SYNTHESIS OF SOME PYRIDINE HYDRAZIDES WITH POTENTIAL BIOLOGICAL ACTIVITY

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A THESIS

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C O N T E N T S

HISTORICAL:

DISCUSSION:

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HISTORICAL

INTRODUCTION:

TUBERCULOSIS is a chronic wasting disease which from archaeological[1] evidence has afflicted mankind since early antiquity. An excellent description of the disease, referred to as phthisis, is due to Hippocrates, and some of the earlier remedies such as inorganic arsenicals, pine-oil, calcium salts, tannins, chaulmoogra oil, mercury and gold salts date from his period. The wide-spread incidence of tuberculosis and the ineffectiveness of the earlier remedies has resulted in an intensive search for substances which would be effective in the clinical control, and eradication of the disease.

The causative organism Mycobacterium tuberculosis was first isolated by Koch in 1882. Various forms of tuberculosis such as miliary tuberculosis (galloping consumption), tubercular meningitis, pulmonary tuberculosis, tuberculosis empyema (characterised by the accumulation of pus in the chest cavity), lupus (tuberculosis of the skin) and scrofula (tuberculosis of the lymphatic glands) are known. The disease begins by ingestion of the invading tubercle bacillus by normal mono-nuclear phagocytes. The cells increase in size, the nuclei enlarge and become epitheloid. Fusion of several epitheloid cells then takes place to give a "Langhan or Giant cell", and this is followed by the formation of nodules visible to the naked eye. With the progressive growth of the nodule adjacent tissue cells are pushed aside and start dying from nutritional deficiencies caused by the

pressure of the expanding tubercle. If multiplication of the bacillus is not checked, the central portion of the tubercles die and become necrotic. The necrotic cells lose structure and outline and the nuclei form a "caseous mass". This process is known as caseation and occurs in practically all cases of the disease. The softening of the caseous area allows imprisoned tubercle bacilli to become free and thus infect new sites. Healing often takes place by toxic irritation stimulating the fibroblaststo increased activity and deposition of calcium phosphate. This process is known as fibrosis and calcification. Such healing will occur only if the resistance of the patient is reasonably high and/or invasion is not too severe. If the resistance is low and invasion great, rapid spread of bacilli may occur, usually through the bronchi or blood stream and this may prove fatal. Pulmonary tuberculosis is the most common form of tuberculosis because infection takes place by direct invasion of the lungs with infected material. Primary infection may be followed by secondary infection after a short period. This often results in a more acute local reaction, probably because the previous invasion leaves the patient hypersensitive to tuberculin protein.

The great decline in mortality due to tuberculosis may be attributed to a number of factors. The major contribution is due to wide-spread X-ray campaigns which facilitate early diagnosis, and to the discovery of several antituberculosis agents. In spite of this great improvement sustained effort is necessary if a corresponding change in morbidity is to be achieved. An ideal chemotherapeutic agent should fulfil the

following requirements:

(I) It should be non-toxic to the patient and should rapidly relieve all symtoms of acute disease, and prevent relapse.

(2) It should be easily administered, preferably orally.

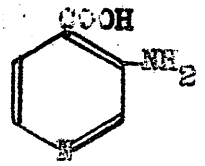
(3) It should be cheap to manufacture, and easily stored without decomposition.

ACID HYDRAZIDES IN THE CHEMOTHERAPY OF TUBERCULOSIS

The activity of the vitamin nicotinamide was first discovered in France by Chorine[2] in 1945, who showed that it had a marked delaying effect on the development of the disease. He further showed that antitubercular and vitamin activity were unconnected; this assumption was borne out by the fact that nicotinic acid, despite its vitamin activity, was not tuberculostatic. This important discovery passed unnoticed until McKenzie[3] and his co-workers in 1948 made a similar observation during the screening of a large number of substances for tuberculostatic activity. In order to settle whether the tuberculostatic effect of nicotinamide and its derivatives was a function of vitamin activity, they prepared a number of derivatives and found that they either exhibited no tuberculostatic activity or decreased activity with a corresponding increase in toxicity. They thus concluded, contrary to Chorine, that the tuberculostatic and vitamin activity were related. As a consequence of this, other vitamins were also examined but all were found to have

no inhibitory action on the tubercle bacillus, with the exception of Riboflavin which showed only slight activity[3].

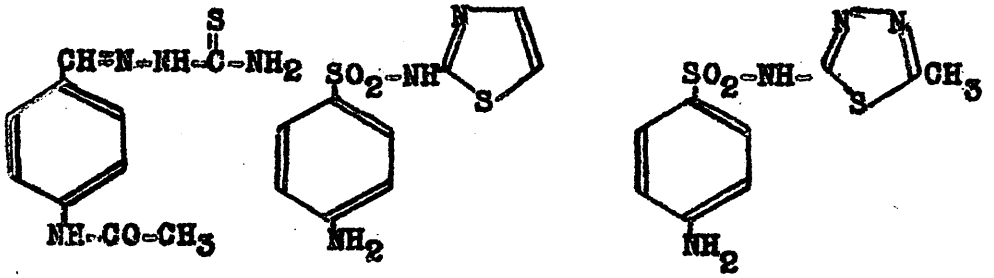
This postulate was subsequently proved wrong when 3-aminoisonicotinic acid (1) and its methyl ester were found to possess antituberculosis activity, although they had no vitamin activity. These compounds not only have a carboxyl group in a different position but also have another substituent in the ring. This suggested that tuberculostatic activity might exist in a wide variety of pyridine derivatives.



(1)

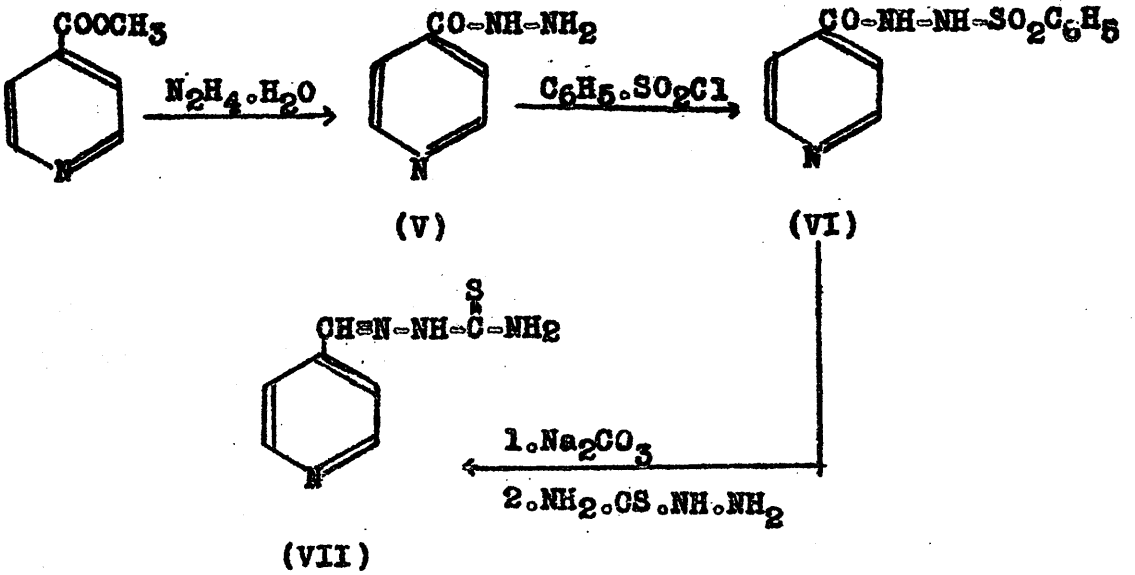
This possibility was investigated by Fox[4] who synthesised a number of pyridine carboxylic acids for testing. Whilst all these compounds proved to be of little value in the treatment of the disease, it revealed that any positional or structural deviation from 3-aminoisonicotinic acid, such as reduction or acylation, completely supressed tuberculostatic activity. Further investigation along this line was therefore abandoned.

With the discovery of p - acetamidobenzaldehyde thiosemicarbazone (II) (Tibione) as an active synthetic tuberculostat, another field of investigation opened up. This investigation started originally with Domagk's[5,6] observation that of the many sulphonamides known only two, viz sulphathiazole (III) and the sulphathiadiazole (IV) showed limited in vivo tuberculostatic activity.



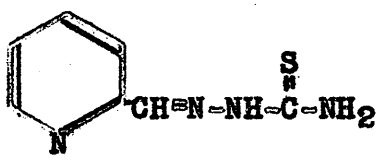
(II) (III) (IV)

The parallel chemical behaviour of nitrobenzene and pyridine derivatives led to the synthesis and study of isonicotinaldehyde thiosemicarbazone by Fox[7,4]. The early attempts to obtain the desired thiosemicarbazone met with failure because of the unstable nature of isonicotinaldehyde. However by a modified Mc adyen and Steven's[8] reaction Fox[4] was able to prepare the compound by the following series of reactions.

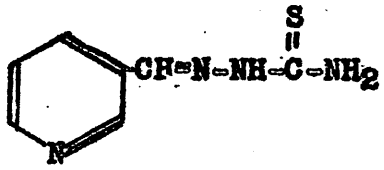


(V) (VI) (VII)

Two positional isomers picolinaldehyde thiosemicarbazone (VIII) and nicotinaldehyde thiosemicarbazone (IX) were prepared in a similar manner.



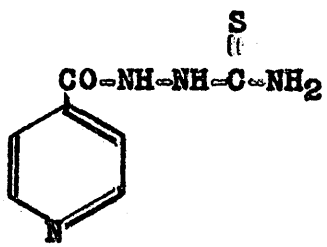
(VIII)



(IX)

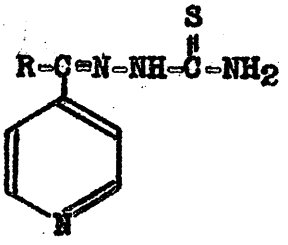
Of the three isomeric thiosemicarbazones, only isonicotinaldehyde thiosemicarbazone and nicotinaldehyde thiosemicarbazone proved to be active antituberculosis agents, the former being more active than Tibione.

In the view of the activity of the β and γ - isomers, it seemed desirable to prepare some closely related compounds in the hope of discovering additional structures with antituberculosis activity. Accordingly, the arylidene group was replaced by an acyl group and thiosemicarbazides of isonicotinic acid (X), nicotinic acid, benzoic acid and p-nitrobenzoic acid were prepared. These compounds showed no significant antituberculosis activity.



(X)

In order to determine the effect of an alkyl group on the antituberculosis activity, methyl 4-pyridyl ketone thiosemicarbazone (XI;R= Me), ethyl 4-pyridyl ketone thiosemicarbazone (XI);R=Et) and propyl 4-pyridyl ketone thiosemicarbazone (XI;R=Pr) were prepared by the method of Kol'off and Hunter[9].



(XI)

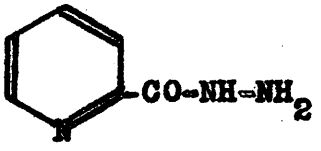
Of these three compounds only compound (XI;R=Me) proved to be active. It could not, however, be used to treat intranasal infection in mice as the therapeutic dose exceeded the maximum tolerated dose. The isosteric semicarbazones were also active. It appeared therefore that the pyridine nucleus offered a promising starting point for synthetic tuberculostats and that the β and γ - positions were the ones of choice for antituberculosis activity among the pyridine aldehyde thiosemicarbazones.

During attempts to establish a structure - activity relationship, two intermediates, isonicotinic acid hydrazide (Isoniazid) (V) and its benzenesulphonyl derivative (VI), were obtained in the preparation of isonicotinaldehyde thiosemicarbazone. These two compounds, because of their relation to the structure under investigation, were also tested for antitubercular activity. The

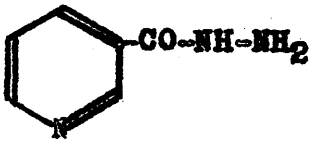
benzenesulphonyl derivative showed no activity, but isonicotinic acid hydrazide was found to be even more active as a tuberculostatic agent than any other known substance whether synthetic or antibiotic.

Bernstein[10], studing the chemotherapy of experimental tuberculosis in mice, found the minimum effective dose of Isoniazid (V) to be one seventh hundredth of that of para-aminosalicylic acid.

From the earlier investigation on tuberculostats of the pyridine series, it seemed likely that positional and structural changes in isonicotinic acid hydrazide would result in diminition or abolition of activity. This was substantiated when picolinic acid hydrazide (XII) and nicotinic acid hydrazide (XIII) were examined.



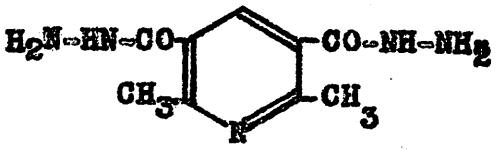
(XII)



(XIII)

The former was found to be active but very toxic whilst the latter was found to be devoid of activity. The addition of another acid hydrazide group in the β -

position of 2, 6 - dimethyl nicotinic acid hydrazide also produced an inactive compound (XIV).

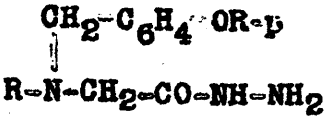


(XIV)

It was, therefore, apparent that the γ - position was the position of choice[11]. Any attempt to replace an amino-hydrogen atom of the hydrazino group by a benzenesulphonyl group also resulted in the production of compounds (XV) devoid of tuberculosis activity.

(XV)

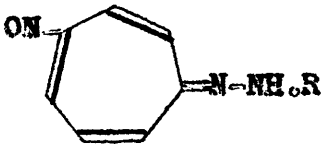
The effect of substituting a benzene for a pyridine ring was studied by preparing a series of benzoic acid hydrazides[12] with subsituents in the ortho-, meta- and para- positions. These compounds proved to be inactive. However Mndzhoyan, Afrikyan and Organesjan[13] have recently prepared a number of benzylalkylamino-acethydrazides of general formula (XVI, R=Me or Et; R^1=Me, Et, n-Pr, n-Bu) which are claimed to be tuberculostatic.



(XVI)

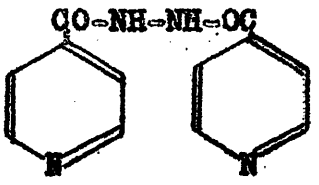
The effect of replacing the amino-hydrogen atoms of the hydrazino moiety by a phosphorus atom has been studied and compounds with the general formula Ar-CONH-N=P-NH-NHCOAr have been prepared[14]. These compounds have also been claimed to be potent tuberculostats.

Recently compounds with the general structure (XVII) (R=acyl) have also been prepared. Of these compounds, 2 - isonicotinylhydrazino-5-nitroso tropone (XVII; R=4-OCC_6H_4N) was found to be an active tuberculostat.[15]

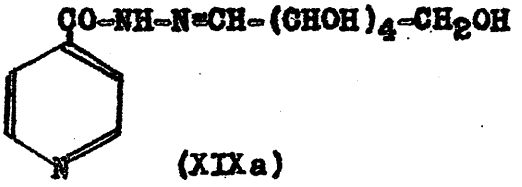


(XVII)

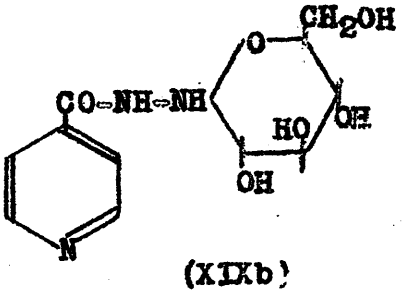
To determine the effect of substitution on the hydrazine moiety, two compounds, 1,2-diisonicotinyl hydrazine (XVIII) and 1-isonicotinyl-2-D-glucosyl hydrazine (XIX), (a or b) were prepared. Both showed high antitubercular activity, the latter being more active and less toxic in mice than the parent compound.



