of decomposition of any organic matter is succeeded by an estimation procedure. Intermediate stages e.g. distillation of selenium as selenium bromide[296], or the preparation of selenocyanate[278], are sometimes adopted as separation procedures. Reduction of the oxidation intermediate to elementary selenium, which is estimated colorimetrically[276] or gravimetrically[280,281], is commonly used.

Critical examination of the various types of method eliminated all but two on the grounds of lengthy procedure[279], type of compounds[272,279], or quantity of material[280]. Gravimetric methods seemed to be of little value for our purpose since a relatively large amount of material was required[280], and collection of the residue was often

incomplete[281]. These two types - volumetric and colorimetric methods - appeared to be worthy of further consideration.

Volumetric Methods.

Many of these methods are combinations or adaptations of existing methods[274]. Probably the commonest is that using potassium iodide, the liberated iodine being titrated with thiosulphate[272-4]. Also used are a potassium permanganate method[275], in which the excess is titrated with ferrous ammonium sulphate, and an argentometric method using chromate indicator[279] which cannot be used for halogen-containing compounds. Two methods use ascorbic acid in which the titration is carried out using photometric[276] and polarographic techniques[297]. A direct titration of selenious acid with 0.01N sodium hydroxide[277], using methyl orange indicator has also been used.

An examination of these methods suggested that the method of Gould[274] was the most useful. Gould conceded that the method of McCullough, Campbell and Krilanovich[272] gave highly accurate results for a number of different types of compound, although it was inapplicable in the presence of bromine, iodine and sulphur. The method evolved by Gould[274] is a combination of the degradative process of Banks and

Hamilton[280] and the titration method of van der Meulen[298], as modified by McCullough, Campbell and Krilanovich[272]. Gould's method[274] is not applicable to compounds containing iodine, since the latter is partially oxidised to iodate which would also liberate iodine from iodide in acid solution.

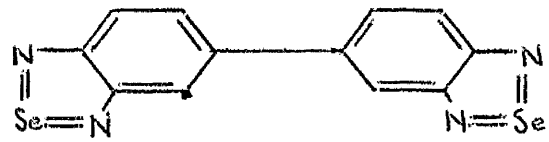
Colorimetric Methods.

Selenium has been shown to give colour reactions with many reagents including codeine sulphate[299], sulphuric acid[300], aqueous sodium sulphide[301], diaminobenzidine[302], and a mixture of o-aminophenylarsonic acid and salicylaldehyde[303]. Two of these reagents, codeine sulphate and diaminobenzidine, have been applied to the quantitative estimation of selenium.

The reaction of codeine sulphate with selenium to give a blue colour was first reported by Schmidt[299], and has been used by Horn[304]. Schmidt showed that Fe^{+++} interfered, as did water from the air. By comparison with freshly prepared standard colours, fairly good results were obtained by Gortner and Lewis[290] for the determination of selenium in tissue and faeces. These workers had considered the method of Robinson, Dudley, Williams and Byers[293], and the modification of Dudley and Byers[305] to be too involved and liable to loss of selenium. Colour development, using 3 per cent

codeine sulphate solution, required seven hours, a period greatly in excess of that expected for a rapid assay.

The diaminobenzidine reaction was first reported by Hoste[302] as a qualitative test in which selenious acid gave a quantitative yield[291] of a yellow insoluble compound in neutral, alkaline and acid media. Unlike most methods of detection of selenium, it does not depend on an oxidation-reduction process. The yellow compound formed was described as diphenyl dipiazselenole (CLVIII)[302], melting



(CLVIII)

at 292°(uncorrected), and analysing correctly for nitrogen and selenium.

If oxidising agents are present, the colour produced is violet. The reaction is stated to be specific for Se^{IV} and Hoste and Gillis[291] modified it to obtain a spectrophotometric method. The reaction was carried out in acid medium, since diaminobenzidine, which is used as the tetrachloride, is rapidly oxidised by the air in neutral or alkaline solutions. The readings were taken on a Beckmann DU spectrophotometer, using a tungsten lamp with a violet filter, below 350mμ in 1 cm. cells, a blank being used.

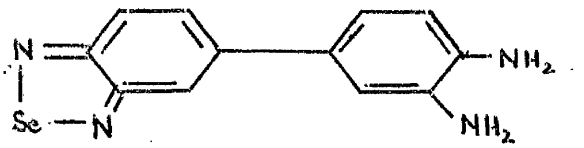
The extinction increased with acidity, but the rate of formation of the yellow selenol was simultaneously slowed. Finally, as a compromise, 0.1N hydrochloric acid was used and the extinction measured at 348m/u, the wavelength of maximum absorption. The Beer-Lambert law was obeyed between 0.25 and 2.5/ug. selenium per ml., but further increase in concentration caused the selenol to precipitate, and led to erratic results. Solutions with an extinction greater than 0.690 were therefore avoided. The apparent advantages of the method were a colour stable over many hours, sensitivity, the molecular extinction coefficient being 10,200, and specificity, since none of the common ions interfered.

Cheng[306] also used diaminobenzidine as a reagent for Se^{IV}. His method involved acidification, to pH 2-3, with formic acid, which gave more rapid development, addition of the reagent, and, after 30-50 minutes, adjusting the pH to 6-7 with 7M. ammonium hydroxide. The coloured complex was then extracted into toluene and the extinction measured at 420m/u against a blank. To eliminate interference from Fe^{+++}, Cu and other metals, ethylenediaminetetraacetic acid (EDTA) was added to the acid solution (pH < 6).

In a recent paper, Parker and Harvey[295] described a method of determining trace amounts of selenium in plant

materials using 3,3'-diaminobenzidine, as did Hoste and Gillis[291] and Cheng[306], to produce a yellow compound, which was determined by means of its fluorescence. In Cheng's method[306], the absorption of the blank limits the sensitivity, but in the fluorescence method, the blank did not emit and the sensitivity was consequently greater.

In investigating the reaction between 3,3'-diaminobenzidine and selenious acid, Parker and Harvey[295] isolated the monoselenium compound (CLIX) as deep red crystals, melting point 202°, when selenious acid reacted with excess of reagent. These workers showed that, although when one molecular equivalent of 3,3'-diaminobenzidine reacted with two molecular equivalents of selenious acid, the dipiazselenol was produced, this was not the case under the assay conditions. The formation of the dipiazselenol was assumed by both Hoste and Gillis[291] and Cheng[306], but the monoselenium compound is in fact formed. It was of interest that the formula (CLVIII) described by Hoste and Gillis[291] and



(CLIX)

by Cheng[306] involved quadrivalent selenium, whereas the formula of Parker and Harvey[295] (CLIX) showed doubly bound selenium. The latter structure seems preferable in view of

the work of Sawicki and Carr[129].

Danzuko and Ueno[307] have also used diaminobenzidine to determine trace amounts of selenium in sulphuric acid spectrophotometrically.

Cheng[306,308] mentioned the disadvantages of redox procedures for detection and determination of selenium, and suggests the use of 4-dimethylamino-1,2-phenylenediamine and 4-methylthio 1,2-phenylenediamine which react with selenious acid to give stable bright red and blue-purple colours respectively, in appropriate media. Cheng suggested that either reagent, possibly with minor modifications, was suitable for the method of Hoste and Gillis[291] for quantitative work.

Many methods involve a final turbid solution produced by reduction of an intermediate, usually selenious acid, to selenium. This can be stabilised and measured by comparison with standards[293]. A novel way of avoiding this turbidity production was described by Franke, Burris and Hutton[309]. The coloured precipitate of colloidal dimensions can be filtered on to a mat of standard barium sulphate on filter paper supported by a filter disc. The colloidal selenium is thus retained as a surface layer against a standard white background. This gives a means of obtaining

stable colour standards and avoids their daily preparation.

Fogg and Wilkinson[310] found that the determination of selenium by the suggested method of the American Public Health Association was unsatisfactory because of low results. They replaced the method of reduction (sulphur dioxide and hydroxylamine) by one reducing agent, ascorbic acid, and obtained the selenium consistently in pink colloidal form instead of in tints varying from yellow to pink. The whole procedure is, however, subject to criticism because of the visual measurement of the turbidity and the relatively complex procedure involved.

A different type of method was used by Goto, Hirayama and Ikeda[286]. This was based on the observations of Feigl and West[285], who reported that the reducing power of alkali sulphide solutions is increased by the presence of selenium. This was tested with chromate, picric acid, dichlorophenolindophenol, cacopheline and methylene blue. The time of fading of the colour of methylene blue was determined in the presence of varying quantities of selenium and a similar method was adopted by Goto, Hirayama and Ikeda[286], and by Lang[287], who used cacopheline or methylene blue, among other reagents, to detect selenious acid.

Another method[296] involves the oxidation of selenium to selenious acid using hydrobromic acid and bromine mixture,

and the oxidation of iodide to iodine which is then estimated by measurement of the extinction at 352 or 615mμ on a Beckman spectrophotometer.

The observation that the reduction of selenious acid in the presence of chlorpromazine hydrochloride gave a golden brown colour instead of a precipitate of selenium, prompted an investigation for assay purposes. When it became evident that a complex of selenium and chlorpromazine was not involved, a comparison of the efficiency of this reagent with that of known stabilisers was also undertaken. This appeared to be desirable in view of the wide variety of stabilisers, some of which would appear too awkward to obtain and to prepare.

