STUDIES IN THE CHEMISTRY OF ANEURIN

(VITAMIN B_1)

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BY

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CONTENTS

The work on the structure and synthesis of aneurin (vitamin B_1) and thiochrome described in this thesis was commenced in the University of Edinburgh on the invitation of Prof. G. Barger, F.R.S. for whose kindness and interest I am deeply grateful. Owing to the highly competitive nature of the work it was necessary to explore all possible avenues of approach to the problem as rapidly as possible, and for this reason I had the assistance of several collaborators in the experimental part of the investigations. The work of these collaborators - which was mainly concerned with model experiments and earlier stages of the syntheses - was throughout inspired by me and carried out under my direction. In addition to much of the preliminary work, all the more crucial parts, e.g. formation of thiochrome from aneurin, the syntheses of 3-pyrimidylthiazolium salts, of aneurin, and of thiochrome and its analogues, were carried out by myself. I am entirely responsible for the composition of the published papers and the thesis.

Introduction and Theoretical.

The earliest recorded investigations relating to the antineuritic vitamin (vitamin B_1) are those of the Japanese admiral Takaki (1885) who recognised that beri-beri, a widespread form of polyneuritis characterised by wasting and paralysis, was due to diet. In the course of a few years he was able to reduce the incidence of the disease in the Japanese navy from 39% to zero, through supplementing the sailors' diet of polished rice by an allowance of meat, fish and vegetables. Takaki considered that the disease was probably due to a deficiency of protein coupled with excess of carbohydrate in diet. Some years later Eijkmann (1890 et.seq.) was able to produce experimental beri-beri in fowls by feeding them on a diet of polished rice, and showed that the condition could be prevented or cured by addition of unmilled rice to the diet. At first he inclined to the view that the polishings contained some antidote to a beri-beri-producing toxin generated in the intestine by rice, but he later adopted the view of his colleague Grijns (1901) - who held that

beri-beri was a deficiency disease - and stated "that there is present in rice polishings a substance of a different nature from proteins, fats or salts which is indispensable to health, and the lack of which causes nutritional polyneuritis". As a consequence of Eijkmann's work, chicks and pigeons have been regularly employed since then in the biological assay of the antineuritic vitamin.

Considerable concentration of the antineuritic factor in rice polishings was effected by Funk (1911), who separated from this material a crystalline fraction active in a dose of about 20mg; this material, as he himself showed later, was not homogeneous. He considered the active principle to be basic in nature and found that it was associated, in his fractionation process, with purines and pyrimidines. The same worker in 1912 introduced the term "vitamine" to describe substances, such as the antineuritic factor, whose presence in a diet is essential to life, in the belief that all such substances were bases. It has since been found that several of the compounds in question are nitrogen-free, but Funk's term modified by omission of the "e",

has been retained as a class name for all substances of this type.

Hopkins (1912) who had for some years been carrying out experiments on the feeding of rats on synthetic diets, showed that normal growth could not take place unless milk were added to the diet. In 1913 Osborne and Mendel,and, independently, McCollum and Davis, as a result of a long series of experiments similar to those of Hopkins, came to the conclusion that there was a factor essential to nutrition associated with certain fats e.g. butter fat, and that without it normal growth could not be maintained. Somewhat later McCollum and Davis (1915) reported that, when fed on a synthetic diet to which butter fat was added to supply the fat soluble vitamin, young rats still required for normal growth an additional food factor. This additional factor was named "water soluble vitamin B" by McCollum and Kennedy (1916), the factor present in butter fat being called "fat soluble vitamin A." Water soluble vitamin B was considered by McCollum and his collaborators to be identical with Eijkmann's

antineuritic factor, since the distribution of both was similar and vitamin B was found to exert a preventive and curative effect in avian polyneuritis.

Much later Smith and Hendrick (1926), also working on the growth of rats, brought forward evidence which showed that water soluble vitamin B was really a complex containing at least two individual vitamins. This conclusion was confirmed and amplified by later workers and it is now recognised that water soluble vitamin B consists of a mixture of several vitamins which have been called vitamins B_1, B_2, B_3 etc. Of these vitamin B_1 is identified with the antineuritic vitamin.

Progress in the isolation of vitamin B_1 after Funk's work was for a time slow, although Suzuki, Shinamura and Ohdake (1912) obtained a partially crystalline product from rice which had a curative effect on polyneuritic pigeons in a dose of about 5mg. The discovery of Seidell (1921) that vitamin B_1 could be adsorbed on fullers' earth from aqueous solution and eluted with baryta, gave great impetus to isolation experiments, as it allowed of a comparatively rapid concentration of the vitamin. Using a method based partly on the work of Seidell,

Jansen and Donath (1926) isolated for the first time, a crystalline substance which was undoubtedly the hydrochloride of vitamin B_1 in practically pure condition; a daily dose of .002mg. of their product was sufficient to protect rice birds (Munia maia) from polyneuritis for three weeks. Curiously enough Jansen and Donath failed to detect the presence of sulphur in their product and the presence of this element as an integral part of the vitamin molecule was not demonstrated for some years (Windaus, Tschesche Ruhkopf, Laquer and Schultz 1931). The empirical formula of the vitamin hydrochloride $C_{12}H_{18}ON_4SCl_2$, proposed by Windaus, Tschesche and Ruhkopf (1932) has since been definitely established. A critical survey of the evidence for this formula is given in a paper by Wintersteiner, Williams and Ruchle (1935).

Since the isolation of the vitamin, various names have been proposed for it e.g. oryzanin, torulin, aneurin. Of these "aneurin" proposed by Jansen (1935), is perhaps the most satisfactory, and it is now in current use as an alternative to the expression "vitamin B_1".

Aneurin is widely distributed in animal and

vegetable foodstuffs, but the amount present in them is always small.

The richest sources are yeast and the pericarp and germ of cereals, but even there the concentration is seldom as high as 0.005%; natural aneurin is thus exceedingly difficult to obtain in any quantity a fact which long delayed chemical investigation. The long and complicated isolation processes are attended by heavy material losses so that from 100kg. of yeast or rice polishings the yield of vitamin is at best about 0.5g. and is usually much less.

Various biological methods may be used to ascertain the amount of aneurin present in a given material.

a) The curative or preventive action on chicks, pigeons or rats may be measured (cf. Kinnersley, Peters and Reader, 1928; 1930).

b) The amount of the material may be determined which must be added to an aneurin-free diet in order to initiate growth in rats (Chick and Roscoe 1929).

c) Advantage may be taken of the fact that the vitamin increases the oxygen-uptake of isolated

brain tissue from polyneuritic pigeons; this is the basis of the so-called "catatorulin" test (Passmore, Peters and Sinclair 1933).

d) The quickest and most convenient test is that described by Birch and Harris (1934). It depends on the fact noted by Carter and Drury (1929) that vitamin B deficiency in rats causes marked bradycardia, the pulse falling from five hundred to about 300 per minute; administration of vitamin-containing material causes the pulse to return to normal, and the duration of the effect is proportional to the amount of vitamin present. On account of the very rapid pulse, measurements are made with the aid of an electrocardiagraph. This method of testing has been used throughout the work described in this thesis.

e) The fact that fungi appear to require aneurin for growth can be used for assay purposes; yeast (Williams 1919) and more especially Phycomyces blakesleeanus (Schopfer and Jung 1935) have been used in this way.

All such tests are carried out in comparison with the International Vitamin B_1 Standard, which is a special fullers' earth adsorbate of the vitamin.

One International Unit (I.U.) corresponds in activity to 10mg. of this preparation. 1mg. crystalline aneurin hydrochloride corresponds approximately to 400 International Units in activity.

Chemical methods for the assay of aneurin concentrates have been proposed by Kinnersley and Peters (1934) using the fact that diazotised sulphanilic acid gives with the vitamin a pink colouration stabilised by formaldehyde, and by Jansen (1936) who utilises the production of the fluorescent thiochrome on oxidation of aneurin in alkaline solution with potassium ferricyanide (see p. 17). Both these methods suffer from the disadvantage that they are only reliable with highly purified vitamin concentrates.