(XVIII)

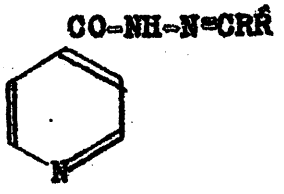


(XIXa)



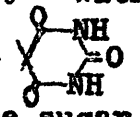
(XIXb)

The high activity of the latter prompted investigation of alkylidene derivatives in the hope of revealing superior tuberculostats and determining, if possible, the structural limits of activity. As a result Fox and Gibas[16,17,18] prepared some twenty alkylidene derivatives (XX) of isonicotinic acid hydrazide by condensation with aldehydes and ketones.

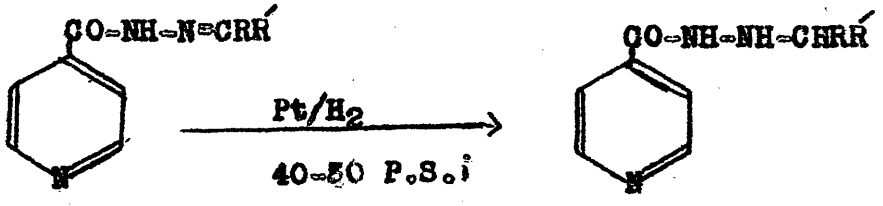


(XX)

All were active, and the *in vivo* activity of several was

greater than any known synthetic tuberculostat except that of the parent compound (V). With the exception of the alloxan derivative (XX; RR′= ) all were readily hydrolysable. In addition some sugar derivatives were also prepared and studied for *in vivo* activity in infected mice. They also were very active and relatively less toxic[19].

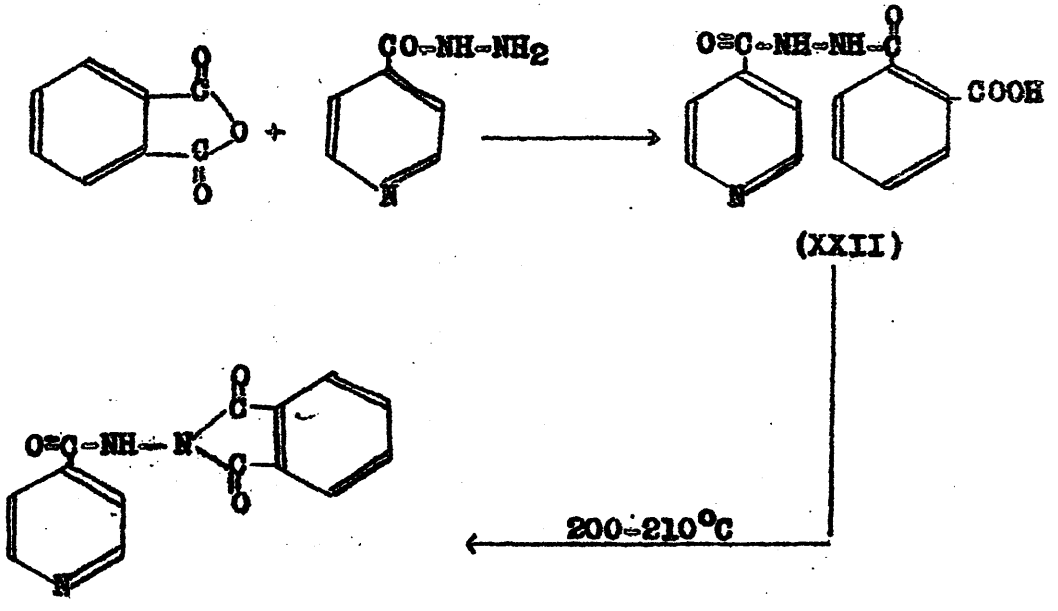
Since scission of the Schiff's base linkage took place even in the solid state, it was decided to eliminate the double bond by hydrogenating the alkylidene derivatives[20,21,22] under mild conditions.



All the alkyl and most of the cyclo-alkyl and aralkyl derivatives were found to be active tuberculostats. When complete reduction of the ring was carried out the resulting compound showed no activity at all. The replacement of the imino hydrogen in the hydrazide moiety by alkyl groups gave compounds much less active than their counterparts with alkyl substituent on the terminal nitrogen.

The fact that 1,2-diisonicotinyl hydrazine has been shown to be an active tuberculostatic *in vivo* indicated

that acyl derivatives might show tuberculostatic activity. Therefore, a series of mono-acyl and diacyl derivatives[23] of isonicotinic acid hydrazide were prepared by the action of the appropriate acid chloride or acid anhydride on the parent compound. The phthalyl derivative could be obtained similarly from isonicotinyl hydrazide and phthalic anhydride to give 1-(isonicotinyl)-2-(o-hydroxy carbonylbenzoyl) hydrazine (XXII) which then cyclised to give 1-isonicotinyl-2-phthalyl hydrazine (XXIIa) by heating at 200-210°C.

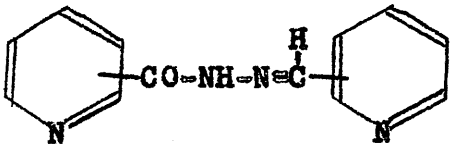


(XXII)

(XXIIa)

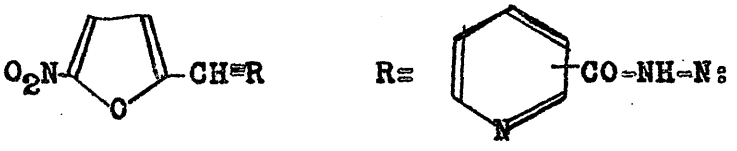
Kakimoto and Yamamoto[24] prepared and examined a series of hydrazones (XXIII) by condensing the three isomeric pyridine carboxylic acid hydrazides with the three isomeric pyridine aldehydes. These derivatives showed high

in vivo activity and were claimed to be superior even to that of isonicotinic acid hydrazide.



(XXIII)

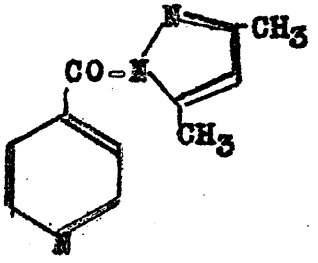
Saikchi, Aramaki and Achi[25] prepared the 5-nitro furfurylidene derivatives (XXIV) by condensing 5-nitrofurfuraldehyde with the three isomeric pyridine carboxylic acid hydrazides. These compounds were also found to possess high tuberculostatic activity.



(XXIV)

Since the structure-activity investigation of isonicotinic acid hydrazide suggested the possibility of tuberculostatic properties in a wide range of structure, Fox and Gibas[25] carried out the preparation of a series of dialkyl derivatives. Two types were possible, one in which both hydrogen atoms on the terminal nitrogen were replaced by alkyl groups or by a cyclic structure, and the other in which one hydrogen on each hydrazide nitrogen was

replaced by an alkyl or equivalent group. Compounds of the first type were prepared by condensing isonicotinyl chloride hydrochloride with the appropriate unsymmetrical[27] hydrazine in the presence of pyridine. The second type were prepared by treating 1-isonicotinyl-2-alkylhydrazines with the appropriate alkyl halide in the presence of sodium ethoxide. 1-Isonicotinyl-3,5-dimethyl pyrazole (XXV) was prepared by condensing isonicotinyl chloride hydrochloride with 3,5-dimethyl pyrazole in the presence of pyridine or alternatively from isonicotinic acid hydrazide and acetylacetone.

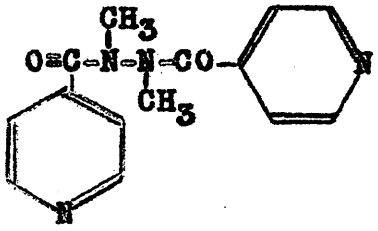


(XXV)

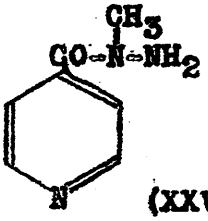
Most of the compounds of the first type were found to possess tuberculostatic activity, probably because these compounds, like alkylidene, phthalyl and diacyl derivatives of isonicotinic acid hydrazide, also have both the hydrogens on the terminal nitrogen atom replaced by substituent groups. However compounds of the second type showed much reduced activity and 1-isonicotinyl-3,5-dimethyl pyrazole in which all the three hydrazino hydrogens are replaced was inactive. It was expected that $\underline{N}^1\underline{N}^2\underline{N}^2$-trialkyl derivatives[28] would

therefore be inactive, and, as anticipated, they were found to be either inactive or weakly active in mice infected with Mycobacterium tuberculosis.

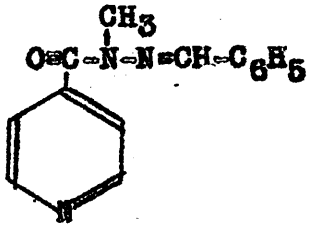
A similar investigation was undertaken by Cymerman-Craig and Willis[29], who prepared N N^1-dimethyl - N N^1- diisonicotinyl hydrazine (XXVI), N-methyl-N-isonicotinyl hydrazine (XXVII), and its benzylidene derivative (XXVIII). All were devoid of tuberculostatic activity.



(XXVI)



(XXVII)

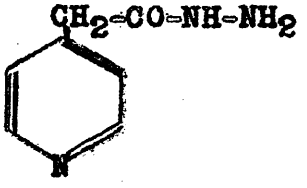


(XXVIII)

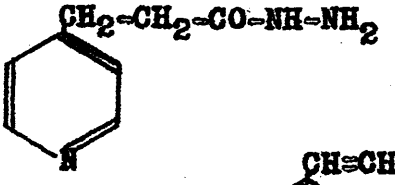
Further modification of the acid hydrazide moiety was carried out by König, Siefkin and Offe[30] by replacing the oxygen function by sulphur. Among the compounds tested against tuberculosis were thioisonicotinic acid hydrazide and its benzylidene and salicylidene derivatives. Tuberculosis activity was greatly diminished compared with isonicotinic acid hydrazide.

In order to determine the effect of separating the acid hydrazide moiety from the pyridine nucleus, Katritzky[31]

prepared 4-pyridylacethydrazide (XXIX), β-4-pyridyl propionhydrazide (XXX), and β-4-pyridyl acrylhydrazide (XXXI) from the corresponding esters. These compounds showed no activity in tuberculosis infected mice. It, however, indicated that the attachment of the - $CO-NH-NH_2$ group to a pyridine nucleus is a necessary requirement for antituberculosis activity.



(XXIX)



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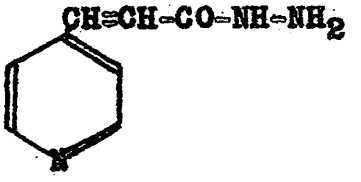
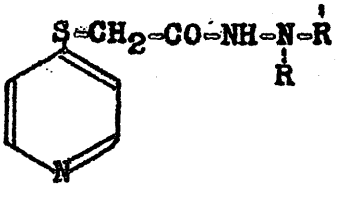


(XXXI)

The effect of separating the acid hydrazide group from the pyridine nucleus by a thiomethylene group was investigated by Japanese workers[32], who prepared α-(4-pyridylthio) acethydrazide (XXXII; R=R^1=H) and its isopropylidene derivative (XXXII; R R^1=:C Me_2). These were also inactive.

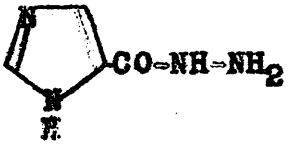


(XXXII)

The high activity of Isoniazid and its derivatives encouraged investigation of other heterocyclic systems[33]. The most active compounds were 2-furoic acid hydrazide (XXXIII), thiophene-2-carboxylic acid hydrazide (XXXIV), and imidazole-4 (5) - carboxylic acid hydrazide (XXXV).

(XXXIII)

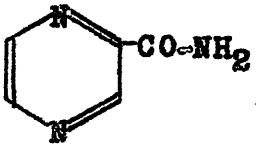
(XXXIV)



(XXXV)

In addition acid hydrazides of various other heterocyclic systems, e.g., pyrrole, pyrimidine and pyrazine[34,35,36] were also prepared and examined against Mycobacterium tuberculosis. While several of these hydrazides showed slight activity in vivo, none appeared to warrant further investigation. Quite recently some oxazolone, thiazolidine, piperidone, indolyl and imidazolone acid hydrazides[37,38] have been prepared and it is claimed they are potential tuberculostatic agent. Although heterocyclic acid hydrazides, in general, did not show any promise, pyrazinamide has proved to be highly active. Pyrazinamide (XXXVI) which is isosteric with nicotinamide was synthesized by Kushner and his associates[39]. Early reports on its

in vivo activity were conflicting. Marked variation of activity was apparent from one culture medium to another, but Tompsett and his associates[40] showed that pyrazinamide was a potentially useful drug.

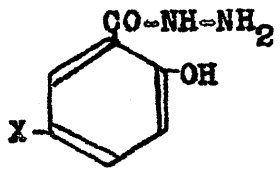


(XXXVI)

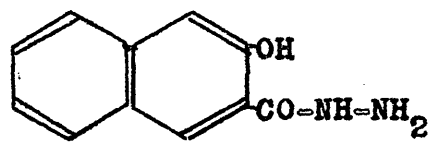
The first chemical trial of pyrazinamide conducted by Yeager and his associates[41] showed that it was moderately antitubercular but its effect was not sustained and resistance developed rapidly. The drug's hepato-toxicity has discouraged its clinical use. However combined with Streptomycin or with Isoniazid it may have particular value, enhancing activity, and delaying onset of resistance to the drug with which it is combined.

Investigation of non-pyridinoid acid hydrazides was carried out by Fox[26], and by Bernstein[42], who prepared a number of chloro-, hydroxy- and amino-benzoic acid hydrazides but none was active. However Buu-Hoi[43] and his coworkers have shown that 5-substituted salicylic acid hydrazides (XXXVII; X=Cl,Br) and 2-hydroxy -3- naphthoic acid hydrazide (XXXVIII) were antitubercular. Misaki[44] has claimed that p-aminosalicylic acid hydrazide (XXXIX) has greater activity than p-aminosalicylic acid (PAS) (XL), but

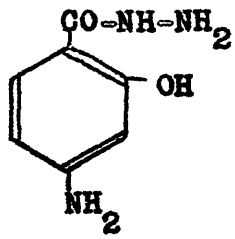
is inferior to Isoniazid.



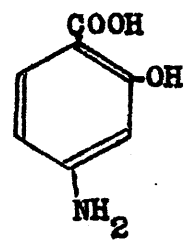
(XXXVII)



(XXXVIII)

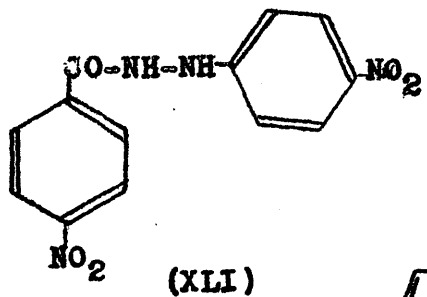


(XXXIX)

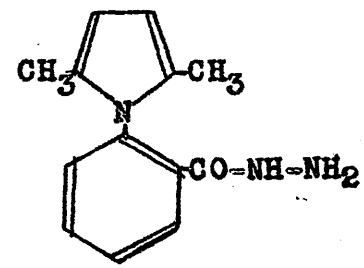


(XL)

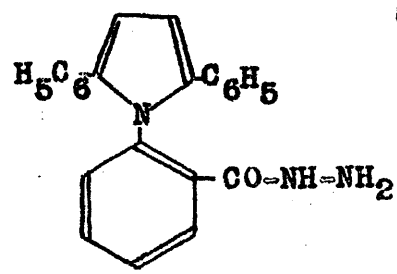
Compounds[35] (XLI, XLII, XLIII) with two or more aromatic groups have also been prepared and found weakly tuberculostatic.