DISCUSSION OF EXPERIMENTAL WORK

STANDARD SELENIUM DIOXIDE

The required product for the formation of a colour is selenious acid, H_2SeO_3, formed when selenium dioxide is dissolved in water. Sublimation of the dioxide always gave traces of red selenium and proved unsatisfactory in preparing a standard. The method using nitric acid, however, gave colourless crystals and was similar to that of Lenher[311], except that condensing funnels were omitted. The product gave satisfactory analyses when examined by four methods, the estimated purity being between 99.2 and 100.0 per cent, whereas the apparent content of SeO_2 in the original material depended upon the method used. The results are deemed to be of sufficient interest to be given in Table XII.

OXIDATION OF ORGANIC MATTER

Oxidation of the organic compounds with a mixture of sulphuric and nitric acids[280,312] proceeded smoothly and without the significant loss of selenium which is liable to occur when such an oxidation mixture is used[310,312]. This is no doubt explained by the very much smaller quantity of organic matter present (less than 10mg.) as compared with 0.2 - 10g. when traces of selenium are determined in vegetable matter. Various other methods mentioned in the literature include the Kjeldahl method as for nitrogen analysis[290,293,304], fusion with sodium carbonate[313], with or without sodium peroxide, Carius tube[279,281], Parr Bomb[273] and a complex mixture used by McNulty[314]. The nitric and sulphuric acid mixture sometimes included hydrogen peroxide[305].

The possibility of loss of selenium during the oxidation procedure[281,290,304,310,315] has given rise to many modifications. Horn[304] used the Kjeldahl method with mercury as a catalyst, in preference to nitric and sulphuric acids, since there is the possibility of double salt formation between mercury and selenium[316] which is consequently fixed. Gortner and Lewis[290] rejected Robinson's method[293] due to the number of stages involved and also adopted the Kjeldahl procedure. Williams and Lakin[315] state that in the Kjeldahl

method, if the evolution of gas is too rapid, there is danger of loss of selenium and suggest a temperature less than 120°, whereas Gould[274] suggests heating with sulphuric acid over a low flame until charring and appreciable fuming occurs. Silverthorn[317] as a further alternative, suggests the use of a glass wool plug in the neck of the distillation flask.

Drew and Porter[281] used nitric and hydrochloric acids in open vessels, but did not remove the nitric acid since it did not interfere with their estimation procedure. Fogg and Wilkinson[310] found that if a large amount of material containing selenium was digested with nitric and sulphuric acids, loss of selenium occurred, but if the process had a little perchloric acid added, selenium recovery was excellent. Perchloric acid could not be used in this instance because selenious acid is oxidised further to selenic acid, which is not reduced under the assay conditions.

Control experiments with selenium dioxide in the presence of glucose and compounds which are difficult to oxidise, gave reasonable recoveries of selenium as can be seen from Table XXII.

REDUCTION OF SELENIOUS ACID

Selenious acid can be reduced by a variety of reagents, of which sulphur dioxide in hydrochloric acid solution[291,293,313], hydrazine salts[280,282], stannous chloride[318], ascorbic acid[276,310,319], and sulphites[305] are most commonly used.

Cheng[306] states that sulphur dioxide, sulphites and ascorbic acid are insensitive and subject to interference from other elements. He had earlier suggested[308] the use of ethylenediamine tetraacetic acid as being more sensitive, because it prevented interference from ferric iron, copper and other metals. It was still not very sensitive, however, and the colour in aqueous solution was unstable.

The products of reduction depend on the reducing agent and the conditions used. The nature of the product is of importance when reduction is used in the production of a colour or turbidity for spectrophotometric determinations. Thus Fogg and Wilkinson[310], as previously mentioned, preferred ascorbic acid to sulphur dioxide and hydroxylamine since the selenium was consistently obtained in pink colloidal form. It is convenient to retain the selenium in colloidal solution, and for this purpose stabilisers are essential. These, however, must be added to the assay

mixture before the ascorbic acid, to avoid precipitation of selenium which was otherwise immediate. If less than 4mg. chlorpromazine hydrochloride was used, no extinction readings could be taken due to turbidity, while at least 6mg. chlorpromazine hydrochloride was necessary for a stable colour to be produced (Table XIII). Rudra and Rudra[319], however, had used ascorbic acid to produce a golden brown sol without the use of a stabiliser.

Reduction of selenious acid to selenium is more conveniently carried out by ascorbic acid than by hydrazine, stannous chloride or sulphur dioxide, as traces of red oxidation products of chlorpromazine were rapidly decolourised. Using 10mg. of ascorbic acid, the colour was fully developed within 20 minutes (figure 7). As a comparison, the full development of colour took approximately 90 minutes using 5mg. ascorbic acid and 15 minutes using 20mg. ascorbic acid (Table XV).

Although reduction can be carried out in strongly acid media (approximately N), the results of the experiments under Effect of Acidity on the Stability and Extinction of Selenium Sols showed that the presence of appreciable traces of nitric acid in the final solution slowed the rate of colour formation and gave erratic results. If more than a trace of nitric acid was present, the solution became turbid

on addition of the ascorbic acid. Neutralisation of the mixture was therefore essential in order that some degree of control could be exercised over the final acidity. One or two drops of hydrochloric acid in excess of that used in the method, caused no marked change in extinction and allowed reasonable latitude in the size of the drops (Table XVII).

The effects of acid when different amounts of chlorpromazine hydrochloride were used, can be seen from the results in Table XIV. These results indicate that the difference between the effect of no acid and 1ml. acid on the extinction decreases with increase in chlorpromazine hydrochloride content. However, a concentration of more than 20mg. chlorpromazine hydrochloride would appear to be undesirable because of solubility effects with sodium sulphate.

From the above discussion, it was concluded that 20mg. chlorpromazine hydrochloride is necessary to avoid too great a variation in extinction with small variations in acid. Also 10mg. ascorbic acid appeared to be sufficient to give a stable extinction reading within a reasonable time and under conditions of acidity which might very slightly under assay conditions. As seen in Table XVI, 20mg. of ascorbic acid gave a reading only slightly greater

than that obtained with 10mg. of ascorbic acid (Table XIV) and so 10mg. of ascorbic acid was adopted for the assay.

Colour.

The colour is attributed to colloidal selenium rather than to a complex of selenium and chlorpromazine, because it is similar to that obtained when other stabilising agents are used. This is confirmed by the precipitation of selenium on addition of ethanol to the reaction mixture.

The sols showed no absorption maximum (figure 6) over the range 370-625mμ and the nominal wavelength of 420mμ was selected because this region is conveniently obtained on a filter absorptiometer, if a spectrophotometer is not available. Also, the extinction of chlorpromazine hydrochloride and ascorbic acid in the blank is small in this region.

COLLOIDAL SELENIUM AND STABILISERS

Coleman and McCrosky[278] state that selenium is an element which lends itself to colloidal dispersion and various methods of preparing colloidal selenium have been described[320-323].

Gutbier and Heinrich[324] investigated the reduction of selenium dioxide with sulphur dioxide to give varying products under different conditions. In conjunction with other workers, Gutbier[325-328] investigated protective colloids to stabilise the selenium in colloidal form. His investigations showed that gelatin[325], especially if a trace of hydrochloric acid or sodium hydroxide were present, saponin[326], mucilage of Plantago psyllium seeds[327] and the glue from the carob bean[328] were effective. Other workers showed that gum arabic in various proportions[278,293], gelatin[276], glycerol[323,329] and in some instances, starch[298,330], were also effective stabilising agents.

Several of the stabilisers tested, were quickly eliminated from consideration by the appearance of the final sols. Glycerol gave turbid solutions in all proportions tested, while starch and gelatin gave slightly opalescent sols, which showed a marked fall in extinction when the concentration of colloid was increased (Table XXI). These

disadvantages could well give rise to difficulties when different batches of stabiliser are used. Cetrimide was more satisfactory, but the Tyndall effect was still evident under assay conditions. The remarkably small effect of cetrimide concentration is of interest (TableXXI); although highly efficient in slightly acid, salt-free solutions, it could not be used without preliminary neutralisation of the oxidation mixture.

Cetomacrogol was very efficient as shown by 1ml. of 0.005 per cent solution being sufficient to stabilise 0.6mg. of selenium dioxide in a slightly acid, salt-free solution and a determined, but unavailing effort was made to confirm cetomacrogol as the stabiliser of choice. Encouraging results were obtained in the presence of sulphuric acid, but as shown in the experimental section, neutralisation of the oxidation mixture could not be avoided. Thus, the selenium sols showed an obvious Tyndall effect at low concentrations of cetomacrogol and an increased concentration was necessary to give the degree of clarity obtained when chlorpromazine hydrochloride was used. Thus cetomacrogol, under the assay conditions, lost two possible advantages viz., elimination of one step in the assay and a low concentration of the reagent. In addition, the composition of cetomacrogol may vary slightly from batch to batch so that a check on the

effect of using different batches of material would be necessary, before suggesting its use in the colorimetric assay.