Regarding the function of vitamin B_1 in the organism, much evidence is available from animal experiments and from clinical observations, but no final conclusion has yet been reached. Both in animals and in man, aneurin-deficiency manifests itself in three ways, namely, by nervous, cardiac, and metabolic disturbances. It would seem from most recent work that it is metabolism, and particularly carbohydrate metabolism, which is

primarily affected, and that the nervous and cardiac disturbances may be secondary symptoms. The remarkable relationship between aneurin-deficiency and disturbance of carbohydrate metabolism can be demonstrated in isolated tissues. Slices of liver, kidney, cardiac and skeletal muscle, and particularly of brain tissue, from avitaminous animals, differ from those of normal animals in having a diminished oxygen-uptake and an increased content of lactic and pyruvic acids. Addition of the vitamin restores normal respiration and causes disappearance of the accumulated intermediate products of carbohydrate breakdown.

The effect is particularly noticeable in the case of pyruvic acid the concentration of which diminishes rapidly (Peters and Thompson 1934). It seems likely that the vitamin may function as a co-enzyme in the breakdown of pyruvic acid in the living organism.

The question as to whether beri-beri is due simply to vitamin deficiency, or primarily to the action of certain toxins, may be regarded as definitely settled in favour of the deficiency hypothesis by the cures effected by administration

of the crystalline vitamin hydrochloride (Williams, Waterman and Keresztesy 1935). Pure aneurin in the form of the chloride has been applied clinically, with marked success, in the treatment of various nervous diseases; best results seem to be obtained by parenteral administration (cf. for example Ritchie Russell 1936). Sufficient work has been done to indicate that aneurin is of great clinical importance, and it will doubtless find increasing application now that the synthetic vitamin is available.

The earlier chemical work on the isolated vitamin hydrochloride by Jansen and Donath (1926) Van Veen (1930-1932), Ohdake (1932), and Windaus and his co-workers (1932) led to the establishment of the empirical formula $C_{12}H_{18}ON_4SCl_2$ and the preparation of several crystalline derivatives e.g. platinichloride, aurichloride, picrate, picrolonate, rufianate. The free base could not be isolated, and it was observed that the vitamin was very unstable to alkali, the sulphur being readily eliminated as sulphide; towards acid it was more stable, though heating with hydrochloric acid to high temperatures caused elimination of ammonia (Van Veen;

Windaus). The absorption spectrum of the vitamin was examined by various workers (cf. Wintersteiner Williams and Ruehle 1935). The results suggesting the presence of a pyrimidine nucleus in the molecule, while crystallographic measurements on the hydrochloride indicated a conjugated ring structure with the sulphur and chlorine atoms not both at the ends of the molecule (Bernal and Crowfoot 1933). That little more was done during the two years following the establishment of the empirical formula was undoubtedly due to the inaccessibility of the vitamin, which was extremely difficult to obtain in quantities large enough for structural determination by organic chemical methods. In the autumn of 1934, however, Windaus, Tschesche and Grewe (1934) by oxidising aneurin with concentrated nitric acid obtained two substances each containing five carbon atoms. The first of these, an acid $C_5H_6O_2N_2$, was isolated as its ethyl ester nitrate $C_7H_{11}O_5N_3$. It was not identical with any of the four iminazole carboxylic acids and a suggestion was made that it might be a methyl-dihydroxy-pyrimidine. The second substance, also an acid, had a formula $C_5H_5O_2NS$, and, based largely on the fact that it gave a positive zinc dust-

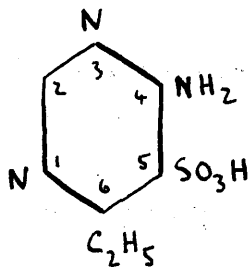
pine splinter reaction, the suggestion was made that it might be a thiopyrrole-carboxylic acid. Owing to lack of material no further work was done with these products.

Such then was the position when the work described in this thesis was commenced. A small quantity of crystalline vitamin hydrochloride was available through the kindness of Prof. B.C.P. Jansen of Amsterdam, and further supplies were extracted from rice polishings by a simplified form of the method described by Williams, Waterman and Keresztesy (1934). Initially attention was directed to the elimination of ammonia by action of acid on the vitamin. It was found that heating with concentrated hydrochloric acid at 100^{o} caused quantitative production of one molecule of ammonia. This behaviour could be shown by an amide, but the general properties of the vitamin suggested that it might well be due to the presence of an amino-group attached to a pyrimidine ring. The elimination of an $-NH_2$ group in 2:6-dihydroxy-4:5-diamino-pyrimidine under the same conditions could be demonstrated. No results of value could be obtained by alkaline hydrolysis of the vitamin. Some support

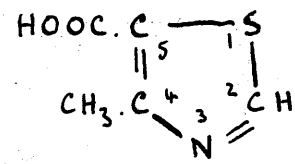
for the view that aneurin contains a quaternary nitrogen compound was obtained by treatment of the chloride with two equivalents of silver oxide and distillation of the amorphous base in a vacuum. Traces of a new blue fluorescent compound were obtained, but further investigation of the product was impossible on account of the very poor yield. The production of a blue fluorescent substance in this way was interesting, as Peters (1935) reported about the same time that aqueous solutions of the vitamin on oxidation develop a blue fluorescence visible in ultra-violet light; it was decided to study oxidation of aneurin in the hope of establishing some connection between the products obtained by this method and those from thermal decomposition.

While these preliminary experiments were in progress a great advance was made in the chemistry of aneurin by Williams (1935) who, early in 1935, announced that treatment of the vitamin at room temperature with sodium sulphite solution containing sufficient excess sulphurous acid to give pH 4-5 caused quantitative fission into two products, a sparingly soluble acid $C_6H_9O_3N_3S$ (A) and a chloroform

soluble base C_6H_9ONS (B). In a series of papers published by Williams and his collaborators during 1935, details of the work and the conclusions from it were published (cf. bibliography). Product (A) had an absorption spectrum similar to that of 4-amino-pyrimidines, which it also resembled in its other properties. Concentrated hydrochloric acid converted it with loss of 1 molecule ammonia into an acid $C_6H_8O_4NS$ having the properties of a 4-hydroxypyrimidine In product A the sulphur atom appeared to be present in a sulphonic acid grouping, presumably derived from the sulphurous acid used in the reaction leading to its formation. It was, on this evidence, provisionally allotted structure (I) by Williams.

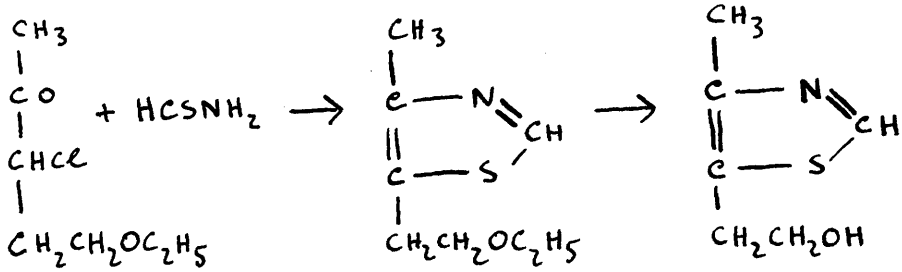


I.



II.

Product (B) gave no iodoform reaction and on treatment with methyl iodide gave a typical quaternary salt; concentrated hydrochloric acid converted it into a base C_6H_8NSCl in which the chlorine was non-ionic. Moreover, oxidation of (B) with nitric acid gave an acid $C_5H_5O_2NS$ identical with the acid of Windaus Tschesche and Grewe (see p.11) and which was shown to be 4-methyl-thiazole-5-carboxylic acid (II), a known substance. For (B) the structure of a 4-methyl-5-β-hydroxyethyl-thiazole (III) was proposed and this was confirmed by its synthesis by Clarke and Gurin (1935); their synthesis from methyl α-chloro-γ-ethoxypropyl ketone and thioformamide took the following route:

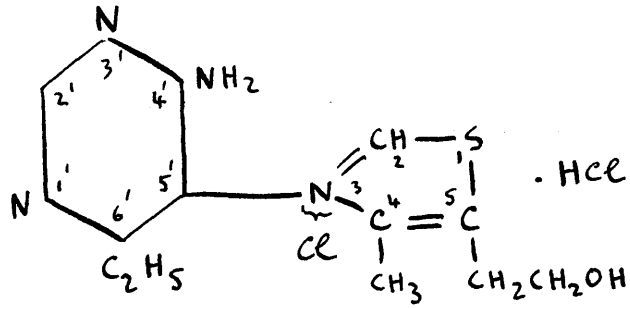


III

Potentiometric titration of the vitamin by Williams and Ruehle (1935) showed that it contained

two basic groups one of which was of the same order of strength as the nitrogen in the quaternary salt of (III) and in 4-methylthiazole ethiodide; also, that both the vitamin and the methiodide of (III) form pseudo-bases in alkaline solution, a behaviour found to be characteristic of quaternary thiazolium salts containing a free 2-position. Similar potentiometric titration curves were obtained by Moggridge and Ogston (1935) and by Birch and Harris (1935), although these workers interpreted their results somewhat differently.