(XLI)



(XLII)



(XLIII)

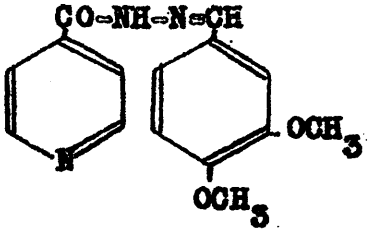
Few aliphatic acid hydrazides[45] are active tuberculostats, an exception being cyanoacetic acid hydrazide (XLIV).

$$CN\text{-}CH_2\text{-}CO\text{-}NH\text{-}NH_2$$

(XLIV)

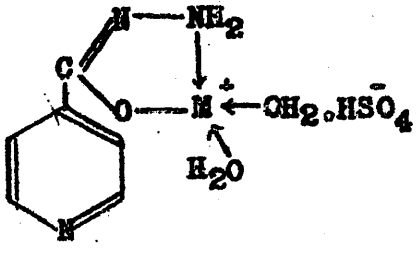
Clinical trial of this compound by Schez[46] yielded results which indicated activity comparable to that of Isoniazid. It is, however, rather toxic and, according to Bernon, Aulanier and Tricoire[47], it is two to three times more toxic than Isoniazid. It may, however, be capable of delaying emergence of resistant strains in patients undergoing treatment with Isoniazid, Streptomycin and para-aminosalicylic acid.

In vitro and in vivo studies of Isoniazid and related hydrazones showed that the latter were significantly less toxic, e.g., Rubbo, Edgar and Vaughan[48] prepared a series of hydrazones to test their relative activity in experimental animals. Comparison showed that verazide (1-isonicotinyl-2-veratrylidenehydrazone) (XLV) exerted marked antituberculosis activity in experimentally infected guinea-pigs and the acute toxicity in mice was about one third that of Isoniazid. Similar experiments carried out by other investigators[49,50] has confirmed this.

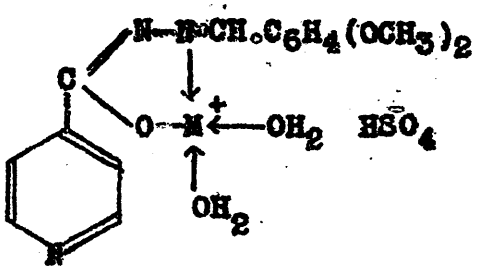


(XLV)

Since Isoniazid has a marked tendency to form chelate compounds, it was thought that chelation with divalent metals might be an essential prerequisite determining the antituberculosis activity of Isoniazid and verazide. In order to test this assumption, the iron, cobalt, zinc and copper complexes of Isoniazid (XLVI,a) and verazide (XLVI,b) were prepared and tested for antituberculosis activity[48]. The results of these tests showed that the activity of all the metal complexes was equal to that of the uncomplexed drug. It did not appear therefore that the role of the potentiator (or antagonizer) of Isoniazid could be assigned to any of the metals examined.

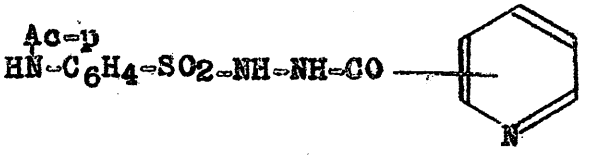


(XLVIa)



(XLVIb)

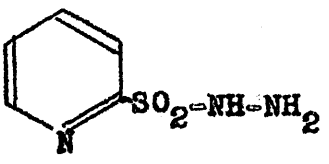
The effect of replacing a hydrogen atom of the primary nitrogen of the isomeric pyridine carboxylic acid hydrazides by a p-acetamidobenzenesulphonyl group was studied by Dornow and Wedekind[51], but these compounds (XLVII) were inactive.



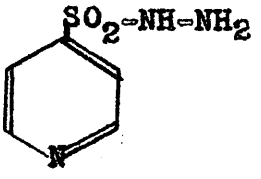
(XLVII)

Several aromatic sulphonylhydrazides were also prepared and subjected to *in vivo* and *in vitro* examinations but were found to be devoid of activity.

To investigate the effect on antituberculosis activity of replacing the carbonyl by a sulphonyl group, Talik and Plazek[52], and Comrie and Stenlake[53], synthesized some pyridine-2, and pyridine-4-sulphonhydrazides (XLVIII,a,b) and their derivatives but none showed any significant activity against *Mycobacterium tuberculosis*.

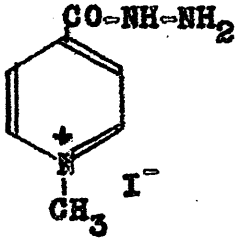


(XLVIIIa)



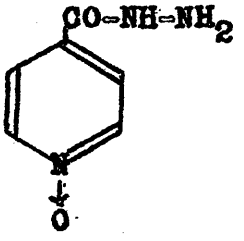
(XLVIIIb)

The effect on the antituberculosis activity of quaternizing the ring nitrogen of Isoniazid was studied by Yale and his coworkers[42], who showed that the compound (XLIX) had lower activity than isonicotinic acid hydrazide.



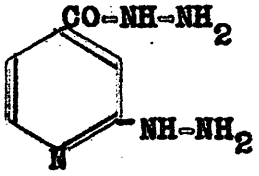
(XLIX)

Again to determine the effect of modifying the ring nitrogen function on the tuberculosis activity, Yale prepared isonicotinic acid hydrazide - 1 - oxide (L). This compound, however, was found to be less active than Isoniazid.

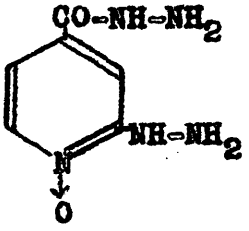


(L)

A similar investigation was carried out by workers[54] in Poland, who prepared 2-hydrazinoisonicotinyl hydrazine (LI) and its N-oxide (LII). These compounds have been found to be only weakly tuberculostatic.

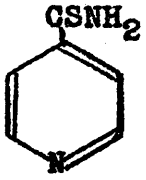


(LI)

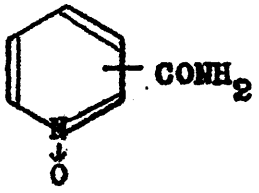


(LII)

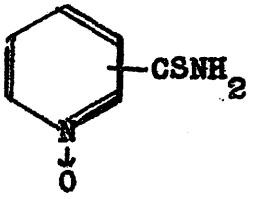
The high activity of thioisonicotinamide (LIII) in experimental tuberculosis prompted Gardner, Wenis and Lee[55] to prepare the N-oxides of the isomeric pyridine carboxamides (LIV) and thiocarboxamides (LV). These compounds were either inactive or less active than thioisonicotinamide.



(LIII)



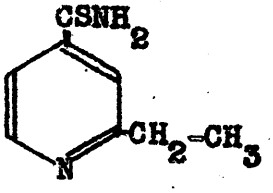
(LIV)



(LV)

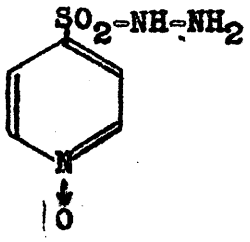
During an attempt to obtain tuberculostats more potent than thioisonicotinamide, alkyl derivatives of this drug were prepared and examined and 2-ethyl thioisonicotinamide (LVI) proved to be an active tuberculostat. This compound was first synthesized by Libermann and the biological studies were carried out by Rist, Grumbach, Moyeux, Cals, Rouaix

and Clavel[57]. High *in vivo* and *in vitro* activity against Isoniazid resistant stains of *Mycobacterium tuberculosis* encouraged its clinical use[58].

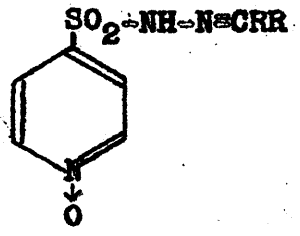


(LVI)

The effect of replacing the carbonyl group in isonicotinic acid hydrazide-1-oxide by a sulphonyl group was studied by Angulo and Munich[59], who prepared pyridine-4-sulphonhydrazide-1-oxide (LVII) and some of its derivatives (LVIII).



(LVII)



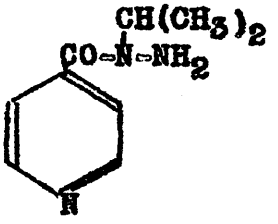
(LVIII)

All these compounds were found to be either inactive or less active than isonicotinic acid hydrazide.

MODE OF ACTION OF ANTITUBERCULOSIS AGENT

At present there is no established mechanism to explain the activity of any antituberculous agent, although a number of hypotheses have been advanced. One of the most widely advocated, postulates that antituberculous activity of compounds such as the thiosemicarbazides, the thioureas, hydroxamic acids, p-aminosalicylic acid, pyridine carboxylic acid hydrazides and their derivatives, is associated with their ability to form stable complexes with metals such as copper and iron which may be essential for the metabolic processes of the tubercle bacillus.[60,61,62,63] Bergel has postulated that copper and iron are "pro-oxidants", which act by catalysing the auto-oxidation of lipids. When copper and iron form chelates with drugs, this role is inhibited. Thus he assumed that the antituberculous activity of a compound is a function of its anti-oxidant activity.

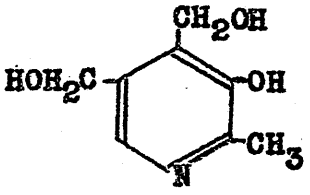
The chelation hypothesis fails to explain the inactivity of nicotinic acid hydrazide although it is as effective as Isoniazid as a chelating agent[64]. Fox and Gibas[28] have, however, shown that at least in one case chelating ability is not essential for antituberculous activity among acid hydrazides, by examining 1-isonicotinyl-1-isopropyl hydrazine (LIX) which, though strongly tuberculostatic, does not form a copper chelate.



(LIX)

Continuing possible correlation between antituberculous activity and chelating ability, Cymerman-Craig and Willis[29] prepared $\underline{N}^1\underline{N}^2$-dimethyl-$\underline{N}^1\underline{N}^2$- diisonicotinyl hydrazine (XXVI) and the benzylidene derivative of $\underline{N}^1$-methyl-$\underline{N}^1$-isonicotinyl hydrazine (XXVIII). Both compounds were inactive and since they are incapable of chelation *via* a pseudo-acid and give neither a colour change nor a precipitate with cupric ions they concluded that a direct connection between chelating ability and antituberculous activity might exist. The evidence is therefore conflicting. Another hypothesis to explain a drug-tubercle relationship postulates that the drug interferes with tubercle enzymes. Pope[65] and Yorida[66] have shown that a number of metabolites of possible significance antagonized the action of Isoniazid. Of these pyridoxine (LX) was the most effective, and there were reasons for believing that essential enzyme systems of the tubercle bacilli containing pyridoxal phosphate as a vital component are inhibited by Isoniazid. Pyridoxal containing enzymes play a prominent role in decarboxylation, transamination and other reactions of amino-acid metabolism. α-Ketoglutaric acid also inhibited the action of Isoniazid. Barclay and his co-workers[67] have shown that tubercle bacilli sensitive to Isoniazid fixed the drug firmly. Thus it is assumed that

after up-take of the drug by the tubercle bacilli normal enzyme activity was hindered. A decrease in the pyridine nucleotide level in tuberculosis infected animals, and subsequent return to normal after the administration of Isoniazid, led Patiala[68] to suggest that Isoniazid inhibited enzymatic reactions in which pyridine nucleotides are involved; this is probably due to the structural similarity of nicotinamide and Isoniazid which replaces nicotinamide in the pyridine nucleotide to produce a biologically inactive compound. Goldman[69] has isolated the Isoniazid nucleotide analogue of diphosphopyridine nucleotide (DPN).



(LX)

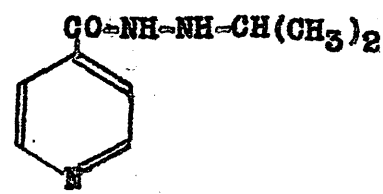
Modification in anabolic activity and reduction in lipid synthesis have also been observed. All these changes show that after treatment with Isoniazid loss of enzymatic activity ensues.

The possibility that antibacterial activity of the drug may be due to its ability to modify surface tension has also been advanced in the case of surface active agents such as polyoxyethylene ethers[70]. The idea is supported by the observation that these substances depress tuberculin sensitivity in guinea-pig[71] and stimulate macrophages to

kill or inhibit growth in the macrophages which have not been exposed to the surface active agent[72].

MONO-AMINE OXIDASE INHIBITORS (MAOI)

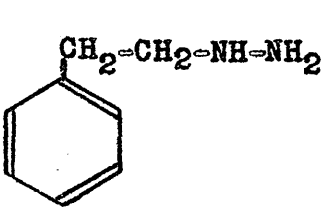
During the search for more potent tuberculostats, Fox and Gibas prepared a large number of 1-alkyl-2-isonicotinyl hydrazine derivatives, one of these being 1-isopropyl-2-isonicotinyl hydrazine (LXI).



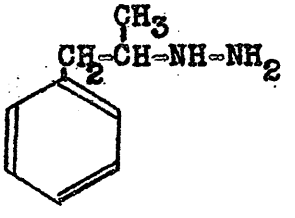
(LXI)

On clinical investigation[73], this compound showed marked central nervous activity, manifested in an improvement of appetite and mental attitude. Simultaneously with these clinical findings, the powerful mono-amine oxidase inhibiting effect of Iproniazid was demonstrated by Zeller and his associates[74,75,76,77] and this led to the synthesis and examination of a large number of hydrazine derivatives. The biological examination of alkyl hydrazines revealed that activity was greatest with alkyl substituents with two to four carbon atoms; increase in the number of carbon atoms led to a decrease in activity which was negligible above eight

carbon atoms[78,79]. The replacement of an alkyl group in an alkyl hydrazine by an aromatic or heteroaromatic group[80,81] often resulted in a loss of antidepressant activity. However in most cases aralkyl hydrazines[82,83] proved to be effective, e.g., β-phenylethyl hydrazine (LXII)(Phenelzine) and β-phenylisopropyl hydrazine (LXIII) are efficient mono-amine oxidase inhibitors which have been used clinically as antidepressants.



(LXII)



(LXIII)

Phenylisopropyl hydrazine is twice as active by the intra-peritoneal route as by the oral route and its action is prolonged. Stereospecificity[84,85] was also observed in these substances, e.g., the optical isomer of β-phenylisopropyl hydrazine which corresponds to D-amphetamine is effective <u>in vivo</u> and <u>in vitro</u>.

The study of symmetrical dialkyl hydrazines[86] showed that they are not generally efficient inhibitors <u>in vivo</u>. Nevertheless with suitable alkyl or aralkyl groups strong mono-amine oxidase inhibitors were obtained, e.g., 1,2,-diisopropyl hydrazine (LXIV) exhibits an effect equivalent to that of isopropyl hydrazine.

$(CH_3)_2CH-NH-NH-CH(CH_3)_2$

(LXIV)

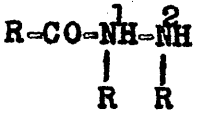
$(CH_3)_2CH-NH-NH-CH_2C_6H_5$

(LXV)

$\underline{N}^1$-Benzyl-$\underline{N}^2$-isopropyl hydrazine (LXV) is inactive *in vitro* but active *in vivo* and is four times as active intraperitoneally as orally. The onset of action of $\underline{N}^1$-benzyl-$\underline{N}^2$-isopropyl hydrazine is fairly rapid and its activity decreased four hours after administration.

The structure-activity relationship of hydrazides showed that by selective acylation[87,88] of alkyl hydrazides it was possible to achieve control over biological potency and distribution of these compounds in the body. The alkyl group in alkyl hydrazides played a prominent role in determining the MAOI activity, e.g., the activity rises to a maximum at three to four carbon atoms and declines markedly at seven to nine carbon atoms.[89] Aralkyl hydrazides often showed higher activity than the corresponding alkyl hydrazides, but the aryl hydrazides were found to be inactive. Acylation of alkyl or aralkyl hydrazides at the nitrogen atom which already carried an alkyl or aralkyl group always led to decrease in antidepressant activity. The introduction of a second alkyl group in the hydrazine moiety of alkyl hydrazides

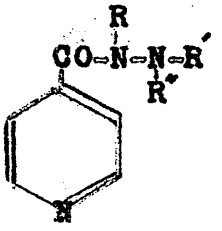
led to two types of derivative, namely, $\underline{N}^1\underline{N}^2$-dialkyl derivatives (LXVI) and $\underline{N}^2$-dialkyl derivatives (LXVII).