Sodium dodecylsulphate was not examined in detail but was included to show the effect of an anionic surface active agent. From Table XXI, it can be seen that the extinction was comparable with those obtained using cetrimide and cetomacrogol, but a high concentration of the stabiliser was required.

Chlorpromazine was therefore retained as the stabiliser of choice and no difference in results was noted when using different batches of reagent. A concentration effect was observed as with other stabilisers, but was not regarded so seriously, since standard solutions can be reproduced more easily than with colloids. On a few occasions, a cloudy precipitate appeared in the assay solution on addition of the chlorpromazine hydrochloride solution. This was easily dissolved by addition of water (1ml.) and shaking, and had no effect on the extinction.

Salt and Temperature Effects.

As might be expected, the presence of sodium sulphate affected the extinction of the sols (Table XVIII). It must therefore be included when preparing the calibration curve.

As previously mentioned, more than a trace of nitric acid slowed the development of colour and even after neutralisation, inconsistent results were obtained. The development time in the presence of small quantities of sodium nitrate was as much as four hours, which can probably be accounted for by the nitrate offsetting the reduction action of the ascorbic acid.

The effect of temperature (Table XIX) indicated that a wide range for development of colour should be avoided and the solutions used were generally at 20°.

The method has been used successfully throughout this work for various types of compounds, using the quantities recommended in Table XXIII. No variation was noted when different samples of chlorpromazine hydrochloride and ascorbic acid were used by several operators using the original calibration curve (figure 8). Table XXIV shows the results obtained, and the theoretical values required, in the assay of the selenium compounds in the synthetic section, together with some test compounds kindly supplied by Smith Kline and French Laboratories.

EXPERIMENTAL

The author wishes to thank Mrs.D.Harvey and Miss M.Buchanan for technical assistance, and Smith, Kline and French, Ltd., for the supply of compounds.

Reagents.

Sulphuric acid.

Nitric acid.

Hydrochloric acid.

Sodium hydroxide solution, 20 per cent $^{w}/_{v}$.

Ascorbic acid solution, 1 per cent $^{w}/_{v}$.

Chlorpromazine hydrochloride solution, 2 per cent $^{w}/_{v}$.

Standard Selenium Dioxide.

Selenium dioxide (reagent grade, 50g.) was treated with nitric acid (25ml.) in an evaporating dish and heated in a heating mantle until the removal of nitric acid was complete. The surface crust of colourless needle crystals (24.5g.) was easily separated from an amorphous grey residue by means of a spatula, stored in a desiccator and used for the preparation of standard selenious acid solutions. These were prepared by dissolving selenium dioxide (0.1g.) in water, adding dilute sulphuric acid (10ml.) and diluting to 100ml. with water. This solution was then diluted as required in the preliminary experiments.

Standardisation of Selenium Dioxide.

Four methods of analysis were used to standardise the selenium dioxide. These involved titration using potassium

permanganate[331], and titration of iodine, liberated from potassium iodide, using sodium thiosulphate[332]. Two gravimetric methods were also used, one using sulphur dioxide[333], and the other hydrazine[282] as reducing agent. The original unsublimed sample was also analysed by each of the four methods. The results are recorded in Table XII.

Table XII.

Percentage of SeO_2 when determined by various chemical methods.

Material	Method			
	Potassium Permanganate (Volumetric)	Iodine/ Thiosulphate (Volumetric-method B)	Sulphur Dioxide (Gravimetric-procedure A)	Hydrazine (Gravimetric)
Original	99.6 99.6	94.6 94.4	93.0 93.1 96.3	91.7
Standard	99.6 99.6	99.7 99.7	99.6 100.0	99.2 99.3

Initial Colour and Absorption Test.

Selenious acid solution (1ml.) containing 0.1mg./ml. of selenium dioxide, was pipetted into a 10ml. graduated flask. To this was added chlorpromazine hydrochloride solution (1ml.), ascorbic acid solution (1ml.) and distilled water to 10ml. The solution became golden brown in colour

almost immediately and remained clear and bright. The extinction of this solution was measured against a blank of reagents over a wavelength range of 370-625 mμ using the SP 500 and Uvispek spectrophotometers. The extinction was then plotted against wavelength. A typical result is shown in figure 6.

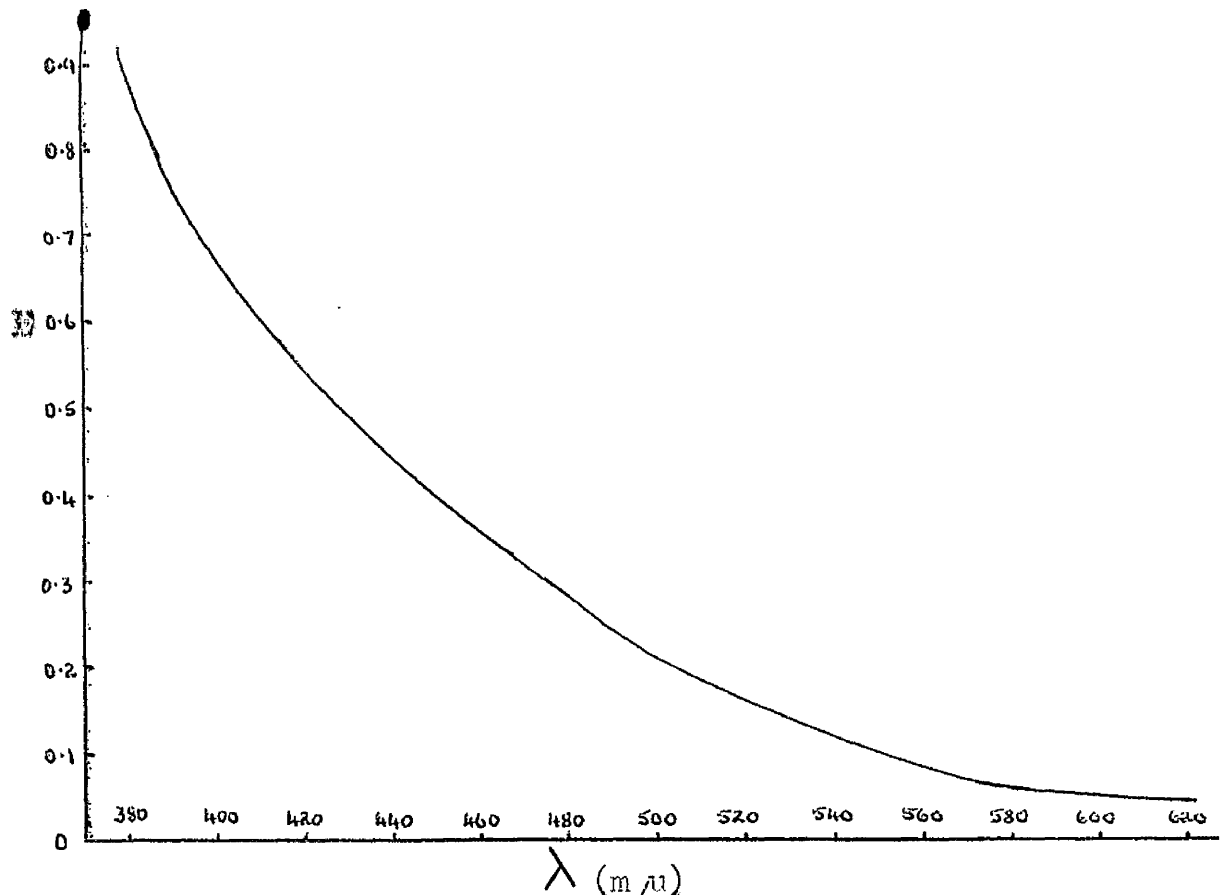


Figure 6 . Extinction of selenium sol.

Time for Development of Colour

Using two solutions, containing 0.05mg. selenium dioxide per ml., and 0.1mg. selenium dioxide per ml. respectively, prepared as in the previous experiment, the

development of the colour produced was followed by measuring the extinction of the two solutions at 420mμ on the SP 600 against a blank of reagents. The readings were taken at intervals of 30-40 seconds until the extinctions reached constant values, followed by two readings at five minute intervals, one after a further fifteen minutes and finally one after a further hour. The initial readings were taken five minutes after preparation. A typical set of results is shown in figure 7 .

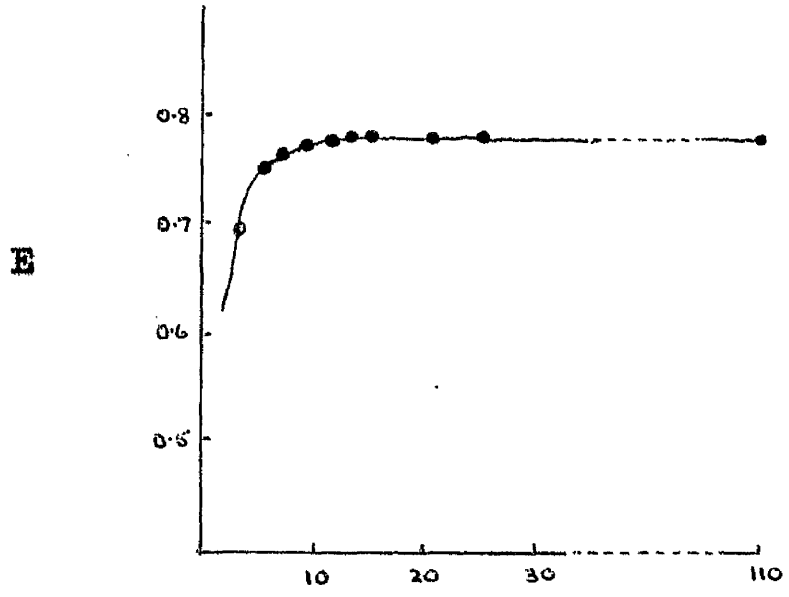


Figure 7 . Development and stability of the extinction of a selenium sol.