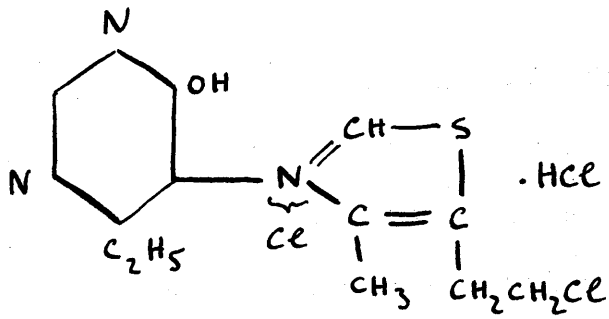
Williams (1935) on the basis of the results described proposed for the vitamin the structure (IV)



IV

The quaternary nature of one nitrogen atom accounts also for the occurrence of the sulphite cleavage, (although an exact analogy is not known) and for the thermal decomposition of the free vitamin base. This formula accounted for most of the known

facts and was generally accepted as a basis for further work. Buchman and Williams (1935) isolated in crystalline form the physiologically inert "chloro-oxyvitamin" produced by heating the vitamin with hydrochloric acid; it had a formula $C_{12}H_{16}ON_3SCl_3$ and as a result of the observations already mentioned regarding the behaviour of the products of sulphite cleavage when similarly treated, it was assigned formula (V)



V

Kuhn, Wagner-Jauregg, van Klaveren and Vetter (1935) isolated from yeast a yellow pigment which showed in neutral or alkaline solution an intense blue fluorescence; for it they established the formula $C_{12}H_{14}ON_4S$ and proposed the name thiochrome. It was suggested by them that it might be a "dehydro-vitamin B_1", since it occurred in yeast, a relatively rich source of vitamin B_1, and its formula was similar to that of the vitamin ($C_{12}H_{18}ON_4SCl_2$). This seemed all the more probable since bluefluorescent degradation products of aneurin had already been reported (cf. p 12).

On the basis of formula (IV) for aneurin, its pseudo-base should contain the grouping $\rangle N-\overset{|}{C}HOH$, and, in this form, it should, by analogy with pyridine derivatives, undergo oxidation by potassium ferricyanide. It was found that by oxidation of the vitamin with potassium ferricyanide in alkaline solution at 15-20° a crystalline substance $C_{12}H_{14}ON_4S$ was produced which was identical in all its properties with thiochrome isolated from yeast; this was confirmed by a direct comparison of the two substances kindly carried out by Prof. R. Kuhn to whom my thanks are due.

For the preparation of thiochrome it is not essential to use pure crystalline vitamin - partially purified concentrates from rice polishings can be utilised, although the yields are considerably less than might be expected on the basis of the vitamin content measured by biological methods. The production of thiochrome can be used as a qualitative test for the presence of aneurin, although, as is also the case with the formaldehyde-azo test, its use as a quantitative method is only possible with highly purified concentrates. Other oxidising agents e.g. hydrogen peroxide, selenium dioxide, potassium permanganate also convert aneurin into thiochrome but the yields are poor. Solutions similar in properties to those of thiochrome are even produced by the action of air or oxygen on solutions of the free vitamin base; this may possibly explain the occurrence of thiochrome in yeast.

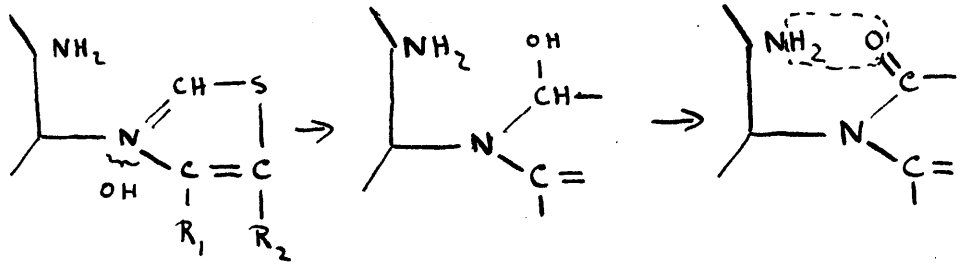
On the assumption that the aneurin molecule contains a quaternary nitrogen atom, then the free base will have the formula $C_{12}H_{18}O_2N_4S$ and therefore thiochrome cannot be a simple dehydrogenation product, its formula differing from that of the vitamin by

$H_2O + H_2$. In accordance with this thiochrome is biologically inactive and it cannot be catalytically hydrogenated to aneurin. Unlike the vitamin, thiochrome can sublime unchanged in a high vacuum at 210-215°. This, together with its solubility in organic solvents, suggests that it no longer contains a quaternary nitrogen atom. Its hydrochloride has the unusual property of separating from solution in colourless crystals, which on filtration and removal of the last traces of solvent gradually develop a yellow colour. It is difficult to give a satisfactory explanation for this change; it is not caused by hydration as drying in a high vacuum does not cause disappearance of the colour. The colouration may have some relation to the fact that the analytical values for thiochrome hydrochloride lie between one and two chlorine atoms i.e. a partial loss of hydrogen chloride seems to occur on standing in air, indicating the presence in the thiochrome molecule of one very feebly basic grouping as well as one of more normal strength.

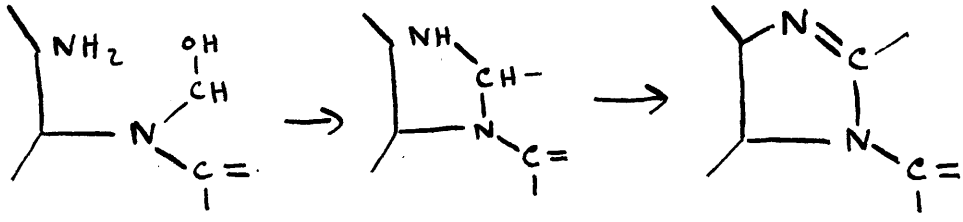
The known facts pointed to the presence in aneurin of a pyrimidine ring bearing an amino group in position 4 which could be quantitatively removed

as ammonia by heating with strong hydrochloric acid. Under similar conditions thiochrome yields no ammonia being indeed, for the most part recovered unchanged. Furthermore, the de-aminated vitamin obtained in solution (and presumably identical with the "chloro-oxyvitamin" of Buchman and Williams) gave no fluorescent product on oxidation with potassium ferricyanide. It follows that the pyrimidine amino group is involved in the production of thiochrome. The blue fluorescence of thiochrome is similar to that of 6:7-dimethylalloxazine. It therefore seemed at first possible that thiochrome might be a pyrimidazine derivative especially as the vitamin formula (IV) is that of a derivative of an σ-diaminopyrimidine. However, closer examination shows the difficulty of formulating the production of a six membered p-diazine ring by potassium ferricyanide oxidation; for this to occur the methyl group in position 4 of the thiazole would need to take part in the ring closure, and this is rather unlikely. By analogy with pyridine derivatives a more likely hypothesis seemed that the vitamin is oxidised in the form of its pseudo-base and that the keto-group so formed condenses with the free amino group. This

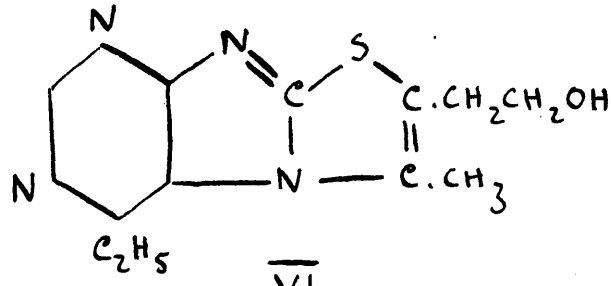
is illustrated by the following scheme.



Another possible mechanism of a similar type was suggested by Kuhn and Vetter (1935); according to it ring closure is considered to occur by intramolecular loss of water at the pseudo-base stage and the resulting compound is oxidised.



On this basis, assuming the Williams formula (IV) for aneurin to be correct thiochrome would be represented by (VI).