(LXVI)

(LXVII)

It was noted that active $\underline{N}^1\underline{N}^2$-dialkyl hydrazides could be obtained from weakly active $\underline{N}^2$-monoalkyl hydrazides by introducing a second alkyl group; the introduction of a second alkyl group as in compound (LXVII) failed to effect improvement. This relationship has been confirmed in the case of the isopropyl group.



Compound	Activity index
R=H; R=H; R=CH Me_2	100
R=CHMe_2; R=H; R=CHMe_2	174
R=CHMe_2; R=R=H	66
R=H; R=R=CHMe_2	88

The diaralkyl hydrazides have not been thoroughly investigated, hence generalization concerning structure-activity relationshipsis not possible. Diacyl hydrazides also showed MAOI activity.[87]

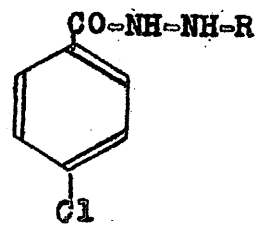
Whilst many aliphatic, arylaliphatic and cycloaliphatic hydrazides have been synthesized and examined, no relatable pattern of structure to activity has been discovered. The higher fatty acid hydrazides are generally less effective, this being attributed to diminished solubility, e.g., N^2-isopropyl stearic acid hydrazide (LXVIII) is devoid of MAOI activity.

$$CH_3\text{-}(CH_2)_{16}CO\text{-}NH\text{-}NH\text{-}CH\text{-}(CH_3)_2$$

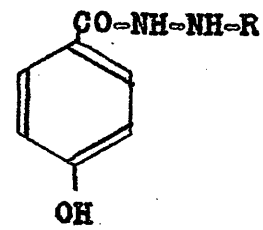
(LXVIII)

Aliphatic hydrazides with branching on the α or β-carbon invariably showed less activity than the corresponding unbranched derivatives, and unsaturated acid hydrazides were more effective than saturated acid hydrazides.

Aromatic and heteroaromatic acid hydrazides[89] usually showed MAOI activity. The introduction of substituents in many cases was unfavourable with respect to activity. Aromatic acid hydrazides with a free phenolic group were less active e.g., compound (LXIX) is an active mono-amine oxidase inhibitor but the replacement of the chlorine atom by a hydroxyl group (compound LXX) abolishes activity.[89]

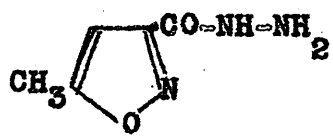


(LXIX)



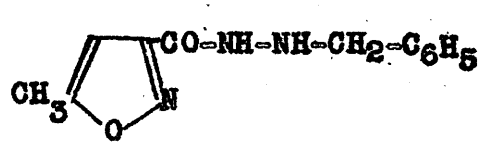
(LXX)

Pyrimidine, pyrazine and pyridazine carboxylic acid hydrazides, generally, yielded potent mono-amine oxidase inhibitors. Among the isoxazole carboxylic acid hydrazides, 5-methyl isoxazole-3-carboxylic acid hydrazide (LXXI) has been found to possess the highest MAOI activity.



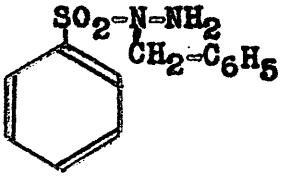
(LXXI)

Recently Isocarboxazole (LXXII) was found to be a potential antidepressant and is now in clinical use.[90]



(LXXII)

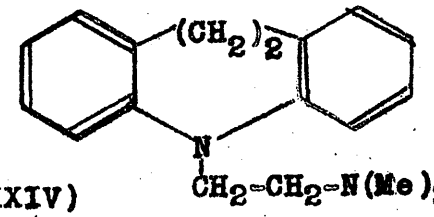
Sulphonation of benzyl hydrazine with an aromatic or an aliphatic sulphonyl chloride gave sulphonyl hydrazides with good mono-amine oxidase inhibiting activity. The N^1-benzyl sulphonyl hydrazide (LXXIII) showed specially high activity.



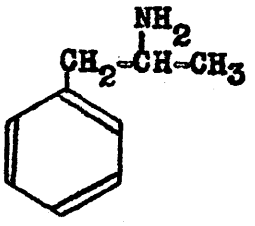
(LXXIII)

MODE OF ACTION OF MONO-AMINE OXIDASE INHIBITORS

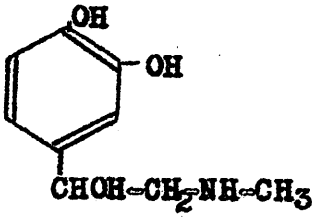
Antidepressant drugs can be divided into three groups: (1) the mono-amine oxidase inhibitors (2) Imipramine (LXXIV) and related compounds and (3) amphetamine (LXXV) like substances. Much of the present day theory depends upon the acceptance of the physiological rôle in the brain of the chemical transmitter substances,[91] notably sympathin which is a mixture of adrenaline (LXXVI), and noradrenaline (LXXVII), dopamine (LXXVIII) and 5-hydroxytryptamine (LXXIX).



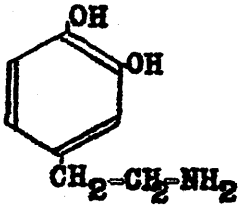
(LXXIV)



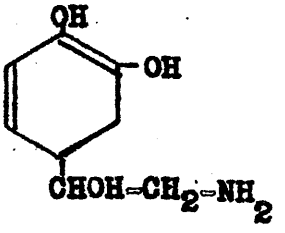
(LXXV)



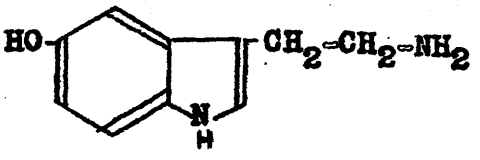
(LXXVI)



(LXXVIII)



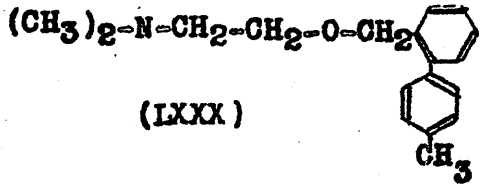
(LXXVII)



(LXXIX)

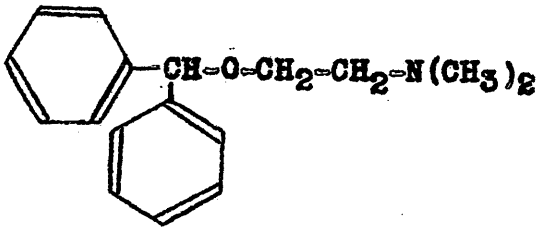
5-Hydroxy tryptamine and tryptamine are believed to be derived from tryptophan and dopamine and nor-adrenaline from tyrosine, and act mainly on the sub-cortical region of the brain. It has been shown that relatively large amounts of these amines are concentrated in the hypothalamus and other cortical regions of the brain. Their chief action is excitation; under the effect of nervous stimuli these substances are liberated at the

receptor sites in the cells. They are not allowed to accumulate at these sites but are oxidized to inactive compounds by mono-amine oxidase which thus prevents their accumulation. Many of the antidepressants have been shown to inhibit the action of mono-amine oxidase and are described collectively as mono-amine oxidase inhibitors. Since one of the function of this enzyme is concerned with inactiviation of 5-hydroxytryptamine and perhaps also of noradrenaline, drugs which impair its activity in any way will interfere with metabolism of 5-hydroxytryptamine and the other amines. This causes the amines to accumulate and leads to exaggerated action. For this reason the characteristic effect of the antidepressants upon the mood of the depressed person has been suggested to be due to the accumulation in the brain of the catecholamines[92,93] (noradrenaline, adrenaline, dopamine or 5-hydroxytryptamine). A number of other potent antidepressant drugs have also been shown to inhibit enzymes. However, there are drugs which are effective antidepressants but which do not inhibit mono-amine oxidase e.g., Imipramine and orphenadrine (LXXX).



(LXXX)

On the other hand the antihistaminic diphenhydramine (LXXXI) is a mono-amine oxidase inhibitor but does not exert antidepressant effects. These facts are difficult to explain and it is possible that the antidepressant drugs and other central nervous stimulants, e.g., the amphetamines, exert their effect primarily by increasing the level of energy-yielding compounds in the brain.



(LXXXI)

All the antidepressants drugs have side-effects.[94-96] Several reactions during therapy are due to individual susceptibility or to incompatibility with certain exogenous substances. The main side-effects are prolonged hypertension and over-stimulation which results in agitation and excitement. Withdrawal symptoms may start after two or three days and may last for several weeks. These symptoms include headache, dreaming, irritability, dizziness, insomnia and somnolence. Sexual function may be disturbed in different ways depending very much on individual susceptibility. Muscle jerking, tremors, fits and peripheral neuritis

have also been reported in susceptible individuals.

DISCUSSION

DISCUSSION

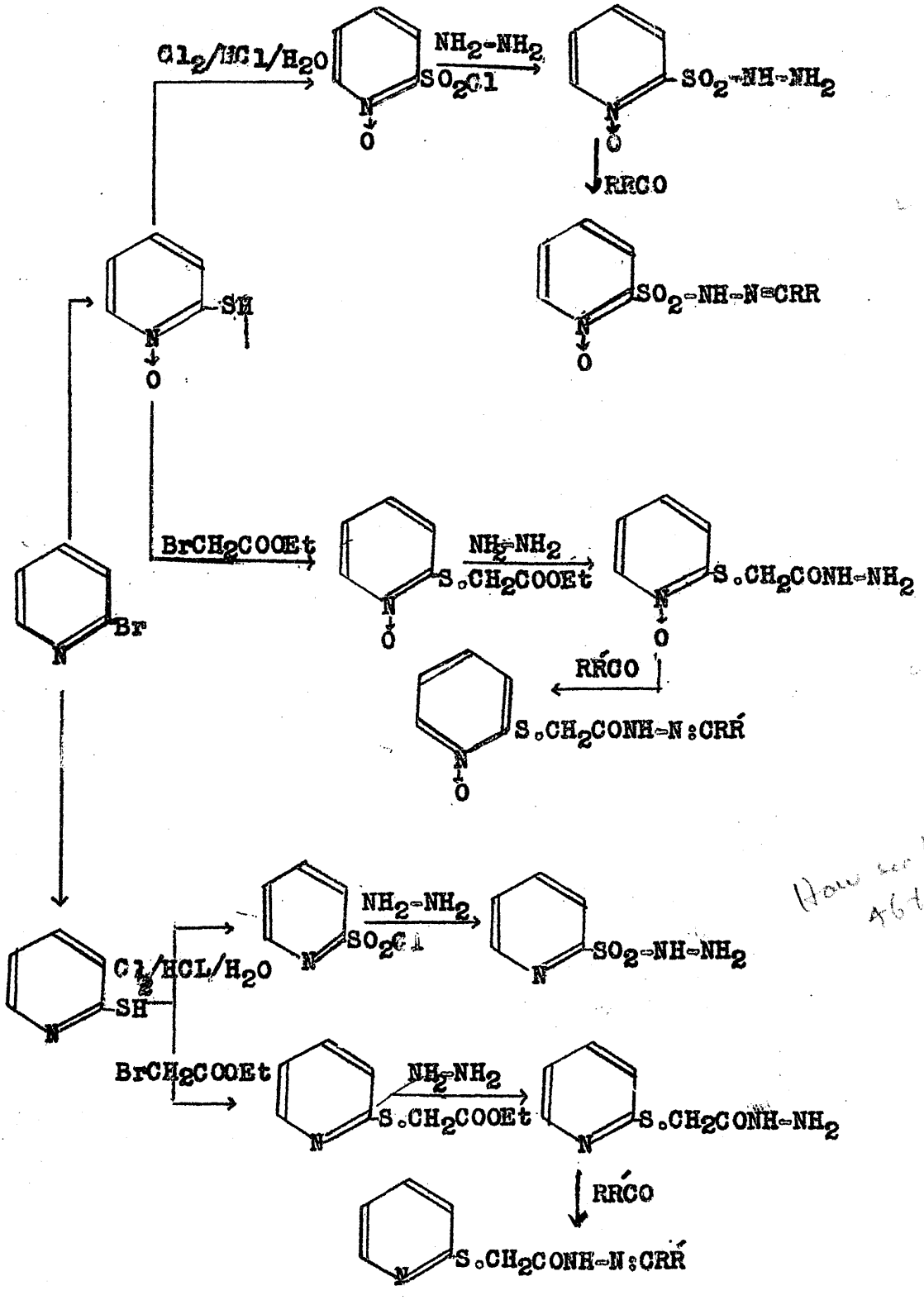
From the widely differing nature of current clinical tuberculostats it would appear that there are a few specific chemical structures which can be associated with tuberculostatic activity, and, within each group provided structural variation is not too radical, it is possible to prepare derivatives in which activity is retained. Thus among the pyridine carboxylic acid hydrazides structural variation with retention of activity was confined to the hydrazide moiety of the molecule, and further the α- and γ- positions were the ones of choice. This structural activity pattern led to the synthesis and examination of a large number of pyridine-2 and -4 carboxylic acid hydrazide derivatives.

A number of aliphatic acid hydrazides, arylaliphatic hydrazides, aromatic acid hydrazides, carbocyclic acid hydrazides and other heterocyclic hydrazides have been prepared and examined for antituberculosis activity.[10,45,97-100] The study of these acid hydrazides showed that the aliphatic hydrazides were often devoid of activity and that aryl hydrazides lacking an amino or hydroxyl group possessed significant activity. Talik and Plazek[52] have shown that 1-isopropylidene-2-(pyridine-2-sulphonyl) hydrazine was tuberculostatic, but they failed to isolate and examine the parent substance. Comrie and Stenlake[53] also found that some of the derivatives of pyridine-4-sulphonhydrazide were very weakly tuberculostati

In view of the tuberculostatic activity of 1-isopropylidene-2-(pyridine-2-sulphonyl) hydrazine, it was decided to prepare pyridine-2-sulphonhydrazide and pyridine-2-sulphonhydrazide-1-oxide and its derivatives. This course of study was also encouraged by the observation of Steinberg[42] and his co-workers who have shown that in general the N-oxides of isonicotinic acid and picolinic acid hydrazide were active and less toxic than most of their other derivatives. Furthermore Angulo and Munic[54] also showed that pyridine-4-sulphonhydrazide-1-oxide and its derivatives were more active than the corresponding pyridine-4-sulphonhydrazide and its derivatives.

Separation of the pyridine nucleus from the hydrazide moiety in isonicotinic acid hydrazide by a thiomethylene group indicated that the resulting compounds were less tuberculostatic than Isoniazid.[32] It was, however, planned to examine the effect of separating the acid hydrazide moiety from the pyridine nucleus in picolinic acid hydrazide and its N-oxide. Four lines of investigation were envisaged:

1. The synthesis of pyridine-2-sulphonhydrazide.
2. The synthesis of pyridine-2-sulphonhydrazide-1-oxide and its derivatives.
3. The synthesis of α-(2-pyridylthio) acethydrazide and its derivatives.
4. The synthesis of α-(2-pyridylthio)acethydrazide-1-oxide and its derivatives.