Effect of Chlorpromazine Hydrochloride on the Extinction and Stability of Selenium Sols.

1) Into each of a series of 10ml. graduated flasks was pipetted selenious acid solution (1ml.) containing selenium dioxide (1mg.). Varying amounts of chlorpromazine hydrochloride solution were added equivalent to 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 mg. of chlorpromazine hydrochloride, together with distilled water (5ml.). Ascorbic acid solution (1ml.) was added to each flask and the solutions made up to volume with distilled water. After 30 minutes development time, the flasks were examined and if the solution was clear and bright, the extinction was measured at 420mμ against a water blank. The experiment was then repeated on the solutions containing 4.0 - 10mg. chlorpromazine hydrochloride. The results are recorded in Table XIII.

Table XIII.

Effect of varying amounts of chlorpromazine hydrochloride on extinction.

Amount of chlorpromazine hydrochloride (mg.)	Appearance of solution after 30 minutes	Extinction at 420 mμ	Notes
0.5	Turbid	-	Immediate turbidity
1.0	Turbid	-	Immediate turbidity
2.0	Turbid	-	Turbidity after 10 minutes
4.0	Clear	0.749; 0.745	Turbidity after 60 minutes
6.0	Clear	0.749; 0.760	Remained clear
8.0	Clear	0.775; 0.781	Remained clear
10.0	Clear	0.801; 0.842	Remained clear

ii) With no acid in the selenious acid solution, solutions were prepared as above containing 6.0, 10.0, 20.0 mg. chlorpromazine hydrochloride, but with the further addition of varying amounts (0.0, 0.4, 1.0ml.) of N hydrochloric acid. The extinction values obtained are recorded in Table XIV.

Table XIV

Effect of varying amounts of chlorpromazine hydrochloride and hydrochloric acid on extinction.

Chlorpromazine hydrochloride added	N Hydrochloric acid added		
	0.0 ml.	0.4 ml.	1.0 ml.
6 mg.	0.909	0.860	0.729
10 mg.	0.860	0.832	0.741
20 mg.	0.810	0.820	0.780

Effect of Ascorbic Acid on Extinction of Selenium Sols.

1) Standard selenious acid solution equivalent to 0.6mg. selenium dioxide was treated with chlorpromazine hydrochloride solution (1ml.) and varying quantities of ascorbic acid. The solutions were made up to volume as before and the extinction was measured after 30 minutes. The results are recorded in Table XV.

Table XV.

Effect of ascorbic acid on extinction.

Ascorbic acid (mg.)	5	10	20
Extinction	0.517	0.507	0.507
Approximate time (in minutes) for complete development of colour.	90	20	15

ii) Using ascorbic acid solution (2ml., equivalent to 20mg. ascorbic acid) and acid-free selenious acid solution, solutions were prepared as before containing 6.0, 10.0, 20.0 mg. chlorpromazine hydrochloride respectively. The extinctions of these solutions, measured after 30 minutes, are recorded in Table XVI.

Table XVI.

Effect of ascorbic acid with varying amounts of chlorpromazine hydrochloride on extinction.

Amount of chlorpromazine hydrochloride added(mg.)	Extinction	Extinction after 90 minutes
6	0.880	0.895
10	0.870	0.883
20	0.830	0.828

Effect of Acidity on Extinction of Selenium Sols.

Traces of nitric acid present

i) Selenium dioxide (2.765mg.) was digested with sulphuric acid (1ml.) and nitric acid (2ml.) in the presence of glucose (5mg.), as described in the Method for Determination of Selenium.

The clear solution was transferred to a 25ml. graduated flask and made up to volume with distilled water. Aliquot

portions (5ml.) of this solution were treated with ascorbic acid solution (1ml.) and chlorpromazine hydrochloride solution (1ml.) as stabiliser. The colour developed slowly over a period of 90 minutes and successive aliquot portions gave erratic extinction readings. Using cetomacrogol as stabiliser, the development was again slow and the extinction readings were even more erratic.

ii) An aliquot portion of the solution obtained from the above i), was neutralised with 20 per cent sodium hydroxide solution to phenolphthalein and the colour developed using hydrochloric acid (1 drop) to acidify the solution before addition of the reagents. The colour developed quickly within 30 minutes and remained stable.

iii) A solution of selenious acid obtained in one of the assays was examined in the presence of one and two drops of hydrochloric acid in excess of that used in the assay process. The results are recorded in Table XVII.

Table XVII

Effect of increased amounts of hydrochloric acid on extinction.

Excess of hydrochloric acid	As in assay	1 drop	2 drops
Extinction	0.883	0.878	0.886

Nitric acid absent.

Aliquot portions of selenious acid solution containing 0.208mg./ml., were treated with sulphuric acid (0.2ml.), chlorpromazine hydrochloride solution (1ml.), ascorbic acid solution (1ml.) and made up to 10ml. with distilled water. The extinction readings became steady within 30 minutes and remained stable.

Effect of Sodium Sulphate on Extinction of Selenium Sols.

Colours were developed from selenious acid solutions in the presence of sodium sulphate (anhydrous, 0.5g.) and in its absence. The extinction readings are recorded in Table XVIII.

Table XVIII

Effect of sodium sulphate on extinction.

Extinction (Na_2SO_4 absent)	0.344	0.528	0.684	0.844
Extinction (Na_2SO_4 present)	0.356	0.538	0.714	0.895

Effect of Temperature on the Extinction of Selenium Sols.

Colours were developed from a selenious acid solution at 13°, 22° and 25°. The extinction readings are recorded in Table XIX.

Table XIX

Effect of temperature on extinction.

Temperature (°C)	13	22	25
Extinction	0.292	0.289	0.284

Calibration Curve.

Using a standard solution of selenious acid, a series of dilutions was made to contain approximately 0.2, 0.4, 0.6, 0.8 and 1.0mg. of selenium dioxide in a final volume of 10ml. Included in each solution was anhydrous sodium sulphate (0.5g.) to correct for the amount produced in the neutralisation process. The colours were developed as described in the method for analysis (page 214). A typical set of results is shown in Table XX.

Table XX

Selenium dioxide content(mg.)	0.238	0.476	0.714	0.952	1.19
Extinction	0.200	0.380	0.590	0.790	0.980

These results when plotted gave the following calibration curve, figure 8a.