VI

Shortly after the completion of this piece of work Kuhn and Vetter (1935) also obtained thiochrome from aneurin by oxidation with porphyrexide and proposed for it a similar structure. Windaus Tschesche and Grewe (1935) also proposed a similar structure, but both these groups of workers preferred a formula in which the pyrimidine part of the molecule in aneurin and thiochrome bore methyl groups in positions 2 and 6 rather than an ethyl group in position 6. Windaus and his co-workers based their view on hypothetical strcutures assigned to nitric acid or barium permanganate oxidation products of aneurin, while Kuhn's claims rested on the result of C-methyl estimations.

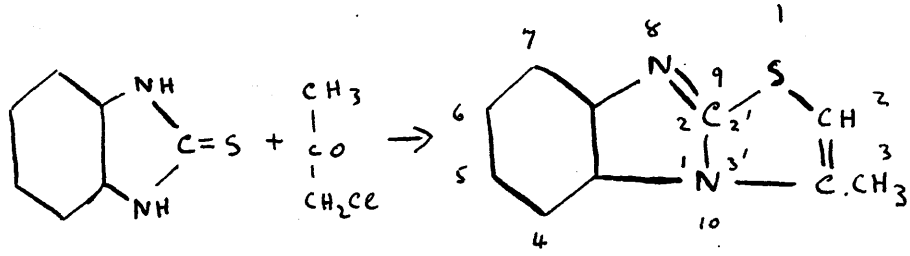
As it was important before going further to settle the question as to the number of alkyl groups in aneurin and thiochrome, a series of C-alkyl determinations was made on both these compounds and on a number of substances of known constitution. The results are shown in the following table.

Name of Compound	Average number of equivalents of volatile acid produced (as acetic acid)
N-Phenyl-2:4-dimethyl thiazolium iodide	1.31
5-Methyl-uracil	0.77
Pyrimidine sulphonic acid from aneurin (Williams)	0.62
3-Methyl-(thiazolo $2^1:3^1$:2:1-benziminazole)	1.0
Thiochrome	1.6
Aneurin (hydrochloride)	1.35

Bearing in mind the fact that each C-alkyl group (ethyl as well as methyl) on oxidation with chromic acid will give rise to an amount of acetic acid varying from 0.6 to 1 equivalent it was deduced from the above table that (1) Williams pyrimidine fragment of the sulphite cleavage contains one alkyl group i.e. an ethyl group; (2) thiochrome and aneurin both contain the same number of alkyl groups i.e. two (3) the higher value for thiochrome as compared with aneurin was reconciled by the fact that in methyl-thiazolobenziminazole (see below) the alkyl group was removed quantitatively; aneurin, on the other hand, gave, like the synthetic thiazolium salt, a

low yield of acetic acid.

It seemed, therefore, that the formulation of aneurin and thiochrome with an ethyl group was preferable. The compound 3-methyl-(thiazolo-2^1:3^1: 2:1-benziminazole) (VII) was prepared as a model for a possible thiochrome synthesis. It was readily obtained by condensation of chloro-acetone with thio-benziminazoline; viewed in ultra-violet light, alcoholic solutions of the product showed feeble blue fluorescence.



VII

The production of a number of fluorescent "quinochromes" similar to thiochrome, on oxidation of aneurin with potassium permanganate, was reported by Kinnersley O'Brien andPeters (1935b) but as none of these products was isolated in a pure state, definite evidence of their existence as individual chemical compounds is lacking. The only further degradative work carried out up to the end of 1935

was done by Windaus, Tschesche and Grewe (1935) who oxidised aneurin with barium permanganate and obtained a base which they suggested might be 2:6-dimethyl 4:5-diamino-pyrimidine.

As a result of the work on thiochrome the position of the original Williams aneurin formula (IV) was strengthened and owing to the difficulty of obtaining aneurin from natural sources it seemed likely that further progress would be best made in the synthetic field. Accordingly experiments were commenced in the direction of synthesis of aneurin and thiochrome.

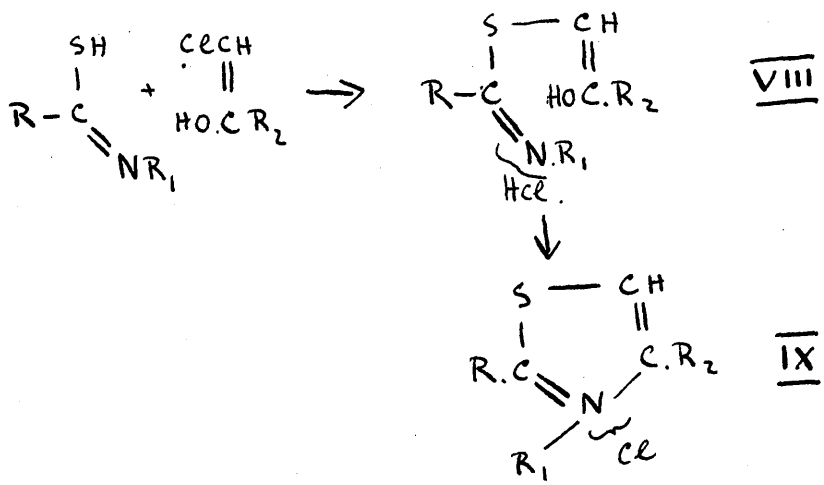
Aneurin according to the Williams formula (IV) was to be regarded as a 3-pyrimidylthiazolium salt, and, before attempting the synthesis of such a structure, it seemed desirable to work out methods for preparing 3-arylthiazolium salts, since such methods could then be applied to the more valuable pyrimidine compounds.

3-Alkylthiazolium salts, including the benzyl compounds, can readily be prepared by the direct addition of alkylhalides to thiazole derivatives.

Despite many efforts under varying conditions

however, it was found impossible to obtain the corresponding 3-aryl compounds from halogenated benzene derivatives. Similar negative results were obtained with pyrimidine derivatives bearing a halogen in position 5.

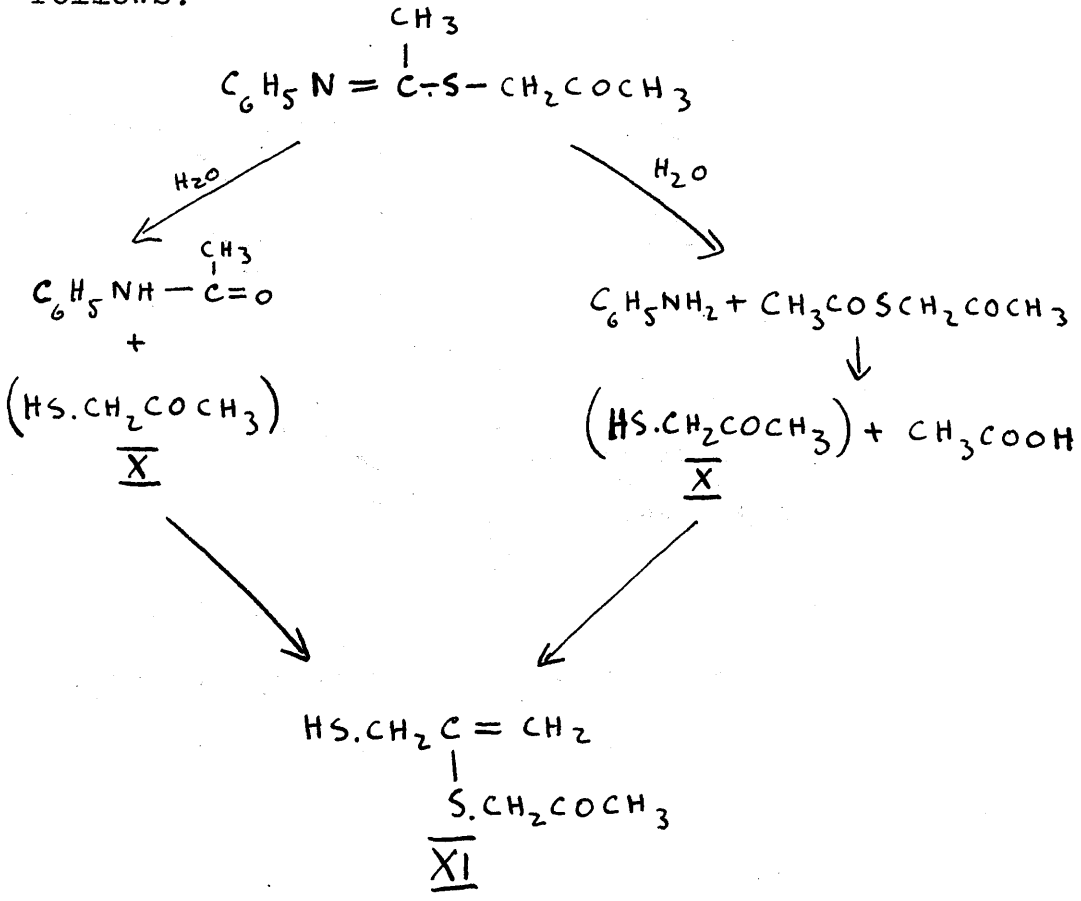
An attempt was now made to modify the well known Hantzsch synthesis of thiazoles in such a way as to lead to the production of thiazolium salts. It seemed possible that the replacement of unsubstituted thio-amides in the Hantzsch synthesis by thioamides of the general formula $R.CS.NHR_1$ might lead to the direct formation of thiazolium salts according to the scheme:



This expectation was realised and the reaction was found to be of general application. A similar result was obtained independently by Clarke and Gurin (1935) in America at about the same time.