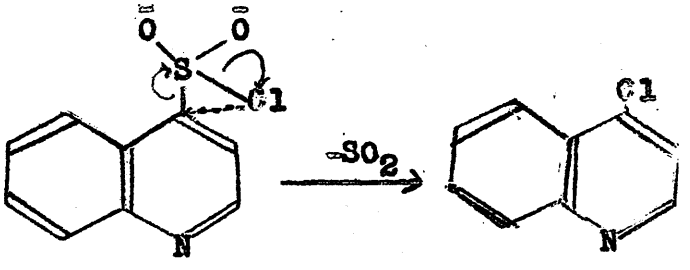

Cl2/HCl/H2O
NH2-NH2
SO2Cl
SO2-NH-NH2
RRCO
SH
SO2-NH-N=CRR
BrCH2COOEt
S.CH2COOEt
S.CH2CONH-NH2
Br
RR'CO
S.CH2CONH-N:CRR'
Cl2/HCL/H2O
SH
BrCH2COOEt
S.CH2CONH-N:CRR'

2-Bromopyridine was obtained in quantitative yield by the method described by Allen and Thirtle,[101] but oxidation of 2-bromopyridine to 2-bromopyridine-1-oxide was unsuccessful. Bernstein and his co-workers[102] obtained the latter compound in good yield using peracetic acid as the oxidising agent, but repetition of this method gave instead white crystalline material which was not homogenous but decomposed over the range of 20° and had none of the properties of the required compound. It was hygroscopic, developed a pyridine-like smell after some time and failed to react with thiourea or form a mercury complex. Oxidation with perbenzoic acid however gave 2-bromopyridine-1-oxide in the reported yield. If the mixture of perbenzoic acid and 2-bromopyridine in chloroform was kept at room temperature for seven days instead of five days as in the method described by Bernstein the yield of 2-bromopyridine-1-oxide was increased. Conversion of 2-bromopyridine-1-oxide into 2-mercaptopyridine-1-oxide was carried out by the method described by Bernstein and Shaw[102], but the literature yield could not be reproduced. The conversion of 2-bromopyridine into 2-mercaptopyridine with thiourea was carried out by the method described by Phillip and Shapiro.[103]

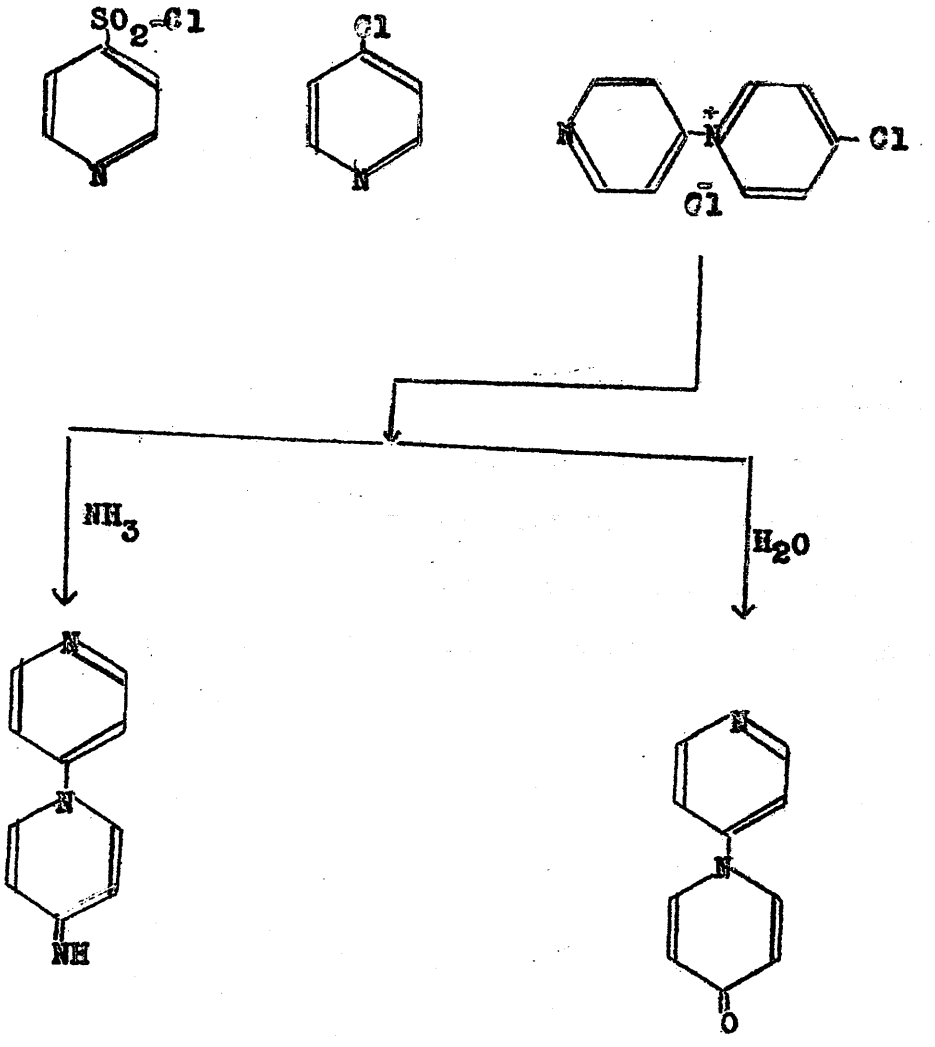
PREPARATION OF PYRIDINE-2-SULPHONYL CHLORIDE.

The difficulty in synthesizing pyridine and quinoline-2- and -4-sulphonyl chlorides from the corresponding

sulphonic acid or its sodium salt, and thionyl chloride, phosphorus pentachloride, chlorosulphonic acid or benzotrichloride has been reported by King and Ware;[104] Caldwell and Kornfeld[105] and by Kwart and Miller;[106] and probably results from the elevated temperature necessary for the transformation, exceeding that of the thermal stability of the sulphonyl chloride. From the nature of the products isolated it appears that a combined desulphonation and nucleophilic replacement takes place. The mechanism of scission of the carbon to sulphur bond has been postulated by Kwart and Miller to take place as follows:



In the case of the corresponding pyridine derivatives the relatively unstable 4-chloropyridine is formed which undergoes a process of self-condensation. The nature of the products formed depends upon their method of isolation.

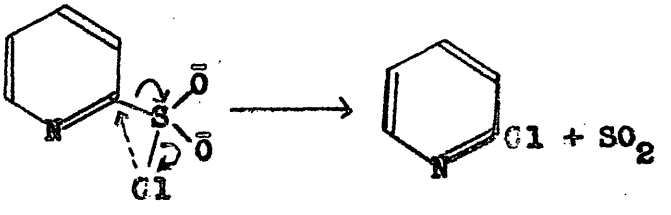


This difficulty has been overcome by the low temperature chlorination of the parent mercaptan under acid conditions.

ATTEMPTED SYNTHESIS OF PYRIDINE-2-SULPHONHYDRAZIDE.

Pyridine-2-sulphonyl chloride was prepared by the chlorination of 2-mercaptopyridine in hydrochloric acid at low temperature and isolated by neutralizing and extracting into chloroform. When the chloroform solution was poured into cold hydrazine hydrate (2 moles) the

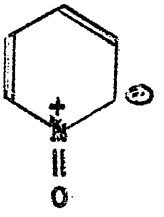
mixture became slightly warmer and gummy material separated out. Attemps to purify the product drastically reduced the yield. The white solid which was obtained only in milligram amounts evolved sulphur dioxide when its aqueous solution was warmed. Extracting the sulphonyl chloride into methylene chloride[105] instead of chloroform failed to increase the yield and varying the experimental conditions was also unsuccessful. Since sulphonamides are more stable and more easily prepared than sulphonhydrazides, preparation of pyridine-2-sulphonamide was attempted to show the formation of sulphonyl chloride but again a poor yield was obtained, probably as a result of desulphonation.



PYRIDINE-2-SULPHONYL CHLORIDE-1-OXIDE.

While the preparation of pyridine -2-sulphonamide and 2-sulphonhydrazide from 2-mercaptopyridine had been unsatisfactory, a parallel series of experiments with 2-mercaptopyridine-1-oxide was more satisfactory. Chlorination took place readily and on passing a dry

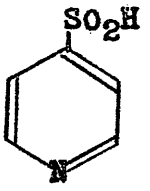
stream of ammonia into a chloroform solution of the sulphonyl chloride-1-oxide, pyridine-2-sulphonamide-1-oxide identical with the product obtained from the peracetic acid oxidation of pyridine-2-sulphonamide[107] was obtained. The greater stability of pyridine-2-sulphonyl chloride -1-oxide is probably due to electron release from the N-oxide to the nucleus increasing electron density at the α and γ-positions, and thus inhibiting the desulphonation and nucleophilic replacement which takes place readily in the deoxygenated parent compound.



PYRIDINE-2-SULPHONHYDRAZIDE-1-OXIDE AND ITS DERIVATIVES.

From the ease with which the sulphonamide-1-oxide was obtained it was expected that the sulphonhydrazide -1- oxide would be obtained by the treatment of the sulphonyl chloride-1-oxide with hydrazine hydrate. Accordingly the chloroform solution was added in small portions to hydrazine hydrate. A mixture of the product and hydrazine hydrochloride was obtained corresponding to an overall yield in the region of 50%, but recovery of pyridine-2- sulphonhydrazide-1-oxide was less than 10%. A somewhat less pure product (m.p.98°) could, however, be obtained in

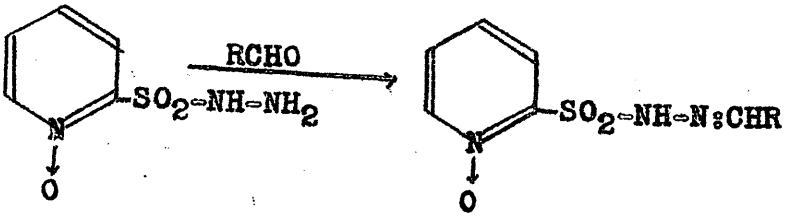
about 15% yield by carefully suspending the product in, and washing with, ice-cold water. This was sufficiently pure for most of the subsequent syntheses undertaken. This product reduced ammonical silver nitrate in the cold and was sparingly soluble in water, methanol and ethanol from which it could be crystallized by caustiously warming to effect solution. Attempts to improve the yield by varying the experimental conditions and method of separation of product from by-product met with no success. It is unstable and loses sulphur dioxide when warmed in aqueous solution. Under the same conditions the isomeric 4-sulphonhydrazide is converted into pyridine-4-sulphinic acid[108] (LXXXII).



(LXXXII)

ALKYLIDENE DERIVATIVES OF PYRIDINE-2-SULPHONHYDRAZIDE-1-OXIDE.

Pyridine-2-sulphonhydrazide-1-oxide reacted readily under mild conditions with aromatic aldehydes to give sulphonhydrazones.



The general method used involved suspension of pyridine-2-sulphonhydrazide-1-oxide in methanol and adding one molecular proportion of the appropriate aldehyde dissolved in methanol. On shaking the reaction mixture the sulphonhydrazide dissolved and, on leaving at room temperature or at 0^{o}, the product eventually precipitated. Analytically pure samples were readily obtained by recrystallization from a suitable solvent. The yields varied from good to excellent. The condensation of pyridine-2-sulphonhydrazide-1-oxide with salicylaldehyde was anomolous and failed to give the expected sulphonhydrazone. The physical and analytical data agreed well with salicylazine and this was confirmed by an undepressed melting point on admixture with an authentic sample of the azine. It is difficult to readily account for the formation of salicylazine under these conditions and the reaction requires further investigation and comparison with the reaction of other phenolic aldehydes with the sulphonhydrazide-1-oxide.

PREPARATION OF DERIVATIVES IN WHICH THE HYDRAZIDE GROUP IS SEPARATED FROM THE NUCLEUS.

The effect of separating the pyridine nucleus and the hydrazine moiety in isonicotinic acid hydrazide by a thiomethylene group has been shown to abolish activity. It was of interest, however, to discover if the same effect obtained in the case of picolinic acid hydrazide which is an active but more toxic tuberculostat.

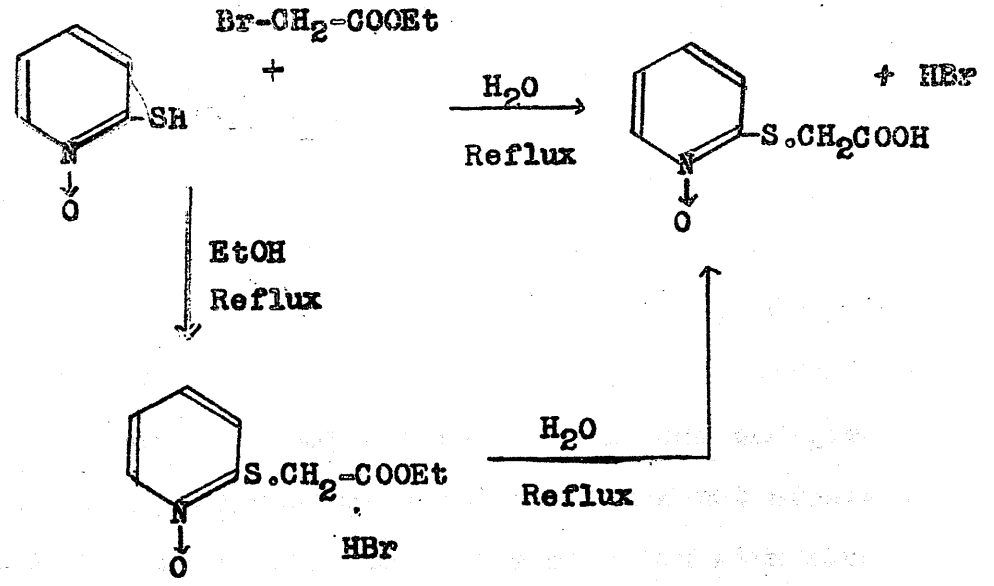
ATTEMPTED SYNTHESIS OF N-(2-PYRIDYLTHIO)CARBONYLHYDRAZINE.

Condensation of 2-mercaptopyridine and ethyl chloroformate was carried out in cold ethanol. The cream-coloured solid which had a fruity smell and was hygroscopic, was refluxed with anhydrous hydrazine (2mole) in dry ethanol. Removal of the solvent gave a viscous oil which failed to crystallize. The oil was extracted with ether till the ether extract was colourless. On concentrating the ether, a yellow solid crystallized out. A mixed melting point with 2-mercaptopyridine did not show any depression. It therefore seemed that hydrazinolysis of the ester had taken place. An analogous reaction sequence has been reported with 4-mercaptopyridine.[109] Attempts to prepare the desired compound by modifying the experimental conditions were unsuccessful. The synthesis of α-(2-pyridylthio)acethydrazide-1-oxide was not considered.

α-(2-PYRIDYLTHIO)ACETHYDRAZIDE-1-OXIDE

The interaction of 2-mercaptopyridine-1-oxide and ethyl bromoacetate on refluxing in aqueous solution gave a crystalline product which failed to analyse for the expected ester. It was acid to limus its infra-red spectrum showed the presence of a carboxyl function and the elementary analysis was satisfactory for α-(2-pyridythio) acetic acid-1-oxide.

The required ester was however obtained as its hydrobromide whon the condesation was carried out in alcohol and when boiled with water it gave the parent acid.