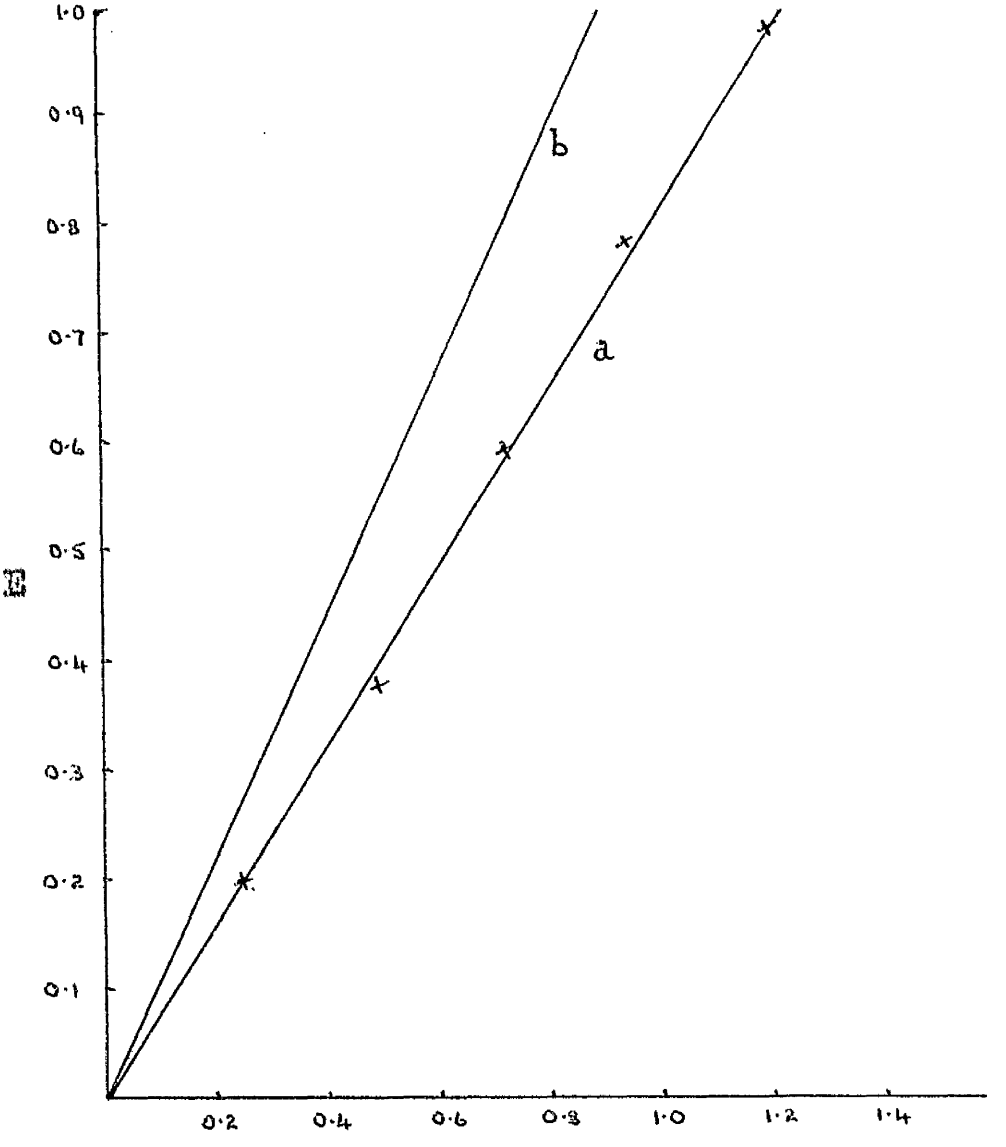


Figure 8 . Calibration curve

For convenience, these results were also plotted in terms of Se (figure 8b) to give the calibration curve for use in the assays.

Comparison of Stabilisers

Varying amounts of stabilisers were added to slightly acid solutions of selenious acid and a sol developed by the addition of ascorbic acid solution (1ml.). The solutions were adjusted to 10ml. with water and the extinctions measured after 30 minutes at 420 mμ in 1 cm.cells. The results are recorded in Table XXI.

Table XXI

Extinctions of selenium sols in the presence of stabilisers

Stabiliser	SeO_2 (mg.)	ml. of 1 per cent solution of stabiliser					
		0.01	0.2	0.5	0.6	1.0	2.0
Chlorpromazine hydrochloride	1.0	-	Turbid	Unstable	0.860	0.832	0.820
Cetomacrogol	0.604	0.490	0.480	0.476	-	0.468	0.463
Cetrimide	0.604	0.479	-	-	-	0.474	-
Gelatin	0.604	-	0.530	0.520	-	0.504	-
Sodium dodecylsulphate	0.604	Turbid	Turbid	-	-	0.468	-
Starch	0.604	-	0.695	0.620	-	0.567	-
Glycerol	0.5	Turbid at all concentrations up to 1ml. in 10ml. of final mixture.					

Effect of Organic Material on the Recovery of Selenium.

Accurately weighed quantities of selenium dioxide were treated as described in the Method for the Determination

of Selenium in the presence of 7-10mg. of organic compounds. The results are recorded in Table XXII.

Table XXII.

Effect of organic material on the recovery of selenium.

Organic Compound	SeO_2 weighed (mg.)	SeO_2 found (mg.)	Recovery (per cent.)
Glucose	3.260	3.21	98.5
Glucose	2.350	2.35	100.0
Methylene Blue	3.360	3.32	98.8
Phenothiazine	3.348	3.28	98.0
Phenothiazine	3.310	3.32	100.3
Phenobarbitone	3.040	3.06	100.7
Thiouracil	2.990	2.97	99.3
"	1.038	1.04	100.2

Method for the Determination of Selenium.

Dissolve an accurately weighed quantity of the organic compound, equivalent to about 2-3mg. of Se, in sulphuric acid (1ml.) and nitric acid (2ml.) in a Kjeldahl flask of about 40ml. capacity. Boil off the nitric acid carefully to leave a colourless residue (7-15 minutes)*.

x The digestion can also be carried out by electrical heating, as used in the normal micro-Kjeldahl process, but a period of 2 hours is necessary to remove the nitric acid.

If the residue acquires a red or brown tint towards the end of the reaction, add 1 drop of nitric acid and remove the excess by heating. Cool the residue, dilute with water (10ml.) and neutralise with sodium hydroxide solution using 1 drop of phenolphthalein solution as indicator. Cool the mixture to 20°, transfer to a 25ml. graduated flask, make up to volume and mix well. Transfer 5ml. of the solution to a 10ml. graduated flask, add hydrochloric acid (1 drop) from a test pipette to make slightly acid, chlorpromazine hydrochloride solution (1ml.), water (2ml. if necessary to give a clear solution) and ascorbic acid solution (1ml.). Make up to volume with water, mix well and measure the extinction of the sol at 420mμ in a 1 cm. cell after 30 minutes, using a blank of reagents treated in the same way. Calculate the percentage of selenium in the compound by reference to a calibration curve prepared by using known amounts of selenium dioxide in the presence of sodium sulphate (0.5g. anhydrous) and hydrochloric acid (1 drop), in a final volume of 10ml.

Amount of Sample Required for Analysis.

Let Se content of compound under examination be x per cent, and amount weighed out M mg.

∴ Amount of Se released (as SeO_2) = $\frac{M\,x}{100}$ mg.

The solution was made up to volume (25ml.) of which an aliquot portion (5ml.) was treated for analysis.

$\therefore$ Amount of Se in assay solution = $\frac{M\,x}{500}$ mg.

For optimum measurement, i.e. E approximately 0.4 - 0.5, the test amount in each 10ml. of assay solution should be approximately 0.4mg. Se.

i.e. $\frac{M\,x}{500} = 0.4$ or $M\,x = 200$

From this, the amounts required for a determination are shown in Table XXIII, assuming the use of 1 cm. cells.

Table XXIII

Amount of sample required.

Se per cent	10	20	30	40	50	60	70
Weight used (mg.)	20	10	7	5	4	3.5	3

Specimen Calculation.

$C_6H_{10}N_2O$ Se requires Se, 38.48%

Amount weighed = 4.796 mg.

Extinction = 0.427

From calibration curve, Se equivalent = 0.368

$\therefore$ % Se = $\frac{0.368 \times 5}{4.796} \times \frac{100}{1} = 38.37$

Table XXIV

Selenium in Organic Compounds.

Compound No.	Se (per cent) Found	Theory	Compound No.	Se (per cent) Found	Theory
1.	31.4	31.63	26	37.9	38.31
2.	16.3	16.41	27	35.3	35.88
3.	21.2	20.82	28	32.6	32.74
4.	45.9	46.44	29	40.9	41.11
5.	30.2	29.8	30	44.1	44.35
6	21.3	21.57	31	38.05	38.31
7	19.5	19.23	32	23.3	23.71
8	39.4	39.58	33	18.4	18.19
9	20.2	20.13	34	38.3	38.50
10	30.8	30.61	35	18.3	18.19
11	19.2	19.44	36	24.8	24.90
12	28.95	29.02	37	26.4	26.13
13	40.9	41.32	38	27.1	26.93
14	18.6	18.79	39	30.75	31.32
15	38.3	38.50	40	29.15	29.45
16	38.4	38.50	41	35.95	36.20
17	26.0	26.32	42	31.8	32.07
18	35.6	36.04	43	26.8	26.84
19	32.6	33.02	44	30.1	29.67
20	40.8	41.11	45	26.9	26.84
21	17.2	17.61	46	29.5	29.67
22	25.8	26.26	47*	31.6	32.0
23	41.6	41.11	48*	27.7	28.15
24	43.9	44.35	49*	24.5	24.1
25	38.1	38.31			

* Compounds supplied by Smith, Kline and French, Ltd.

PHARMACOLOGICAL RESULTS

The author would like to thank Smith, Kline and French Laboratories, Ltd., for testing the compounds submitted.

In view of the diverse biological-activity of the oxygen and sulphur analogues, representative selenium compounds were tested for a wide range of activity. 5-Ethyl-2-imino-4-selenazolidone hydrobromide and 5-ethylselenazolid-2,4-dione were tested and gave the results shown in Table XXV.

Table XXV.

Test	Route	Results	
		5-Ethylselenazolid-2,4-dione	5-Ethyl-2-imino-4-selenazolidone hydrobromide.
Dose Range	Oral	LD50:24mg./Kg. Severe clonic convulsions prior to death.	LD50:250mg./Kg. Depression 125mg./Kg. Death after several hours.
	S.C.	LD50:24mg./Kg. Severe clonic convulsions prior to death.	LD50:750mg./Kg. Depression 125mg./Kg.
Analgesic	S.C.	-ve at 10mg./Kg.	-ve at 10mg./Kg.
Max.Electroshock seizure	Oral	-ve at 10mg./Kg.	-ve at 100mg./Kg.
Conditioned Response	Oral	-ve at 9.6mg./Kg.	-ve at 100mg./Kg.
Max.Metrazol Seizure	Oral	sl.+ve at 10mg./Kg. (3/5 protected)	-ve at 100mg./Kg.
Anti-Histamine		+ve at 100/ug. in 20ml.	+ve at 100/ug. in 20ml.
Anti-Amphetamine	Oral	-ve at 1-9mg./Kg.	-ve at 10-90mg./Kg.
Anti-Tremorine	Oral	-ve at 9.6mg./Kg.	-ve at 100mg./Kg.
Sympathetic Block	S.C.	sl.+ve at 5mg./Kg.	-ve at 50mg./Kg.
Diuretic	Oral	-ve at 1-9mg./Kg.	-ve at 10-90mg./Kg.
Parasympatholytic		-ve at 100/ug. in 20ml.	+ve at 100/ug. in 20ml.