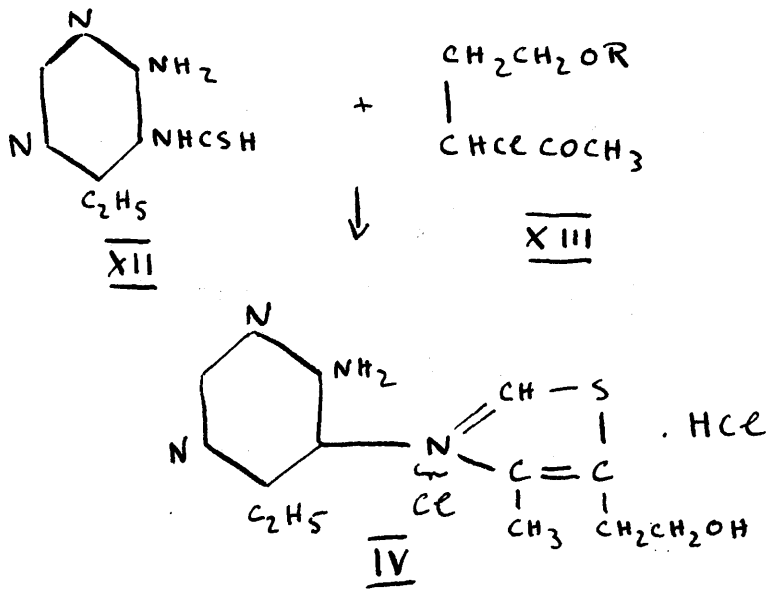
In a preliminary experiment N-methyl thioacetamide was found to react even at room temperature with chloro-acetone producing 3-methyl-2:4-dimethylthiazolium chloride (IX; R = R_1=R_2=CH_3) in quantitative yield. When thioacetanilide was heated on the water-bath with chloro-acetone either alone or in absolute alcoholic solution 3-phenyl-2:4-dimethylthiazolium chloride (IX; R = R_2=CH_3; R_1=C_6H_5) was similarly produced. If, however, the substances were allowed to react at room temperature an unstable crystalline substance was obtained which could be converted into the expected thiazolium salt by heating for a short time. The conclusion that this compound was the hydrochloride of S-acetonyl-thioacetanilide (VIII; R = R_2=CH_3; R_1=C_6H_5) was verified by analysis and by its behaviour on hydrolysis. Dissolved in water or dilute mineral acids the substance underwent hydrolysis slowly in the cold and more rapidly on warming yielding aniline, acetanilide and a sulphur containing compound which is presumably derived from a hypothetical thiol-acetone (X). As this sulphur compound forms a semicarbazone, gives a colourless precipitate with mercuric chloride and answers the tests for -SH groups, it may possibly

have structure (XI). The production of both aniline and acetanilide in the hydrolysis is interesting as it suggests that fission occurs not only between N and C but also between C and S, as follows:



Condensation of thioacet-σ-toluidide and σ-nitro-thioacetanilide with chloracetone gave in similar fashion 3-σ-tolyl-and 3-σ-nitro-2:4-dimethylthiazolium chlorides; in both cases the corresponding intermediate products could be isolated.

As a result of this work on 3-arylthiazolium salts it seemed likely that synthesis of the supposed aneurin (IV) could be effected by condensation of 4-amino-5-thioformamido-6-ethylpyrimidine (XII) with a suitable α-halogenated ketone (XIII. R = H)



For convenience the syntheses of the intermediate compounds (XII) and (XIII) and their subsequent condensation will be dealt with in turn.

In their synthesis of 4-methyl-5-β-hydroxyethyl thiazole (cf. p. 14) Clarke and Gurin condensed methyl α-chloro-γ-ethoxypropyl ketone (XIII; R=C_2H_5) with thioformamide and subsequently de-alkylated the product by heating to a high temperature with strong hydrochloric acid. Since such treatment is known to de-aminate aneurin, the above mentioned ethoxy-ketone may be regarded as useless in a vitamin

synthesis. Accordingly methyl α-chloro-γ-hydroxypropyl ketone (XIII; R = H) was synthesised according to the scheme:

$$CH_3COOCH_2CH_2Br + CH_3COCHNaCOOC_2H_5$$

$$\downarrow$$

$$CH_3COOCH_2CH_2CH(COOC_2H_5)COCH_3$$

$$\downarrow SO_2Cl_2$$

$$CH_3COOCH_2CH_2CCl(COOC_2H_5)COCH_3$$

$$\downarrow \text{hydrolysis}$$

$$HOCH_2CH_2CHClCOCH_3$$

In this synthesis the initial step, namely, condensation of β-bromoethyl acetate with ethyl sodioacetoacetate, is rather unsatisfactory; the method described in the literature by Haller and March (1905) gives very poor results and the use of benzene as a diluent although leading to an improvement, still gives rather low yields. For this reason the more recent synthesis of Buchman (1936) which starts from ethylene oxide and ethyl

sodioacetoacetate is preferable for general work.

Methyl α-chloro-γ-hydroxypropyl ketone condensed readily with thioformamide to give 4-methyl-5-β-hydroxy-ethyl thiazole whose picrate gave no depression in m.p. when mixed with a specimen (m.p. 162°) prepared from aneurin by sulphite cleavage.

Efforts were also made to prepare methyl α-halogeno-γ-phenoxypropyl ketones in the hope that the phenoxyethyl thiazoles resulting from their condensation with thioamides would yield the corresponding hydroxy compounds under relatively mild conditions. At first, direct halogenation of methyl γ-phenoxypropyl ketone (Boyd Barrett and Robinson 1932) was tried under a variety of conditions but no homogeneous product could be isolated. The synthesis of methyl α-chloro-γ-phenoxypropyl ketone (XIII; R = C_6H_5) was, however, effected by a method analogous to that employed for the corresponding hydroxy compound (XIII; R = H); the substance could not be purified completely by distillation, but it condensed with thioamides readily to yield phenoxyethyl thiazoles. Further work with phenoxy-compounds was discontinued as it was found impossible to replace the phenoxy-group by hydroxyl

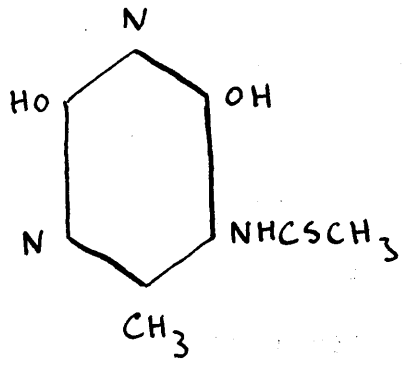
save under conditions which destroy aneurin.

Before attempting the synthesis of 4-amino-5-thioformamido-6-ethylpyrimidine (XII) a series of model experiments was made with more accessible pyrimidine derivatives. It was known that an amino group in position 5 of the pyrimidine nucleus is unique in that it is readily acetylated; amino groups in other positions are not. Thus formylation of a 4:5-diaminopyrimidine yields 4-amino-5-formamidopyrimidine and not the diformyl derivative (Gabriel and Colman 1901; Johns 1908).

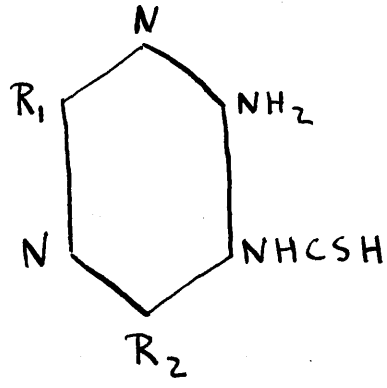
Thioacylamidopyrimidines had not been described in the literature, and it was found impossible to prepare them by the action of phosphorus pentasulphide on the corresponding acyl derivatives. A similar lack of success was encountered on attempting to replace the 5 amino-group by an isonitrile group with a view to subsequent addition of hydrogen sulphide according to Hofmann (1878).