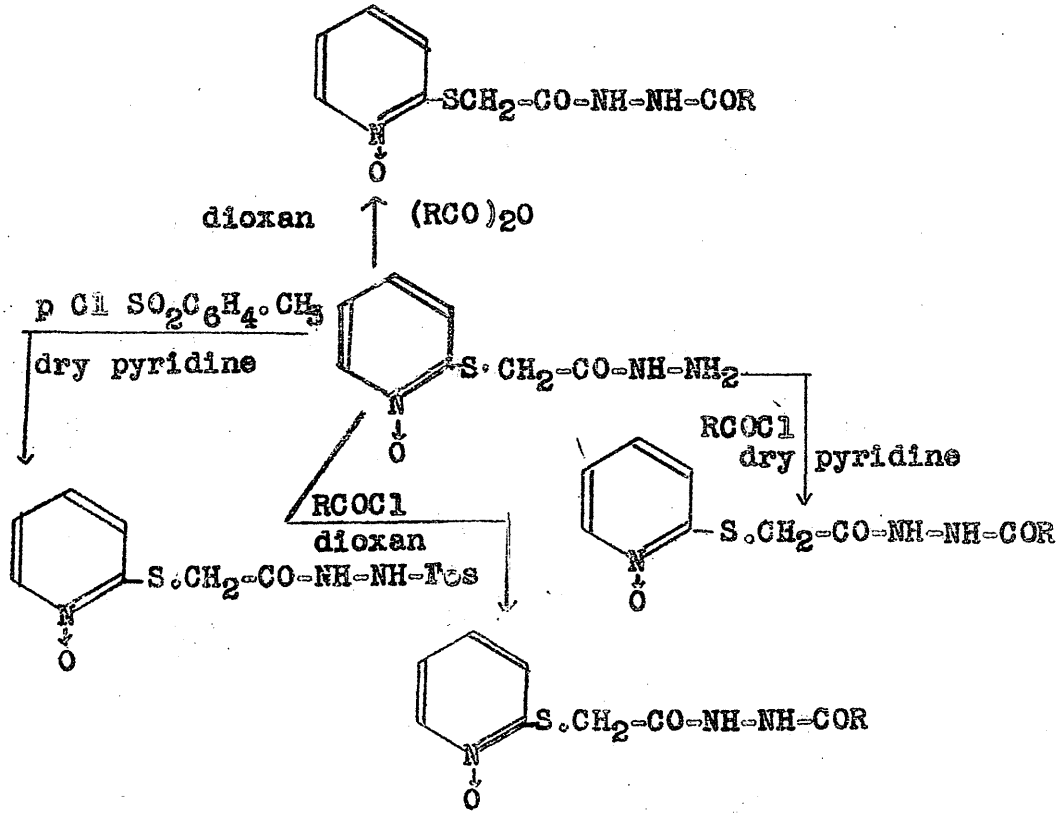


ARALKYLIDENE AND ALKYLIDENE DERIVATIVES OF ∝-(2-PYRIDYLTHIO) ACET HYDRAZIDE-1-OXIDE.

∝-(2-Pyridylthio) acethydrazide-1-oxide reacted readily under mild condition with aliphatic and aromatic aldehydes and ketones, as well as heterocyclic carbonyl compounds. The general method involved suspension of the ∝-(2-pyridylthio)acethydrazide-1-oxide in methanol, heating if necessary to effect solution and adding one molecular proportion of the appropriate carbonyl compound dissolved in methanol. On shaking the reaction mixture vigorously for some time and leaving it at room temperature, a crystalline substance eventually separated out. Analytically pure samples were obtained by recrystallization from a suitable solvent to constant decomposition point and removing adherent solvent in vacuo. The yields varied from good to excellent. The reaction with acetophenone was slow as might be expected for a ketone and the mixture had to be refluxed on a water-bath for one hour before the product separated. On the other hand the reaction with acetaldehyde and butanal at room temperature was vigorous and gave semi-solid resinous material which did not crystallize from the usual solvents. The reaction with glucose, fructose and ribose gave solids which were very hygroscopic and liquified even during filtration. All attempts to isolate the sugar derivatives were unsuccessful.

ACYL AND SULPHONYL DERIVATIVES OF ∝-(2-PYRIDYLTHIO) ACETHYDRAZIDE-1-OXIDE.

∝-(2-Pyridylthio) acethydrazide-1-oxide reacted smoothly with acid chlorides, acid anhydrides and sulphonyl chlorides giving well-defined crystalline derivatives in good yield, according to the following scheme.



Toluene-p-sulphonyl chloride in dry pyridine was added to a suspension of ∝-(2-pyridylthio)acethydrazide-1-oxide in the same solvent. The ∝-(2-pyridylthio)acethydrazide-1-oxide dissolved, and the mixture which had turned orange was heated for half an hour on a water-bath. The product was isolated by the addition of water and the crude product

purified by recrystallization from ethanol.

CONDENSATION OF ETHYL α-(2-PYRIDYLTHIO) ACETATE-1-OXIDE WITH HYDROXYLAMINE.

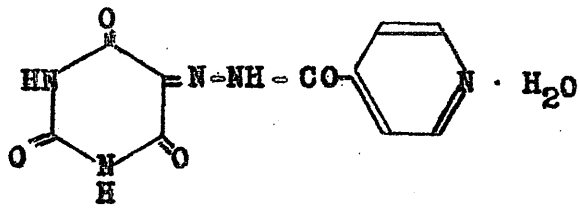
The required compound was prepared by interaction of ethyl-α-(2-pyridylthio) acetate with hydroxylamine hydrochloride in the presence of sodium ethoxide. The product, obtained in moderate yield, gave a positive test for a hydroxamic acid.

PREPARATION OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE AND ITS DERIVATIVES

Condensation of 2-mercaptopyridine and ethyl bromoacetate was carried out in ethanol. The cream coloured solid which had a fruity smell and was hygroscopic, was refluxed with hydrazine (2moles) in dry ethanol. Removal of the solvent gave a viscous oil which crystallized after some time. It was further purified by suspending the crude product in, and washing with ice cold water and used as such as was the case with α-(2-pyridylthio) acethydrazide-1-oxide. It reduced ammonical silver nitrate in the cold, and was sparingly soluble in water, insoluble in ether, chloroform and benzene, soluble in methanol and ethanol from which analytically pure samples could be obtained by recrystallization.

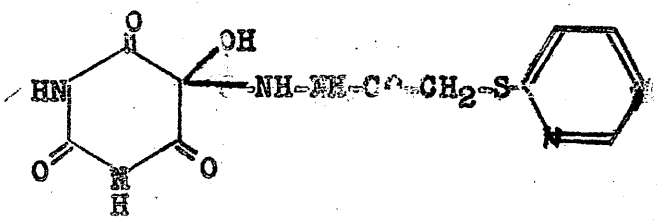
ALKYLIDENE AND ARALKYLIDENE DERIVATIVES OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE.

As with its 1-oxide, α-(2-pyridylthio)acethydrazide reacted at room temperature with aliphatic and aromatic aldehydes and ketones, as well as heterocyclic carbonyl compounds. The general method involved suspension of the α-(2-pyridylthio)acethydrazide in methanol, heating if necessary to effect solution and adding one molecular proportion of the appropriate carbonyl compound dissolved in methanol. On shaking the reaction mixture for some time and leaving it at room temperature, a crystalline product eventually separated out. Analytically pure samples were obtained by recrystallization from a suitable solvent to constant melting point and removing the last traces of adherent solvent *in vacuo*. The reaction with acetaldehyde and butanal was vigorous, and as in the case of the 1-oxide, gave a resinous material which did not crystallize from the common solvents. Preparation of sugar derivatives again proved difficult and the product separating was too hygroscopic to be isolated. The structure of the alloxan (hexahydro-2,4,5,6-tetraoxypyrimidine) derivative of Isoniazid N^2-(2,4,6-trioxy-5-pyrimidylidene)-N^1-isonicotinylhydrazine has been formulated as (LXXXIII) by Fox and Gibas.[16]

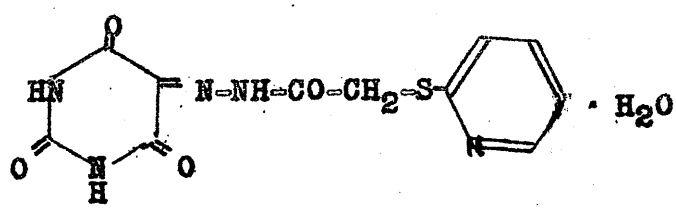


(LXXXIII)

From the physical evidence for the structure of alloxan,[100] the derivative with α-(2-pyridylthio)acethydrazide is probably better represented by structure (LXXXIV) rather than structure (LXXXV).



(LXXXIV)

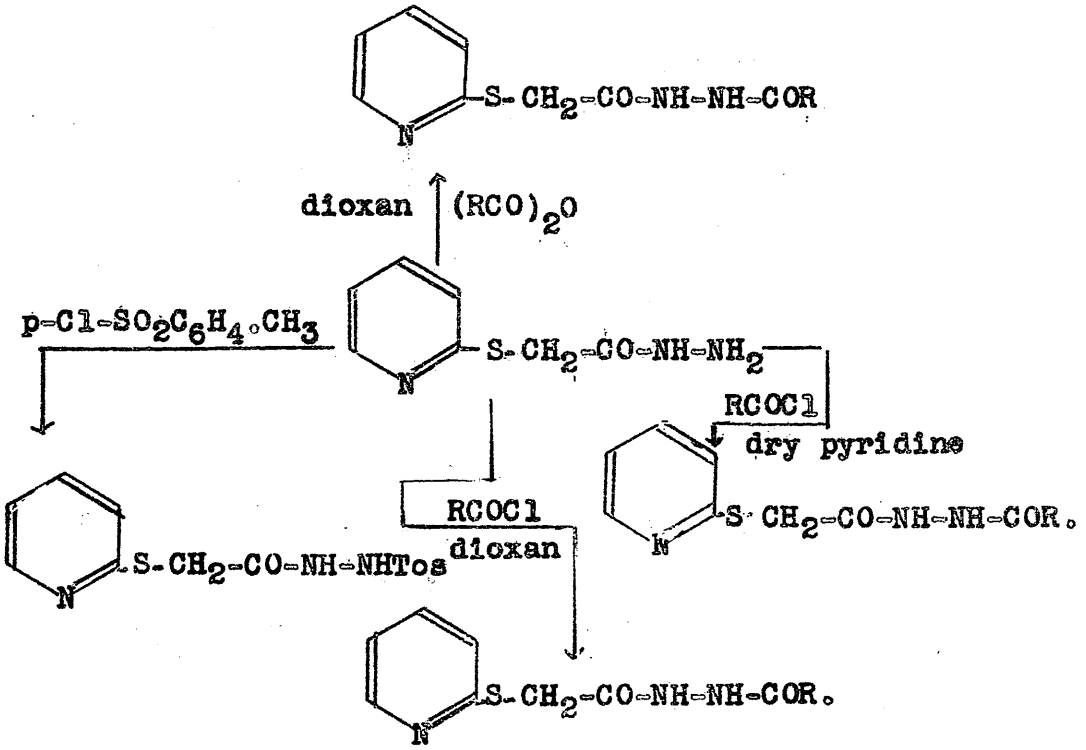


(LXXXV)

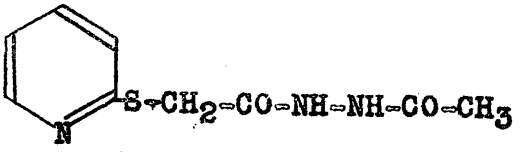
The condensation between alloxan and α-(2-pyridylthio) acethydrazide thus parallels that with Isoniazid and also pyridine-4-sulphonhydrazide.

SYNTHESIS OF ACYL AND SULPHONYL DERIVATIVES OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE.

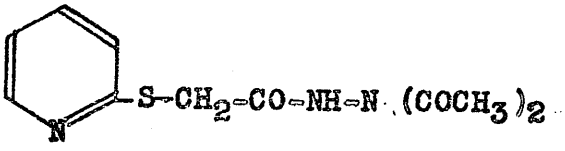
α-(2-Pyridylthio)acethydrazide reacted smoothly with acid chlorides, acid anhydrides and sulphonyl chlorides, giving well-defined crystalline derivatives in good yield, according to the following:



Two derivatives were obtained with acetic anhydride, depending upon the relative amount of anhydride added. With one molecular proportion and using methanol or dioxan as solvent the monoacetyl derivative (LXXXVI) was obtained whilst treatment of the hydrazide with excess acid anhydride gave the diacetate (LXXXVII).



(LXXXVI)



(LXXXVII)

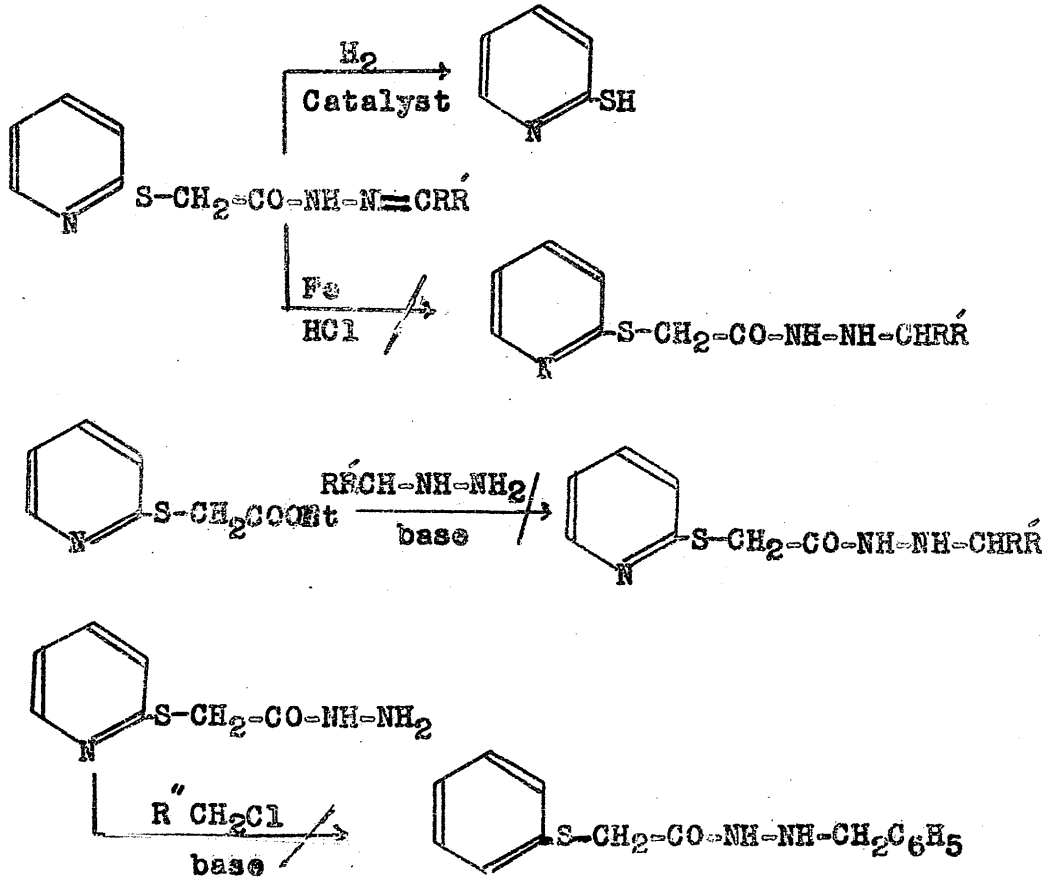
α-(2-Pyridylthio) acethydrazide reacted with toluene-p-sulphonyl chloride in the presence of pyridine to give a well-defined crystalline product. As before the mixture turned orange, and was heated for 1 hour on a water-bath to complete the reaction. The product was isolated as before by the addition of water and purified by recrystallization from ethanol.

ATTEMPTED SYNTHESIS OF ALKYL AND ARALKYL DERIVATIVES OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE.

In view of the antitubercular and MAOI activity of substituted hydrazides it was considered of interest to attempt the synthesis of some alkyl and aralkyl derivatives of α-(2-pyridylthio)acethydrazide.

Catalytic reduction of alkylidene and aralkylidene derivatives of isoniazid takes place smoothly to give the alkyl and aralkyl derivatives in good yield. Reduction of the Schiff's base linkage of the corresponding

derivatives of α-(2-pyridylthio)acethydrazide however resulted in hydrogenolysis and regeneration of 2-mercaptopyridine. Several other methods were attempted without success (vide infra).



SEMICARBAZIDES OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE AND ITS 1-OXIDE.

Allylisothiocyanate and phenyl isocyanate condensed with α-(2-pyridylthio)acethydrazide and its 1-oxide respectively in acetonitrile to give the appropriate semicarbazides.

R.NH.NH.C(:X)NH.R' (X= O or S)

EXPERIMENTAL

PREPARATION OF STARTING MATERIALS.

2-Mercaptopyridine was prepared from 2-bromopyridine and thiourea by the method described by Phillip and Shapiro,[103] and Thirtle.[112]

2-Mercaptopyridine-1-oxide was prepared from 2-bromopyridine-1-oxide and perbenzoic acid by the method described by Shaw, Bernstein, Losee and Lott.[102]

Perbenzoic acid was prepared by the method described in Organic synthesis.[112]

Anhydrous Hydrazine:- Hydrazine Hydrate (75g., 1·5 mole) was added to sodium hydroxide (80g., 2 mole) in toluene (6·5 mole). After twenty four hours the mixture was refluxed at 120-130° for one hour, access of moisture being prevented by a calcium chloride drying tube attached to the condenser outlet. The mixture was then distilled, the distillate (b.p. 94-95°) collected, and the lower layer consisting of anhydrous hydrazine (50g.) drawn off.