The two compounds tested above, together with 5-benzylideneselenazolid-2,4-dione and 5-ethylselenazolid-2,4-dione-2-isopropylidenehydrazone were also tested for antibacterial activity. The results so far obtained are recorded in Table XXVI.

Table XXVI

5-ethyl-2-imino-4-selenazolidone hydrobromide	Staph. aureus	Strep. pyogenes	E. coli	Proteus vulgaris
0.01 %	+	+	+	+
0.005%	+	+	+	+
0.001%	+	+	+	+

+ = growth after 24 hours.

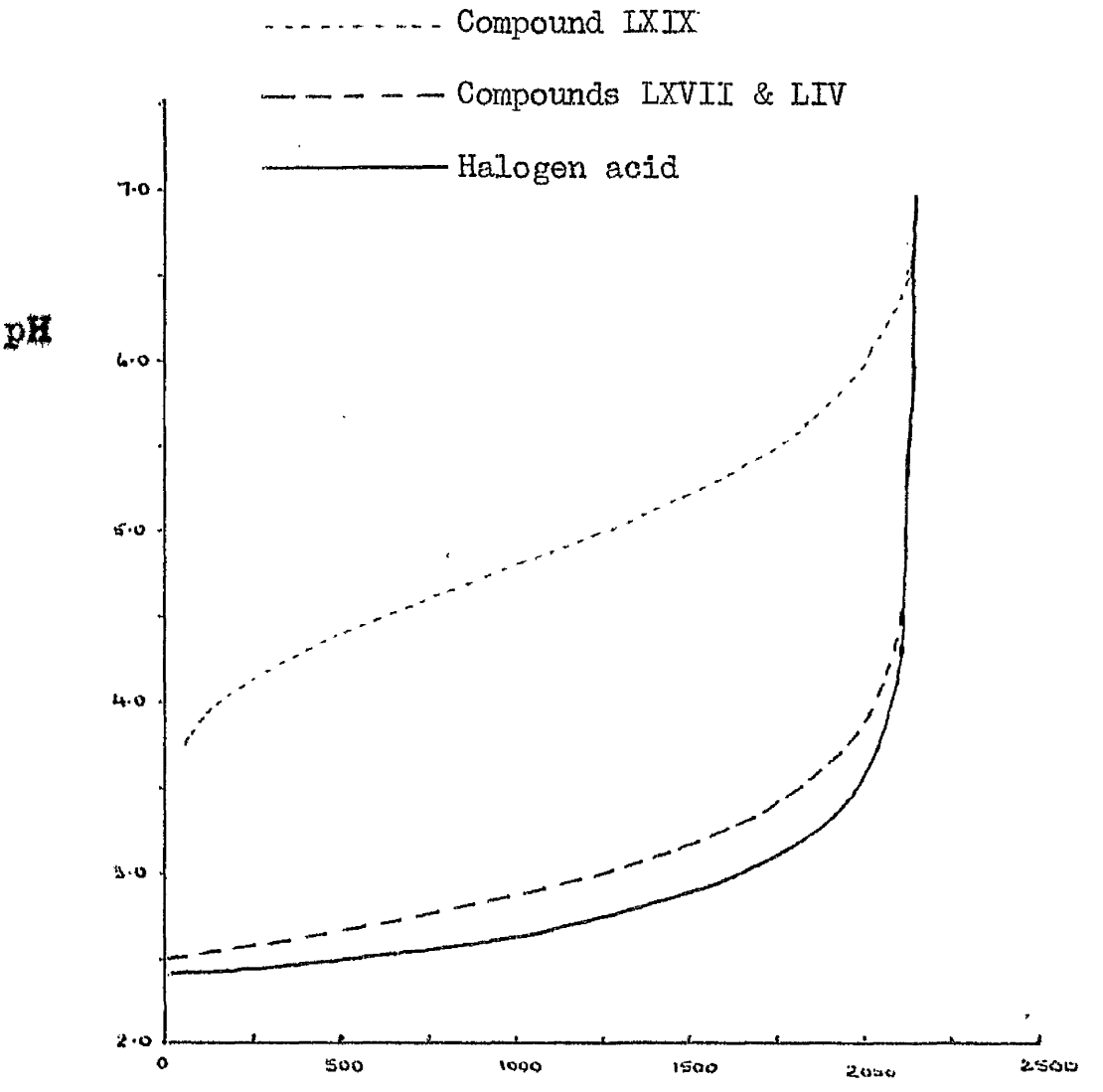
- = no growth after 48 hours.

Conclusions.

2-Imino-4-selenazolidones and selenazolid-2,4-diones showed no promising biological-activity.

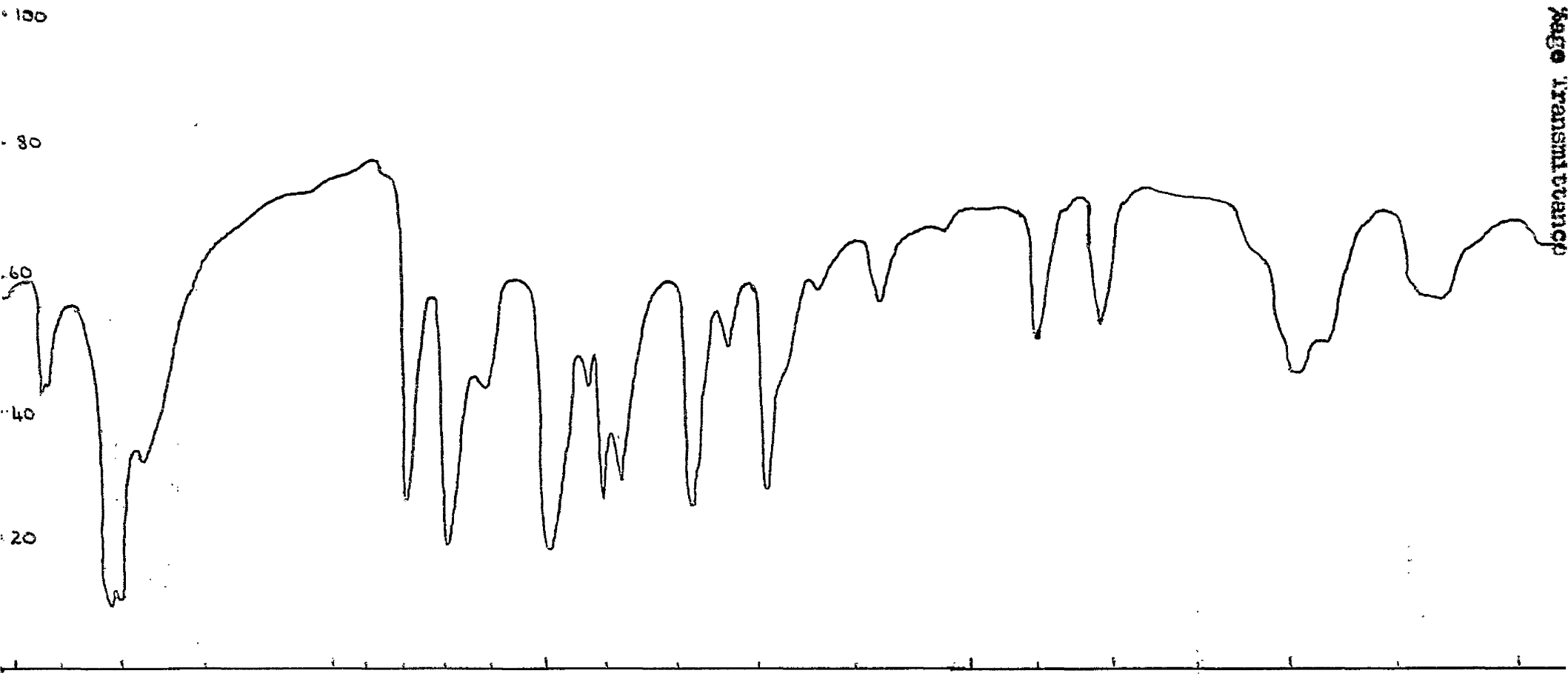
Appendix A.