Thioacetic acid reacts readily with primary amines to give the corresponding acetyl derivatives (Pawlewski 1898, 1902); thioacylation was therefore attempted by heating amines with thiol-thionic acids (RCSSH). With dithioacetic acid this was completely

successful. 5-thioacetamido-6-methyluracil (XIV) being readily obtained from 5-amino-6-methyluracil.



XIV



XV

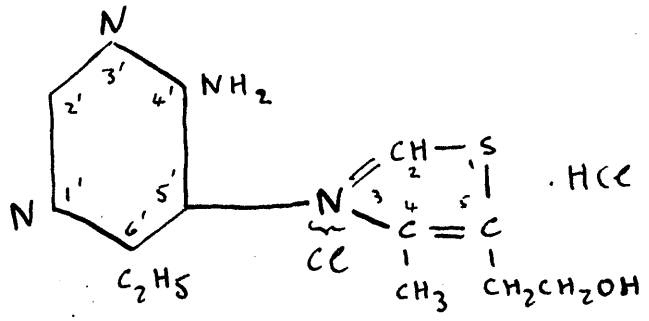
Dithioformic acid (HCSSH) acted in a similar way, but the thioformyl-derivative was obtained in poor yield and was difficult to purify. It was found, however, that thioformylation could be readily effected by mixing aqueous solutions of 5-amino-pyrimidines and potassium dithioformate; at room temperature in an atmosphere of carbon dioxide the thioformyl derivatives normally separate in almost pure condition, the yield being nearly quantitative. Amino groups in positions 2, 4 and 6 of the pyrimidine nucleus did not react under these conditions. The ease with which thioformylation occurred in the case

of 5-aminopyrimidines has led to the application of the method to all classes of amines (Todd Bergel Karimullah and Keller 1937); as a result, it may be that thioformyl derivatives will play an important part in the separation and identification of amines in general.

Using potassium dithioformate and 4:5-diamino-6-ethyl pyrimidine, 4-amino-5-thioformamido-6-ethyl-pyrimidine (XV; R_1=H; R_2= C_2H_5) necesaary for the projected vitamin synthesis was obtained. In similar fashion 4-amino-5-thioformamido-6-methyl-pyrimidine (XV; R_1=H, R_2=CH_3) and 2:4-diamino-5-thioformamido-6-methylpyrimidine (XV; R_1=NH_2; R_2=CH_3). All three are crystalline compounds which evolve hydrogen sulphide above the melting point and yield the corresponding purines. On heating with chloroacetone they yield the corresponding 3-pyrimidylthiazolium salts.

In course of these syntheses a considerable number of aminopyrimidines were prepared; most of these are known compounds, but 2-amino-4-hydroxy-6-ethylpyrimidine and 2:4-diamino-6-ethylpyrimidine had not hitherto been described. Neither could be thioformylated with potassium dithioformate.

As wasmentioned above 3-pyrimidyl-thiazolium salts can be synthesised by heating 5-thioformamido-pyrimidines with chloroacetone. This simple method cannot, however, be applied when chloroacetone is replaced by methyl α-chloro-γ-hydroxypropyl ketone owing to the low reactivity of the latter substance. The difficulty can be surmounted by using, in place of the free thioformamido-compound, its sodium salt. This condenses readily in absolute-alcoholic solution with α-halogenated ketones and the product, treated with hydrogen chloride, yields the desired 3-pyrimidyl-thiazolium salt. In this way the sodium salt of 4-amino-5-thioformamido-6-ethylpyrimidine (p. 34), condensed with methyl α-chloro-γ-hydroxy-propyl ketone (p. 30), yielding 3-(4^1-amino-6^1-ethyl-pyrimidyl-5^1)-4-methyl-5-β-hydroxyethyl-thiazolium chloride hydrochloride (IV).



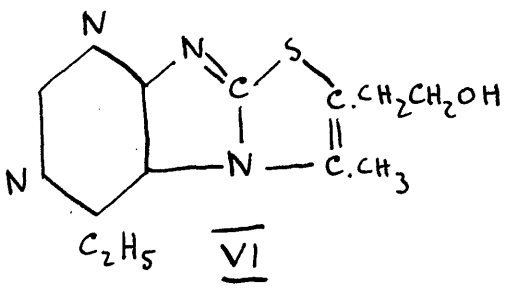
IV

According to the original suggestion of Williams

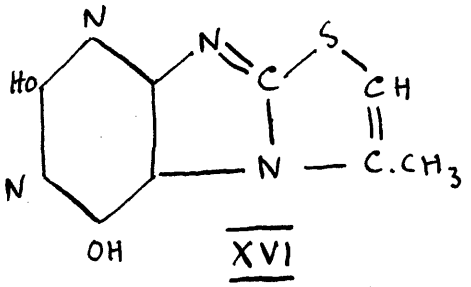
(1935) this compound should have been identical with the hydrochloride of aneurin. This was not the case; in appearance and general solubilities the synthetic substance resembled the natural vitamin hydrochloride, but it melted much lower (220° as compared with 250°) and when tested on rats by the electrocardiagraphic method of Birch and Harris (1934) it showed no measurable physiological activity. Several other synthetic 3-pyrimidylthiazolium salts described in the experimental section were tested biologically with similar negative results, and none of them underwent fission with sodium sulphite in acid solution. The formaldehyde-azo test is given by (IV) as well as by the vitamin. A positive result in this test seems to depend in some way on the presence of a β-hydroxyethyl group in position 5 and a hydrogen atom in position 2 of the thiazole nucleus. Thus 3-pyrimidyl thiazolium salts without the hydroxyethyl group, the oxychlorovitamin of Buchman and Williams (1935) and thiochrome all give negative results.

Any possibility that the vitamin might be represented by a closely related structure bearing two methyl groups instead of one ethyl group in the pyrimidine could be excluded on the following grounds.

When synthetic 3-pyrimidylthiazolium salts containing an amino-group in position 6[1] are oxidised with potassium ferricyanide under the conditions used for preparing thiochrome from aneurin, they yield solutions which, though blue-fluorescent in ultra-violet light, show no fluorescence whatever in visible light, in which thiochrome solutions fluoresce strongly. Evidence pointing in the same direction was obtained in experiments carried out with a view to synthesising thiochrome for which on the basis of structure (IV) for aneurin the formula (VI) had been proposed (p. 21).



VI

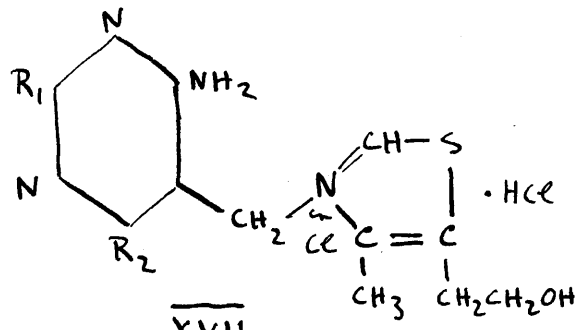


XVI

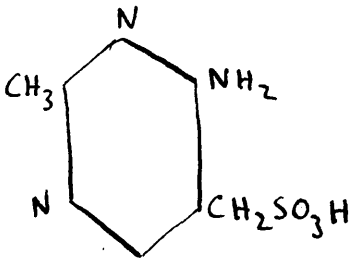
By analogy with methyl-thiazobenziminazole (p. 24) thiazolopurines of type (VI) should be capable of synthesis from 8-thiopurines and α-halogenated ketones. By this means the substance (XVI was prepared from 2:6-dihydroxy-8-thiopurine (Fischer 1898) and chloroacetone; the compound had a feeble, though distinct, fluoreseence in

ultra-violet light but none in visible light. In continuation 8-thio-6-methylpurine (Gabriel 1901) and 8-thio-6-ethylpurine (prepared in similar fashion from 4:5-diamino-6-ethylpyrimidine) were condensed with chloroacetone and with methyl α-chloro-γ-hydroxypropyl ketone; the products were not completely purified, but in neutral or alkaline solution they showed feeble blue fluorescence only when viewed in ultra-violet light. The non-fluorescence of thiazolopurines in visible light has also been noted independently by Ochiai (1936). The conclusion that thiochrome is not a thiazolo-purine is inevitable, and on the evidence of these synthetic experiments it is clear that the vitamin hydrochloride is not a 3-pyrimidyl-thiazolium salt.