2-Sulphonamidopyridine-1-oxide. 2-Mercaptopyridine-1-oxide (1·2g., 0·01 mole) was dissolved in a cold mixture of concentrated hydrochloric acid (7·5 ml) and water (2 ml) and cooled in a mixture of crushed ice and salt. When the temperature had dropped to -10°, chlorine gas

was bubbled through at such a rate that the temperature was maintained at about - 5°. When the temperature started to drop, the flow of chlorine through the mixture was increased, and, towards the end of the reaction, it was passed vigorously. The reaction was complete when the temperature did not rise despite the rapid stream of chlorine passing through the solution. The solution was treated at - 5° with calcium carbonate added in small portions to effect neutralization. Cold chloroform (60 ml) was added during neutralization and after vigorously shaking the clear chloroform solution was decanted from the white sludge which was further washed with cold chloroform (40 ml). The combined chloroform extracts were dried (Na_2SO_4) at 0-5°, filtered, and a steady stream of dry ammonia gas passed through. The crude product was filtered, washed with cold water and dried in a vacuum desiccator. Recrystallization from methanol gave 2-sulphonamidopyridine-1-oxide as shining white crystals (0.4g., 33%) m.p. 228° (Literature[10] m.p. 228°).

PREPARATION OF PYRIDINE-1-OXIDE-2-SULPHONHYDRAZIDE AND SOME ARALKYLIDENE DERIVATIVES.

Pyridine-1-oxide-2-sulphonhydrazide. 2-Mercaptopyridine-1-oxide (1.27g., 0.01 mole) was converted into pyridine-1-oxide-2-sulphonyl chloride as above. The cold, dry chloroform solution of pyridine-1-oxide-2-sulphonyl chloride was added in portions (10-20 ml) to hydrazine hydrate (1g., 0.02 mole) and vigorously shaken after each addition.

The mixture was left overnight at 0° and the solid filtered and washed with a little ether. The dry product was suspended in ice-cold water and sucked dry before transferring to a vacuum desiccator. The product m.p. 96-98°, crystallized from methanol as the monohydrate. It was sparingly soluble in water, methanol and ethanol, and insoluble in non-polar solvents. (Found: C, 28.8; H, 3.9. $C_5H_9N_3O_4S$ requires C, 28.9; H, 4.3%).

1-Benzylidene-2-(pyridine-1-oxide-2-sulphonyl) hydrazine. Benzaldehyde (0.106g., 0.001 mole) in methanol (5 ml) was added to pyridine-1-oxide-2-sulphonhydrazide (0.189g., 0.001 mole) in methanol (5 ml) and the mixture vigorously shaken till only a faint smell of benzaldehyde remained. The white solid which separated was filtered, washed with a little methanol and ether, and dried in a vacuum desiccator. Recrystallization from methanol gave the product (0.16 g.) as white needles m.p. 145-147° (decomp.) (Found: C, 52.6; H, 4.1; N, 15.4. $C_{12}H_{11}N_3O_3S$ requires C, 52.0; H, 4.0, N, 15.2%).

1-Veratrylidene-2-(pyridine-1-oxide-2-sulphonyl) hydrazine Pyridine-1-oxide-2-sulphonhydrazide (0.189g., 0.001 Mole) was treated with veratraldehyde (0.166g., 0.001 mole) as described above. Recrystallization from ethanol gave the veratrylidene derivative (0.15 g.) as needles. m.p. 146-148° (decomp.) (Found: C, 49.2; H, 4.1; N, 12.7.

$C_{12}H_{11}O_3N_3S$ requires C, 49.8; H, 4.4; N, 12.45%).

Salicylazine: Pyridine-1-oxide-2-sulphonhydrazide (0.189g., 0.001 mole) reacted with salicylaldehyde (0.122g., 0.001 mole) as above. The crystalline solid separating was recrystallized from ethanol to give salicylazine (m.p. 210-212°) (Found: C, 69.8; H, 4.8. Calc. for $C_{14}H_{12}N_2O_2$ C, 70.0; H, 5.0%).

Attempted synthesis of N'-(2-pyridylthio) carbonylhydrazine

Method I

The chloroformic ester (0.011 mole) was added dropwise to an ice-cold solution of 2-mercaptopyridine (0.01 mole) in water (25 ml) containing sodium bicarbonate (0.11 mole) and vigorously shaken till only a faint smell of the chloroformic ester remained. The mixture was extracted with ether and the ether dried (Na_2SO_4) then removed under reduced pressure to give a viscous oil which did not crystallize. The oil (0.1g) showed maximum absorption at 253 mμ.

Method II

2-Mercaptopyridine (0.01 mole) was dissolved in N sodium hydroxide (10 ml) and the solution evaporated to dryness under reduced pressure. The residue was dissolved in dry alcohol (20 ml) and ethyl chloroformate (1.1g., 0.011 mole) added. The mixture was filtered and the filtrate evaporated to dryness, leaving a viscous oil (0.05g., 50%) which failed to crystallize. Distillation

at 3.0 -2.0 mmHg gave a white viscous oil which showed maximum absorption at 253 mu and an ester peak at 1736 cm.

Method III

2-Mercaptopyridine (1.11g., 0.01 mole) was added to a solution of ethyl chloroformate (1.11g., 0.01 mole) in dry ethanol (30ml). The mixture was vigorously shaken with occasional warming on a water-bath till a faint smell only of ethyl chloroformate remained, and then it was evaporated to dryness under reduced pressure. The residue was redissolved in dry ethanol (20ml) and the solution refluxed with anhydrous hydrazine (0.5g.) for three hours. Removal of the solvent under reduced pressure gave a viscous oil which crystallized from ether as a yellow solid (1g.) m.p.118°. A mixed melting point with 2-mercaptopyridine showed no depression.

PREPARATION OF α-(2-PYRIDYLTHIO) ACETIC ACID -1-OXIDE ITS ACID AMIDE AND HYDRAZIDE.

α-(2-Pyridylthio) acethydrazide-1-oxide. 2-Mercapto pyridine-1-oxide (3.8g., 0.03 mole) was added to a solution of ethyl bromoacetate (4.01g., 0.03 mole) in dry ethanol (50ml). The mixture was refluxed for 1½ hour, and then evaporated to dryness under reduced pressure. The cream-coloured solid (3.9g.) was refluxed with anhydrous hydrazine (0.9g.) in dry ethanol (40ml) for five hr., and

then evaporated to dryness under reduced pressure to give a crystalline residue which was suspended in ice-cold water (15ml), quickly filtered, washed with more ice-water (10ml), and sucked dry before transferring to a vacuum desiccator. It was washed with a large excess of ether and again dried in a vacuum desiccator. The hydrazide (2.5g., 73%), m.p. 200-201° (decomp.) crystallized in colourless needles from ethanol. α-(2-Pyridylthio)acethydrazide-1-oxide is insoluble in benzene, ether and chloroform, sparingly soluble in ethanol and soluble in methanol and hot water. (Found: C, 42.2; H, 4.4; N, 20.5. $C_7H_9N_3O_2S$ requires C, 42.2; H, 4.5; N, 20.6%).

2-Pyridylthioacetamide-1-oxide. 2-Mercaptopyridine-1-oxide (1.27g., 0.01 mole) was added to a solution of ethyl bromoacetate (1.68g., 0.01 mole) in dry ethanol (10ml). The mixture was refluxed for 1½hr., and then evaporated to dryness under reduced pressure. The cream coloured salt of ethyl (2-pyridylthio)acetate (1.6g.) was dissolved in ethanol (10ml), treated with excess of concentrated ammonia at room temperature, vigorously shaken and evaporated to dryness under reduced pressure. The amide (1g.), m.p. 215° (decomp.), crystallized in white needles from methanol. (Found: C, 45.6; H, 4.3; N, 15.4. $C_7H_8N_2O_2S$ requires C, 45.65; H, 4.3; N, 15.4%).

2-Pyridylthioacetic acid-1-oxide. 2-Mercaptopyridine-1-oxide (1.27g., 0.01 mole) was converted into ethyl (2-pyridylthio) acetate-1-oxide as described above. The cream coloured solid obtained was refluxed for 1½hr with water (10ml). The product (1g.), m.p.288° (decomp.) was recrystallized from aqueous methanol. (Found: C, 45.6; H, 4.0; N, 7.7. $C_7H_7NO_3S$ requires C, 45.4; H, 3.8; N, 7.6%). The infra-red spectrum showed absorption maxima at 1700$cm.^{-1}$ and 3400$cm.^{-1}$

PREPARATION OF ALKYLIDENE AND ARALKYLIDENE DERIVATIVES OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE-1-OXIDE.

N-Benzylidene-α-(2-pyridylthio)acethydrazide-1-oxide. α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05 mole) in methanol (5ml) was added to benzaldehyde (0.212g.) in methanol (5ml) and the mixture vigorously shaken till only a faint smell of benzaldehyde remained. The mixture was then left overnight in the refrigerator. The white crystalline solid which separated was filtered, washed with a little methanol and ether, and dried in a vacuum desiccator. Recrystallization from methanol gave the benzylidene derivative as white needles (0.4g.) m.p. 202-203° (decomp.) (Found: C, 58.5; H, 4.55; N, 14.95. $C_{14}H_{13}N_3O_2S$ requires C, 58.5; H, 4.5; N, 14.7%).

N'-Salicylidene-α-(2-pyridylthio) acethydrazide-1-oxide.

α-(2-Pyridylthio)acethydrazide-1-oxide (0.398g., 0.05mole) was treated with salicylaldehyde (0.244g., 0.05 mole) in the manner described for N-benzylidene -α-(2-pyridylthio) acethydrazide-1-oxide. The white crystalline solid which separated on standing was filtered, washed with a little methanol and ether and dried in a vacuum desiccator. Recrystallization from methanol gave the salicylidene derivative as needles (0.41g). m.p. 230-232° (decomp.) (Found: C, 55.55; H, 4.0; N, 13.9. $C_{14}H_{13}N_3O_3S$ requires C, 55.45; H, 4.3; N, 13.9%).

N'-Isopropylidene-α-(2-pyridylthio) acethydrazide-1-oxide.

α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05 mole) was treated with excess acetone. The mixture was vigorously shaken for some time and then evaporated to dryness under reduced pressure. The residue was crystallized from ethanol to give the isopropylidene derivative (0.4g.) m.p. 210-211°(decomp.) (Found: C,50.3; H, 5.3; N, 17.7. $C_{10}H_{13}N_3O_2S$ requires C, 50.3; H, 5.4; N, 17.6%).

N'-Piperonylidene-α-(2-pyridylthio) acethydrazide-1-oxide.

α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05 mole) was treated with piperonal (0.3g., 0.05 mole) as described for N'-salicylidene-α-(2-pyridylthio) acethydrazide-1-oxide. The product (0.41g.) m.p.207-208°,

was obtained by recrystallizing the precipitate from methanol. (Found: C, 54·2; H, 3·9; N, 12·1. $C_{15}H_{13}N_3O_4S$ requires C, 54·4; H, 3·9; N, 12·7%).

N'-(α-Phenylethylidene)-α-(2-pyridylthio) acethydrazide-1-oxide. Acetophenone (0·132g., 0·05 mole) in methanol (5 ml) was added to α-(2-pyridylthio) acethydrazide-1-oxide (0·398g., 0·05 mole) in methanol (5 ml) and refluxed for one hour. The mixture was concentrated and left overnight at room temperature to crystallize. The product (0·42g.) m.p. 201-203° (decomp.) was obtained by recrystallizing the precipitate from methanol. (Found: C, 60·5; H, 5·0; N, 13·5. $C_{15}H_{15}N_3O_2S$ requires C, 59·9; H, 5·0; N, 13·8%).

N'-Veratrylidene-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio)acethydrazide-1-oxide (0·398g., 0·05mole) was treated with veratraldehyde (0·166g., 0·05 mole) as described under the benzylidene derivative. The product (0·42g.) m.p. 213-214° (decomp.) was obtained by recrystallizing the precipitate from methanol. (Found: C, 54·8; H, 5·1;. $C_{16}H_{17}N_3O_4S$ requires C, 55·0;, H, 5·0%).

N'-Dimethylaminobenzylidene-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio)acethydrazide-1-oxide (0·398g., 0·05 mole) was treated with p-dimethylaminobenzaldehyde (0·298g., 0·05 mole) as above. A crystalline solid had separated after four days. Recrystallization from ethanol

gave the product (0.38g.) as needles m.p. 218-220°(decomp.). (Found: C,58.6; H, 5.5, N, 16.2. $C_{16}H_{18}N_4O_2S$ requires C, 58.2; H, 5.45; N, 16.6%).

N'-Hexahydro-2,4,6-trioxy-5-pyrimidylidene-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05 mole) was treated with hexahydro-2,4,5,6-tetroxypyrimidine (0.33g., 0.05 mole) as before. The solid which had separated after three days was filtered, washed with a little methanol and recrystallized from methanol to give the product (0.4g.) m.p. 183°(decomp.) (Found: C, 37.8;, H, 3.8; N, 19.5. $C_{11}H_9N_5O_5S$ requires C, 38.0; H, 3.5; 20.0%).

N'-Cinnamylidene-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05 mole) in methanol (5 ml) was treated with cinnamaldehyde (0.132g., 0.05 mole)in methanol (10ml). The product (0.42g.) m.p. 220-223°(decomp.) was obtained by recrystallizing the precipitate from methanol. (Found: C, 61.55;, H, 4.9; N, 13.3. $C_{16}H_{15}N_3O_2S$ requires C, 61.55; H, 4.8; N, 13.5%).

N'-Vanillylidene-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05mole) in methanol (5ml) was treated with vanillin (0.304g., 0.05 mole) in methanol (5ml). The solid which separated was filtered, washed with a little methanol and ether and dried in a vacuum desiccator. Recrystallization from

methanol gave the product (0.3g.) as needles m.p. 198-200° (decomp.) (Found: C, 54.2; H, 4.55; N, 12.8. $C_{15}H_{15}N_3O_4S$ requires C, 53.9; H, 4.6; N, 12.3%).

N'-(B-Phenylethylidene)-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio) actehydrazide-1-oxide (0.398g., 0.05 mole) in methanol (5ml) was treated with freshly distilled phenylacetaldehyde (0.212g.) in methanol (10ml). After four days the solid which had separated was filtered, washed with methanol and ether. Recrystallization from methanol gave the product (0.31g.) as needles m.p. 190-191° (Found: C, 60.0;, H, 5.2; N, 10.0. $C_{15}H_{15}N_3O_2S$ requires C, 59.8; H, 5.0; N, 10.3%).

N'-Acetylisopropylidene-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05 mole) was treated with acetylacetone (0.2g., 0.05 mole) as described above. The white crystalline solid which separated was filtered, washed with methanol and ether and dried in a vacuum desiccator. Recrystallization from ethanol gave the product (0.32g.) as fine needles. m.p. 175° (decomp.) (Found: C,51.25; H, 5.7; N, 15.4. $C_{12}H_{15}N_3O_3S$ requires C, 51.25; H, 5.3; N, 14.9%).

N'-Furfurylidene-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05mole) was treated with furfuraldehyde (0.192g., 0.05 mole)

in methanol (10ml), and the solid separating filtered, washed with methanol and ether and dried in a vacuum desiccator. Recrystallization from ethanol gave the product (0.4g.) as fine needles. m.p. 180-182° (Found: C, 51.9; H, 4.2; N, 15.8. $C_{12}H_{11}N_3O_3S$ requires C, 52.0; H, 4.0; N, 15.4%).

CONDENSATION OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE-1-OXIDE WITH ACID ANHYDRIDES, ACID CHLORIDES AND SULPHONYL CHLORIDES.