Titration curves



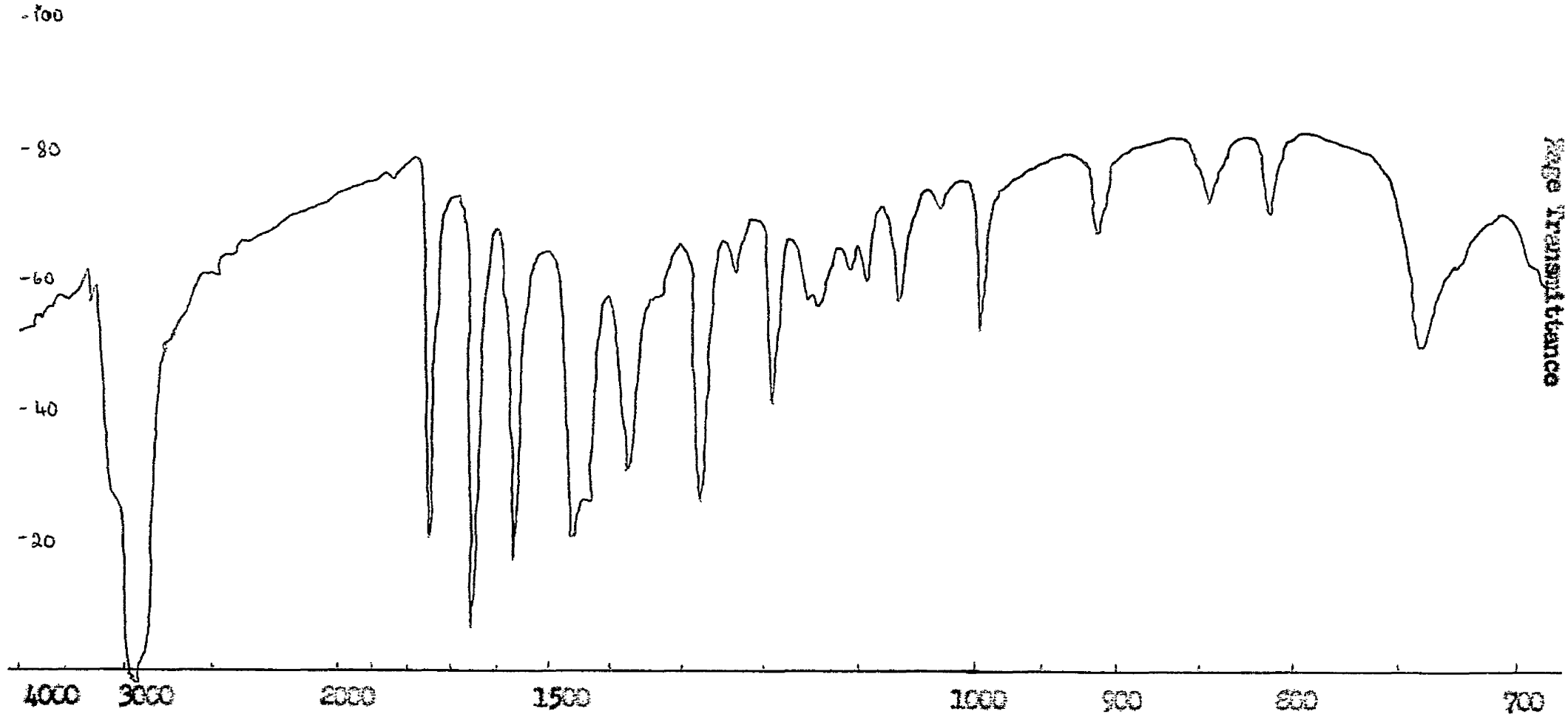
Volume of potassium hydroxide (micrometer units)

Figure 5.

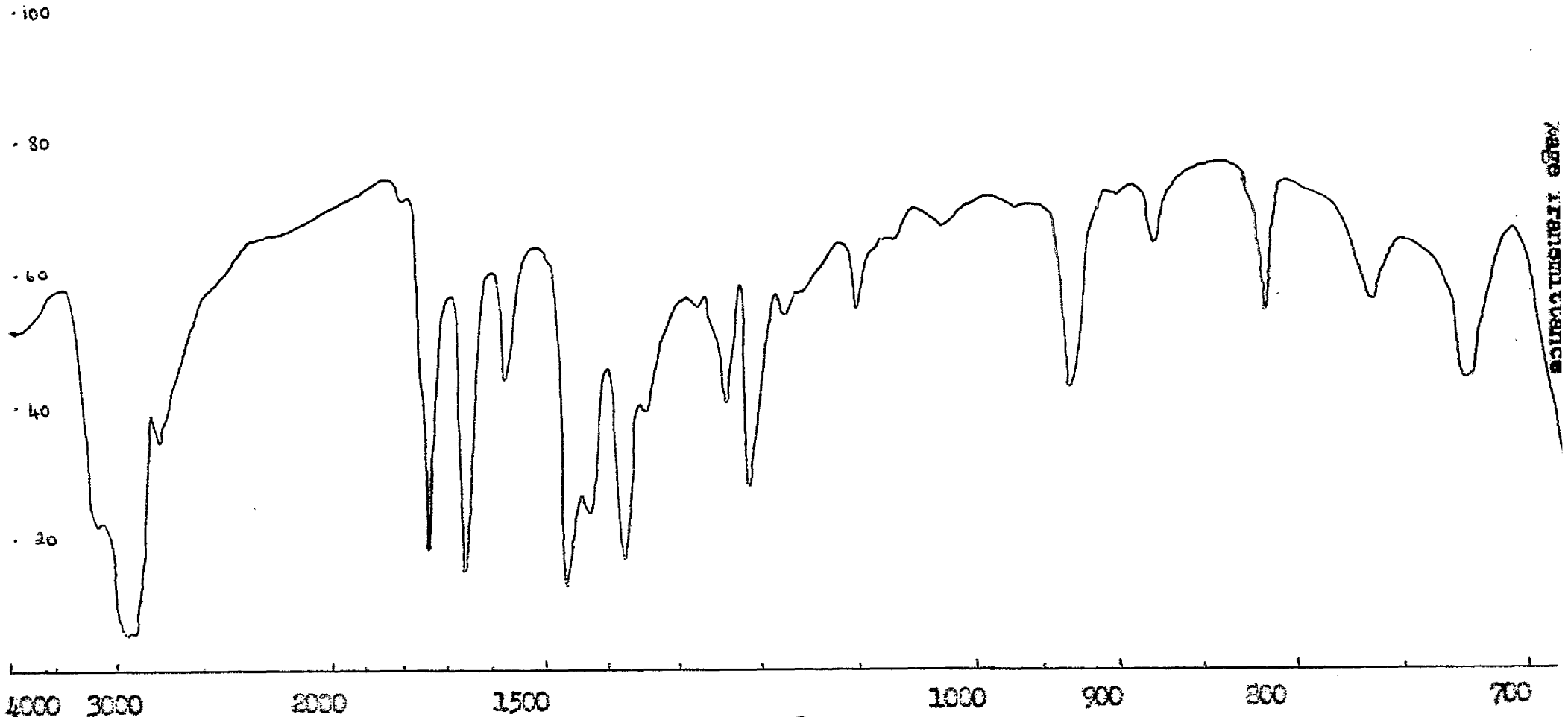


Infrared absorption spectrum of LXVII.

Appendix B.



Infrared absorption spectrum of XXIX.



Infrared absorption spectrum of LIV.

Change of ultraviolet absorption spectra with pH change.