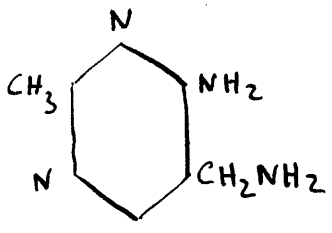
If the aneurin molecule contains a pyrimidine nucleus then the only possible structures for it are (XVII; R_1=H; R_2=CH_3) or the closely related (XVII; R_1=CH_3; R_2=H). The former structure was indeed proposed on purely theoretical grounds by Makino and Imai (1936). A distinction between the two alternatives is impossible on the synthetic evidence described above.



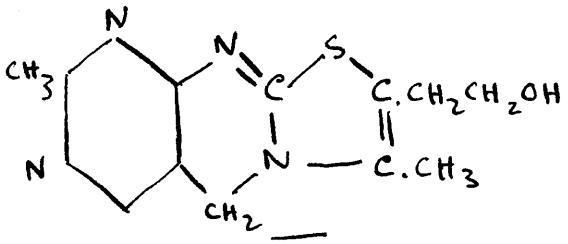
XVII



XVIII



XIX

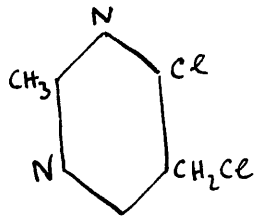


XX

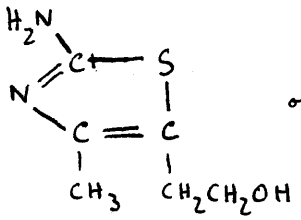
Simultaneously with the completion of the portion of the work described Williams (1936) announced that he had proved by synthesis the structure (XVIII) for the acidic product of the sulphite cleavage; details of the synthesis have only recently been published (Cline, Williams, Ruehle and Waterman 1937). Consequently aneurin must be represented by (XVII; R_1=CH_3; R_2=H). A synthesis of (XVIII) was also reported later (during the course of the work described below) by Grewe (1936); in the same paper he also described a synthesis of (XIX) the barium permanganate oxidation product of aneurin, and mentioned that aneurin itself had been synthesised by Andersag and Westphal in the Elberfeld laboratories

of the I.G. Farbenindustrie A.G. and had been shown to have structure (XVII; R_1=CH_3; R_2=H).

On the basis of the vitamin formula (XVII; R_1=CH_3; R_2=H), thiochrome should have the structure (XX) and attention was now directed to its synthesis. It seemed likely that compounds similar to (XX) might be synthesised by condensing 4-chloro-5-chloromethylpyrimidines with 2-aminothiazoles; thiochrome itself would be synthesised in this way from 4-chloro-5-chloromethyl-2 methyl-pyrimidine (XXI) and 2-amino-4-methyl-5-β-hydroxy-ethylthiazole (XXIIa or XXIIb).

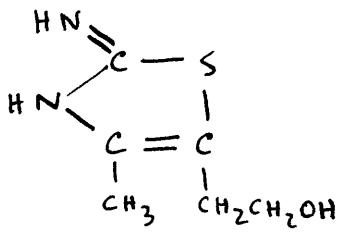


XXI

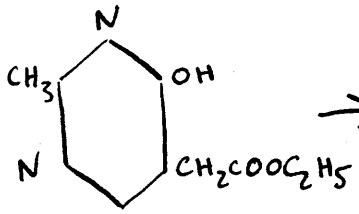


XXII a

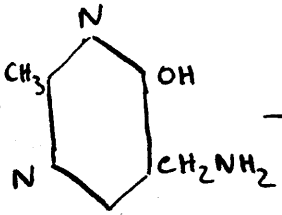
or



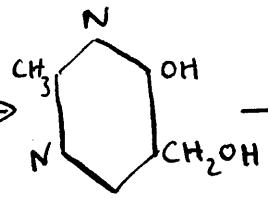
XXII b.



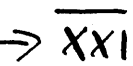
XXIII



XXIV



XXV



XXI

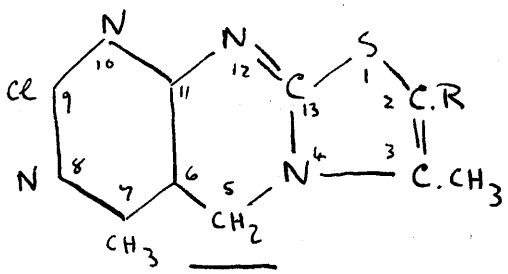
For the synthesis of (XXI) various methods seemed possible; the following was adopted as the earlier intermediates were necessary for attempts to synthesise aneurin that were being commenced at the same time. Condensation of acetamidine with ethyl formylsuccinate yielded ethyl 4-hydroxy-2-methylpyrimidine-5-acetate (XXIII), from which, by Curtins degradation, 4-hydroxy-5-aminomethyl-2-methyl-pyrimidine (XXIV) was obtained in good yield. Various methods of carrying out this degradation were tried, the most successful being direct conversion of the hydrazide to the urethane (cf. Jackson and Kenner 1928) and subsequent hydrolysis with concentrated hydrochloric or hydrobromic acid. The amine (XXIV) was characterised as its hydrochloride thioformyl, and acetyl derivatives. Replacement of the amino-group by hydroxyl was effected by nitrous acid, and the resulting 4-hydroxy-5-hydroxymethyl-2-methylpyrimidine (XXV), on boiling with phosphoryl chloride, yielded the required chloro-compound (XXI). 2-Amino-4-methyl-5-β-hydroxyethyl-thiazole (XXII) was obtained by condensing methyl

α-chloro-γ-hydroxypropyl ketone with thiourea and characterised as its picrate.

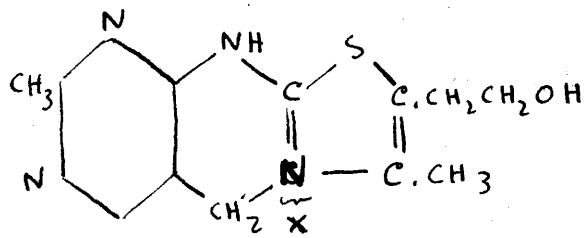
When a mixture of (XXI) and (XXII) was heated at 110° for a short time, reaction occurred with the formation of a thick resin, which was in the main soluble in water; the aqueous solution, when made alkaline, deposited a considerable amount of an insoluble substance, presumably formed by interaction of the chloromethyl group of the pyrimidine with the 2-amino-group of the thiazole. The filtered alkaline solution had the intense blue fluorescence characteristic of thiochrome solutions; from it was isolated a yellow crystalline substance having all the properties of thiochrome prepared from aneurin. The identity of the synthetic material was established by careful comparison with a specimen of thiochrome prepared from the vitamin; both had the same melting point, the mixed melting point showed no depression, and no divergences in other properties could be detected. Thiochrome therefore has structure (XX). For the ring system present in thiochrome the name thiochromine is proposed; thiochrome itself is then 3:9-dimethyl-2-β-hydroxyethylthiochromine.

In a similar fashion 9-chloro-3:7-dimethyl-

-thiochromine (XXVI; R=H) and 9-chloro-3:7-dimethyl-2-β-hydroxyethylthiochromine (XXVI; R=CH_2CH_2OH) were prepared by condensation of 2:4-dichloro-5-chloromethyl-6-methylpyrimidine with 2-amino-4-methylthiazole and 2-amino-4-methyl-5-β-hydroxethylthiazole respectively; both these compounds are similar in properties to thiochrome, and exhibit almost identical blue fluorescence in neutral or alkaline solution.