N'-Diacetyl-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05 mole) was added in small portions to acetic anhydride (5ml) and warmed till solution was complete. On cooling the crystalline solid separating was filtered, washed with a large excess of ether to remove acetic anhydride and dried in a vacuum desiccator. It was twice recrystallized from methanol to give the diacetyl derivative (0.4g.) as needles m.p. 135° (decomp.) (Found: C, 44.5;, H, 4.8; N, 14.9. $C_{11}H_{13}N_3O_4S$ requires C, 45.2; H, 4.6; N, 14.3%).

N'-β-Hydroxycarbonylpropionyl-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05 mole) was added to succinic anhydride (0.2g., 0.05 mole) in methanol (10ml). The solid separating was twice crystallized from methanol to give the product (0.4g.) as needles. m.p. 200-201°(decomp.) (Found: C,44.4; H, 4.55;

N, 14·85. $C_{11}H_{13}N_3O_5S$ requires C, 44·1; H, 4·35; N, 14·05%).

N-Toluene-p-sulphonyl-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio) acethydrazide-1-oxide (0·398g., 0·05 mole) in dry pyridine (10ml) was treated with toluene-p-sulphonyl chloride (0·05 mole) and the mixture heated on a water-bath for 15 min. The mixture was cooled and an excess of distilled water added. After three hours a white crystalline solid had separated. This was filtered, washed with a little water and dried in a vacuum desiccator. Recrystallization from ethanol gave the toluene-p-sulphonyl derivative (0·29g.) as needle m.p. 242° (decomp.) (Found: C, 47·4; H, 4·5; $C_{14}H_{15}N_3O_4S_2$ requires C, 47·6; H, 4·2%).

N-B-Hydroxycarbonylacryloyl-α-(2-pyridylthio) acethydrazide-1-oxide. α-(2-Pyridylthio) acethydrazide-1-oxide (0·398g., 0·05 mole) was treated with maleic anhydride (0·05 mole) as described for N-β-hydroxycarbonylpropionyl-α-(2-pyridylthio)acethydrazide-1-oxide. The crystalline solid separating was filtered, washed with a little methanol and ether and dried in a vacuum desiccator. Recrystallization from methanol gave the product (0·3 g.) m.p. 110-113°. (Found: C, 44·8; H, 4·2; N, 14·9. $C_{11}H_{11}N_3O_5S$ requires C, 44·4; H, 4·0; N, 14·15%).

1-α-(2-Pyridylthio) acetyl -4-phenylsemicarbazide-1-oxide.

α-(2-Pyridylthio) acethydrazide-1-oxide (0.398g., 0.05 mole) was treated with phenylisocyanate (0.2g., 0.05 mole) in dry acetonitrile (10ml). The mixture was vigorously shaken for 1 hr., and evaporated to dryness under reduced pressure. The white crystalline solid remaining was recrystallized from ethanol to give the semicarbazide (0.2g.) as needles m.p. 194-195°. (Found: C, 52.7; H, 4.2; N, 18.0. $C_{14}H_{14}N_4O_3S$ requires C, 52.8; H, 4.4; N, 17.7%).

α-(2-Pyridylthio) acethydroxamic acid-1-oxide.

Method I

Ethyl (2-pyridylthio) acetate -1-oxide (1.6g.) was added to an 85% solution of potassium hydroxide (3ml) in methanol (15 ml) containing hydroxylamine hydrochloride (0.6g.). The potassium chloride was filtered off, washed with a little methanol and the filtrate and the washings left at room temperature to crystallize. The solid separating was dissolved in a mixture of water (10ml), neutralized with acetic acid but the product failed to separate.

Method II

Ethyl (2-pyridylthio) acetate-1-oxide (1.6g.) was added to a solution of hydroxylamine hydrochloride (1.1g.) in methanol (15ml) containing sodium methoxide (1.1g.). The precipitate was quickly filtered and washed with a

little methanol. The combined filtrate and washings were concentrated to small volume under reduced pressure and left at room temperature to crystallize. The hydroxamic acid (0.5g.) separated in fine needles. m.p. 195-197° (decomp.) (Found: C, 41.9; H, 4.2, $C_7H_8N_2O_3S$ requires C, 42.0; H, 4.0%).

PREPARATION OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE AND ITS DERIVATIVES.

α-(2-Pyridylthio) acethydrazide. 2-Mercaptopyridine (1.11g., 0.01 mole) was added to a solution of ethyl bromoacetate (1.67g., 0.01 mole) in dry ethanol (30ml), refluxed for two hr., and evaporated to dryness under reduced pressure. The off-white solid (1.3g.) was refluxed with anhydrous hydrazine (0.99g.) in dry ethanol (40ml) for 5-6hr., and again reduced to dryness under reduced pressure. The residue was suspended in ice-cold water (10ml) and then sucked dry before transferring to a vacuum desiccator. The dry solid was liberally washed with ether and crystallized from ethanol to give the product (1.2g.) as needles. m.p. 90-92° (Found: C, 46.0; H, 4.9; N, 22.9. $C_7H_9N_3OS$ requires C, 45.9; H, 4.9; N, 22.2%).

ALKYLIDENE AND ARALKYLIDENE DERIVATIVES OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE.

N-Benzylidene-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0.183g., 0.001 mole) in

methanol (5ml) was added to benzaldehyde (0.106g., 0.001mole) in methanol (5ml) and vigorously shaken till only a faint smell of benzaldehyde remained. It was left overnight to crystallize and the solid washed with a little methanol and ether and dried in a vacuum desiccator. Recrystallization from methanol gave the benzylidene derivative (0.2g.) as needles. m.p. 191-192°. (Found: C, 62.0; H, 4.7; N, 14.5. $C_{14}H_{13}N_3O$ S requires C, 62.0; H, 4.75; N, 14.9%).

N-Salicylidene-α-(2-pyridylthio) acethydrazide.

α-(2-Pyridylthio) acethydrazide (0.183g., 0.001 mole) in methanol (5ml) was added to salicylaldehyde (0.122g., 0.001 mole) as described above. Recrystallization from ethanol gave the salicylidene derivative as needles (0.2g.) m.p. 200-201° (decomp.) (Found: C, 58.25; H, 4.3; N, 14.6. $C_{14}H_{13}N_3O_2S$ requires C, 58.5; H, 4.5; N, 14.6%).

N-Isopropylidene-α-(2-pyridylthio) acethydrazide.

α-(2-Pyridylthio) acethydrazide (0.183g., 0.001 mole) was allowed to react with acetone (10ml) and the solid recrystallized from ethanol to give the product (0.2g.) as needles. m.p. 128-130°. (Found: C, 53.5; H, 5.8; N, 19.0. $C_{10}H_{13}N_3O$ S requires C, 53.8; H, 5.8; N, 18.8%).

N-(α-Phenylethylidene)-α-(2-pyridylthio) acethydrazide.

α-(2-Pyridylthio) acethydrazide (0.183g., 0.001 mole) in methanol (5ml) was added to acetophenone (0.1g.,

0.001 mole) in methanol (10ml) and the solid separating recrystallized from ethanol to give the product (0.21g.) as fine needles. m.p. 142-144°. (Found: C, 63.1; H, 5.55; N, 15.0. $C_{15}H_{15}N_3O$ S requires C, 63.15; H, 5.4; N, 14.8%).

N'-Piperonylidene-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0.183g., 0.001 mole) was mixed with piperonal (0.15 g., 0.001 mole) in the manner described for the salicylidene derivative above and the light cream-coloured solid obtained recrystallized from ethanol to give the product (0.19g.) m.p. 167-170° (Found: C,57.4; H, 4.3;. $C_{15}H_{13}N_3O_3S$ requires C, 57.1; H, 4.1%).

N'-Veratrylidene-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0.183g., 0.001 mole) was added to veratraldehyde (0.166g., 0.001 mole) as described above. Recrystallization from ethanol gave the product (0.199g.) m.p. 138-141° (Found: C, 57.6; H, 5.4. $C_{16}H_{17}N_3O_3S$ requires C, 57.9; H, 5.2%).

N'-Hexahydro-2,4,6-trioxy-5-pyrimidylidene-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0.183g., 0.001 mole) was treated with hexahydro-2,4,5,6-tetroxypyrimidine (0.001 mole) in methanol (5ml). The mixture was vigorously shaken and left to crystallize. Recrystallization from methanol gave the product (0.2g.) m.p. 290°(decomp.) as the monohydrate. (Found: C, 40.8; H, 3.9; N, 21.9.

$C_{11}H_{11}N_5O_5S$ requires C, 40·9; H, 3·4; N, 21·6%).

N-Dimethylaminobenzylidene-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0·183g., 0·001 mole) in methanol (5ml) was treated with p-dimethylaminobenzaldehyde (0·001mole) as described above. Recrystallization from methanol gave the product (0·29g.) as needles. m.p. 186-188°. (Found: C, 60·9; H, 5·7. $C_{16}H_{18}N_4O$ S requires C, 61·0; H, 5·7%).

N-Cinnamylidene-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0·183g., 0·001 mole) was treated with cinnamaldehyde (0·139g., 0·001 mole) as before to give the product (0·16g.) as needles. m.p. 135-136°. (from methanol). (Found: C, 64·2; H, 4·9; N, 15·7. $C_{16}H_{15}N_3O$ S requires C, 64·6; H, 5·0; N, 15·1%).

N-Vanillylidene-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0·181g., 0·001 mole) was treated with vanillin (0·152g., 0·001 mole) as before to give the product as the hemihydrate. m.p. 165-167° (Found: C, 55·75; H, 5·4. $C_{15}H_{15}N_3O_3S$, $\frac{1}{2}H_2O$ requires C, 55·4; H, 4·95%).

N-(β-Phenylethylidene)-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0·183g., 0·001 mole) was treated with freshly distilled phenylacetaldehyde (0·001 mole) as before to give the product (0·198g.) m.p. 152-153°(from methanol). (Found: C, 62·9; H, 5·4;

N, 15·4. $C_{15}H_{15}N_3OS$ requires C, 63·0; H, 5·3; N, 14·7%).

N'-Cyclohexylidene-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0·183g., 0·001 mole) was treated with cyclohexanone (0·96g., 0·001 mole) as before to give the product (0·19g.) as needles. m.p. 112-114° (from methanol). (Found: C, 59·35; H, 6·6. $C_{13}H_{17}N_3O$ S requires C, 59·35; H, 6·45%).

N'-p-Methoxybenzylidene-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0·183g., 0·001 mole) was treated with p-methoxybenzaldehyde (0·136g., 0·001 mole) in methanol (10ml) and the solid separating recrystallized from methanol to give the product (0·1g.) as needles m.p. 136-138° (Found: C, 59·5; H, 5·1. $C_{15}H_{15}N_3O_2S$ requires C, 59·8; H, 5·0%).

N'-p-Hydroxybenzylidene-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0·183g., 0·001 mole) was treated with p-hydroxybenzaldehyde (0·122g., 0·001 mole) as before to give the product (0·15g.) m.p. 194° (decomp.) (from methanol). (Found: C, 56·7; H, 4·7. $C_{14}H_{13}N_3O_2S$ requires C, 56·3; H, 4·7%).

CONDENSATION OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE WITH ACID ANHYDRIDES, ACID CHLORIDES AND SULPHONYL CHLORIDE.

N'-Diacetyl-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0·183g., 0·001 mole) was added in small portions to freshly distilled acetic anhydride (5 ml). The mixture which solidified

immediately was sucked dry at the pump, washed with a large quantity of ether and dried in a vacuum desiccator. Recrystallization from ethyl acetate gave the diacetyl derivative (0.19g.) as needles m.p. 141-143°. (Found: C, 47.9; H, 4.9. $C_{11}H_{13}N_3O_3S$ requires C, 47.75; H, 5.1%).

N'-β-Hydroxycarbonylpropionyl-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0.183g., 0.001 mole) in methanol (5ml) was treated with succinic anhydride (0.116g., 0.001 mole) in methanol (5ml). The crystalline solid which separated gave the product (0.2g.) as needles m.p. 150-151° (from isopropanol) (Found: C, 46.2; H, 4.7. $C_{11}H_{13}N_3O_4S$ requires C, 46.55; H, 4.6%).

N'-Acetyl-α-(2-pyridylthio) acethydrazide. α-(2-Pyridylthio) acethydrazide (0.183g., 0.001 mole) in pyridine (5ml) was treated with acetic anhydride (0.18g., 0.001 mole) in dry pyridine (5ml). The solid obtained was recrystallized from ethyl acetate to give the acetyl derivative (0.1g.) as needles. m.p. 138-140°. (Found: C, 48.0; H, 5.1; N, 17.3. $C_9H_{11}N_3O_2S$ requires C, 48.0; H, 5.0; N, 18.1%).

N'-Toluene-p-sulphonyl-α-(2-pyridylthio) acethydrazide. To α-(2-pyridylthio) acethydrazide (0.366g., 0.05mole) in dry pyridine (10ml) was added toluene-p-sulphonyl chloride (0.38g., 0.05 mole). The mixture was heated on a water-bath for 10 min. and allowed to cool to room temperature. Water (50ml) was added and the white solid which had separated after 1hr., was filtered, washed and

dried in a vacuum desiccator. Recrystallization from ethanol gave the product (0.1g.) m.p. 165-167°. (Found: C, 49.3; H, 4.6. $C_{14}H_{15}N_3O_3S_2$ requires C, 49.8; H, 4.45%).

1-α-(2-Pyridylthio)acetyl-4-allylthiosemicarbazide. α-(2-Pyridylthio) acethydrazide (0.366g., 0.05 mole) in acetonitrile (5ml) was added to allylisothiocyanate (0.198g., 0.05 mole) and the mixture heated on a water-bath for 10 min., then allowed to cool to room temperature. Recrystallization from ethanol gave the product (0.32g.)(m.p. 116-117°) (Found: C, 46.3; H, 5.0., $C_{11}H_{14}N_4OS_2$ requires C, 46.8; H, 5.0%).

ATTEMPTED SYNTHESIS OF ALKYL AND ARALKYL DERIVATIVES OF α-(2-PYRIDYLTHIO) ACETHYDRAZIDE.

A. Method I.

N-Isopropylidene-α-(2-pyridylthio) acethydrazide (1g., 0.0045 mole) was dissolved in methanol (20 ml) and hydrogenated at room temperature and atmospheric pressure, using Adam's platinum oxide catalyst (0.1g.). There was no uptake of hydrogen and on filtration and evaporating the solution to dryness starting material was recovered.

Method II.

N-Benzylidene-α-(2-pyridylthio) acethydrazide (1g., 0.004 mole) was dissolved in N methanolic

hydrochloric acid (20 ml) and hydrogenated under pressure, using Adam's platinum oxide catalyst (0.1g.). On filtering and evaporating the solution to dryness a yellow solid was obtained (m.p. 118°). A mixed melting point with 2-mercaptopyridine showed no depression.

Method III.

Finely devided iron powder (1.0g.) was added to a boiling solution of N-benzylidene-α-(2-pyridylthio) acethydrazide (1g., 0.004 mole) in ethanol (15 ml). Concentrated hydrochloric acid (10ml) was added dropwise over a period of 30 min., and the mixture refluxed for a further 1½hr., before taking down to dryness under reduced pressure. The solid was redissolved in water and neutralized with ammonia. Removal of the solvent gave a white solid (m.p. 90°). A mixed melting point with authentic α-(2-pyridylthio)acethydrazide showed no depression.

Method I.

Ethyl α-(2-pyridylthio) acetate hydrobromide (1g., 0.0035 mole) in ethanol (20 ml) was treated with sodium ethoxide (0.34g.). The sodium bromide was removed and the solution heated at 120° under reflux with unsymmetrical diisopropylhydrazine (0.48g.) for six hr. Removal of the solvent gave a viscous oil which did not crystallize.

Method II.

∝-(2-Pyridylthio) acethydrazide (1g., 0.0052 mole) in pyridine (20 ml) refluxed with benzyl chloride (0.005 mole) for six hr., gave a white solid which was not identified.

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