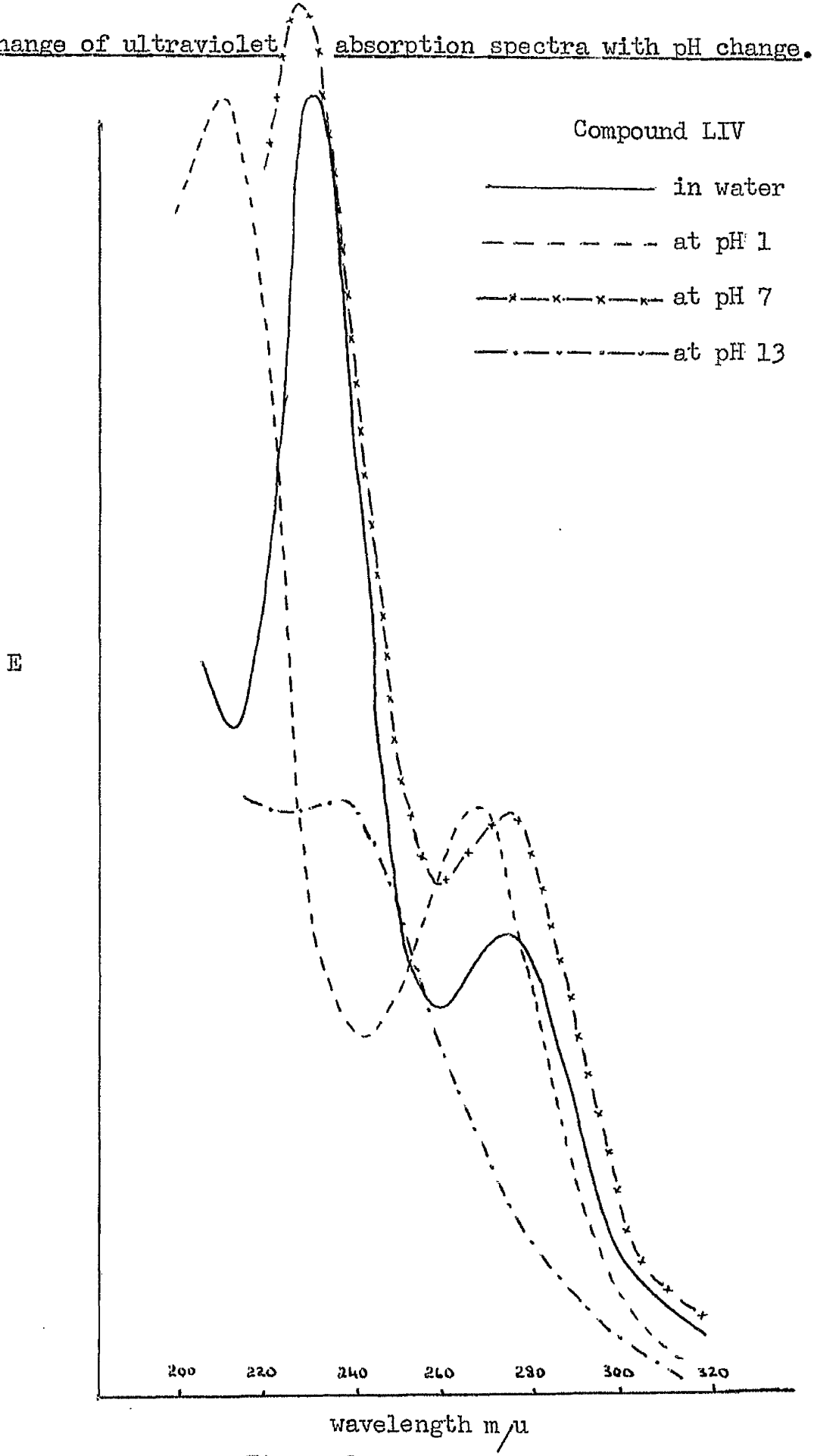


Figure 2.

Change of ultraviolet absorption spectra with pH change.

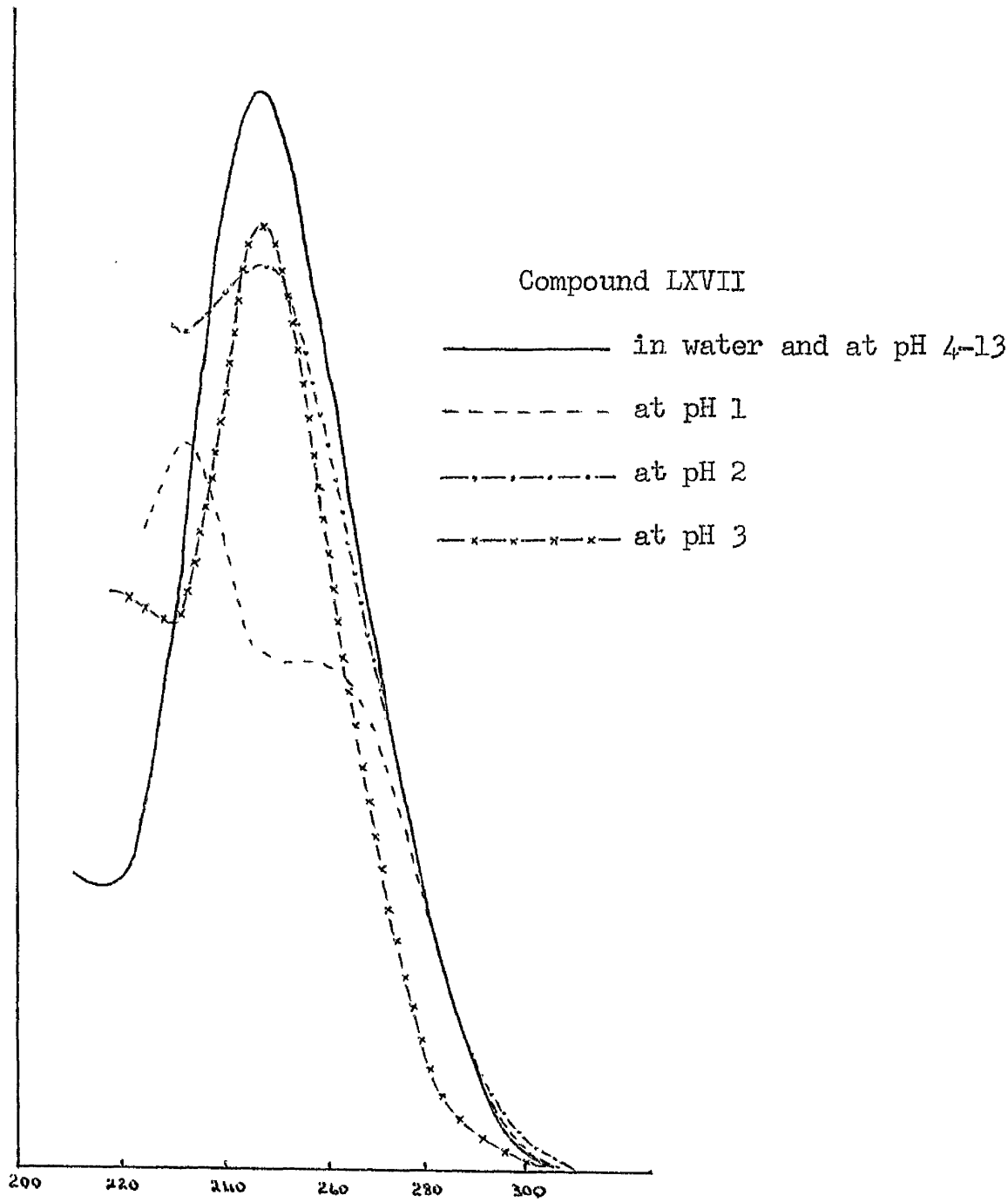


Figure 3.

Change of ultraviolet absorption spectra with pH change.

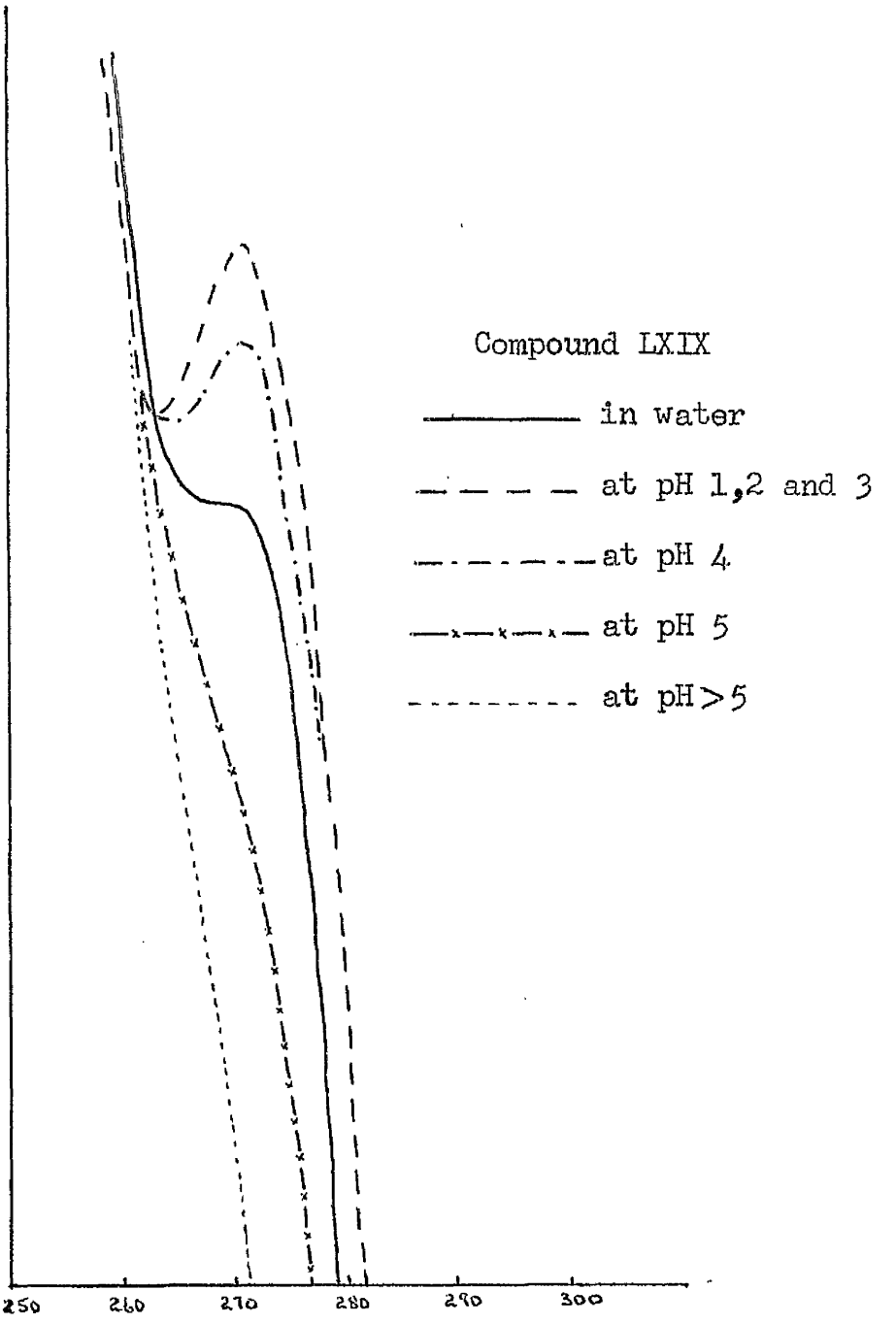


Figure 4.

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