XXVI



XXVII

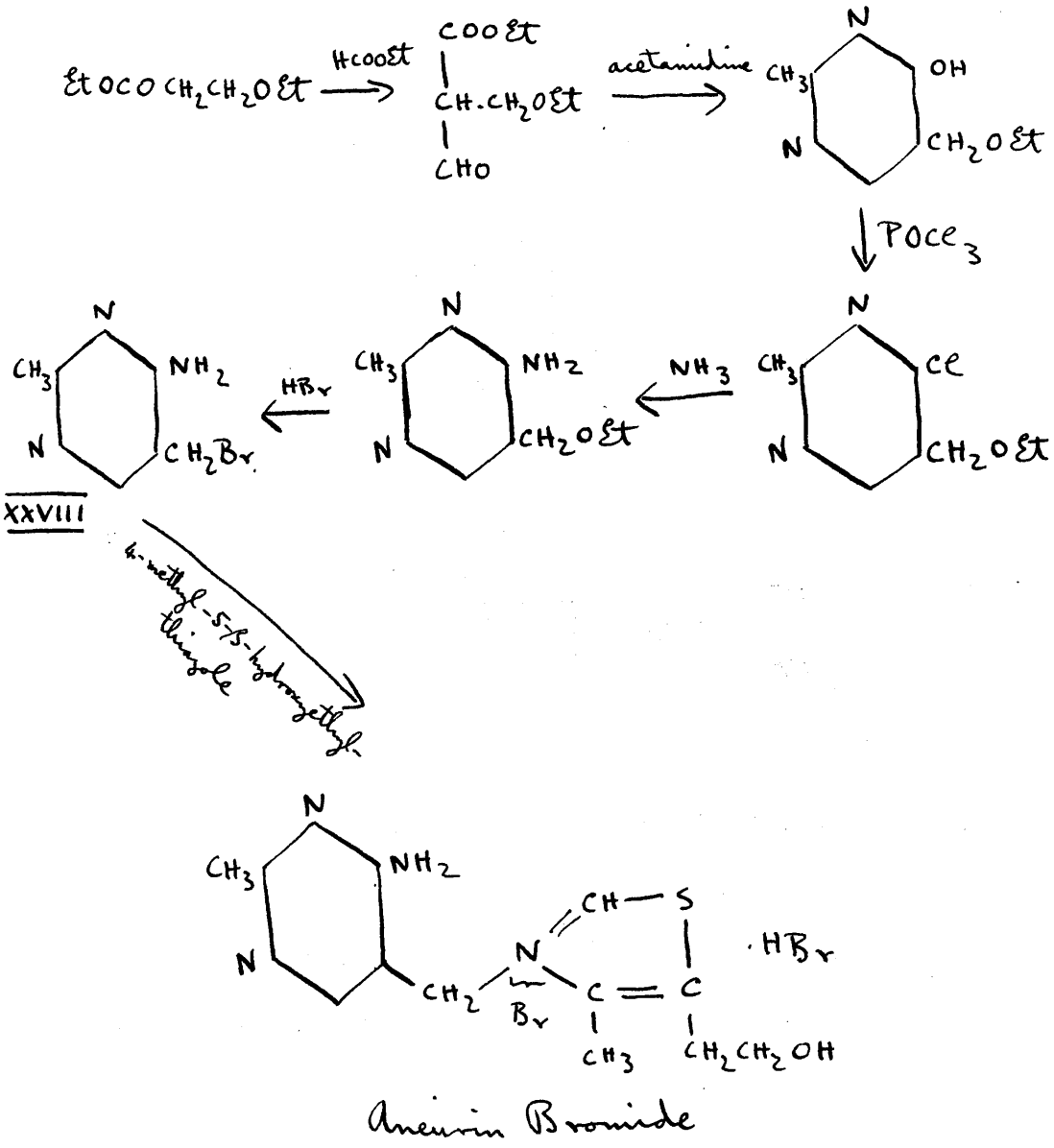
That salts of thiochrome on electrometric titration behave as if they contained a quaternary nitrogen atom has been reported by Ogston and Peters (1936), although thiochrome itself has the properties of a tertiary base (p. 19). A possible explanation

for this anomaly is that thiochrome may be in reality an anhydro-base and that its salts may have structure (XXVII), it being assumed that the quaternary base liberated from such salts is very unstable and passes at once into the anhydro-form by loss of water.

Although the synthetic method employed in the synthesis of thiochrome leaves no doubt as to the structure of the final product, it is impossible to say whether the 2-amino-thiazole reacts as such (XXIIa) or in the tautomeric 2-imino-thiazoline form (XXIIb). The synthesis may also be regarded as indirect proof of the structure of aneurin (XVII; R_1=CH_3; R_2=H).

Simultaneously with the work just described on the synthesis of thiochrome experiments were set afoot with a view to the complete synthesis of aneurin so as to establish its structure conclusively. In August 1936 however, just when the thiochrome synthesis was complete Williams and Cline (1936) in a letter to the Editor of the Journal of the American Chemical Society announced that they had succeeded in synthesising aneurin.

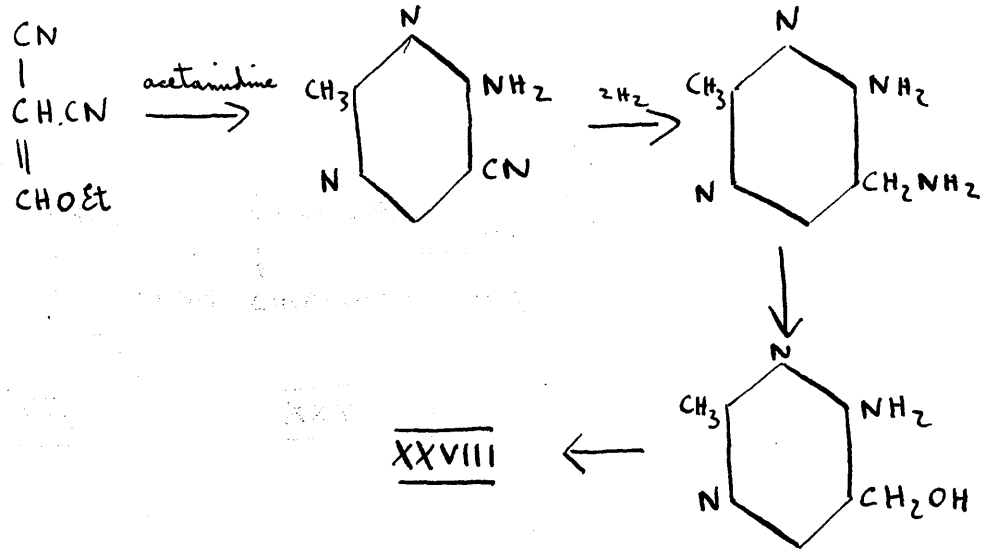
They indicated schematically the course of their synthesis but no details were published. The following scheme is reproduced from their letter:



Aneurin Bromide

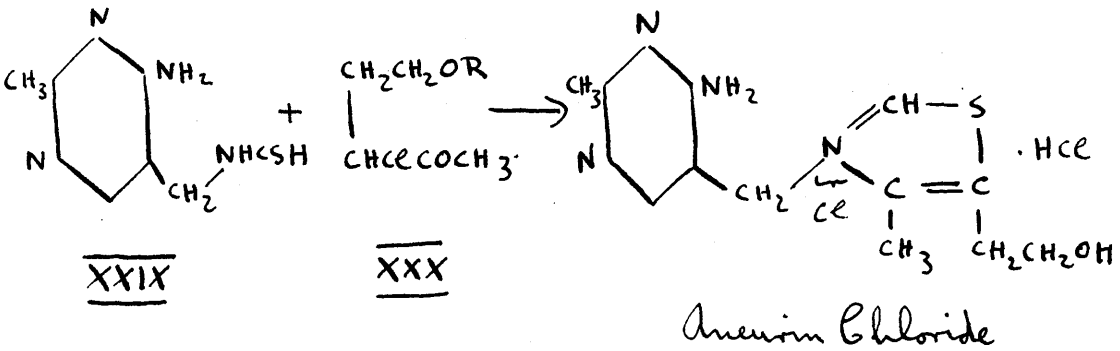
At about the same time Grewe (1936) mentioned in a publication that the I.G. Farbenindustrie A.G. had synthesised the vitamin. Few details of this

synthesis are available (cf. Engl.Pat.456, 735. Centr., 1937, 1, 2818) but it may be deduced from Grewe's paper that it differs from that of Williams and Cline only in the fact that the following route (devised by Grewe) is used for the synthesis of the necessary intermediate (XXVIII).



It was decided to continue the work already commenced on the synthesis of aneurin for several reasons. In the first place the projected synthesis depended on an extension of the synthetic method worked out for 3-pyrimidyl-thiazolium salts and was thus distinct from either of those mentioned above; furthermore the great importance of aneurin in clinical medicine made it desirable that the synthetic product should be made readily available.

The synthesis of aneurin to be described was based on a series of investigations in which many avenues of approach were tried. The method adopted depends on the condensation of 4-amino-5-thioformamido-methyl-2-methylpyrimidine (XXIX) with methyl α-chloro-γ-hydroxypropylketone (XXX; R=H) or one of its derivatives.



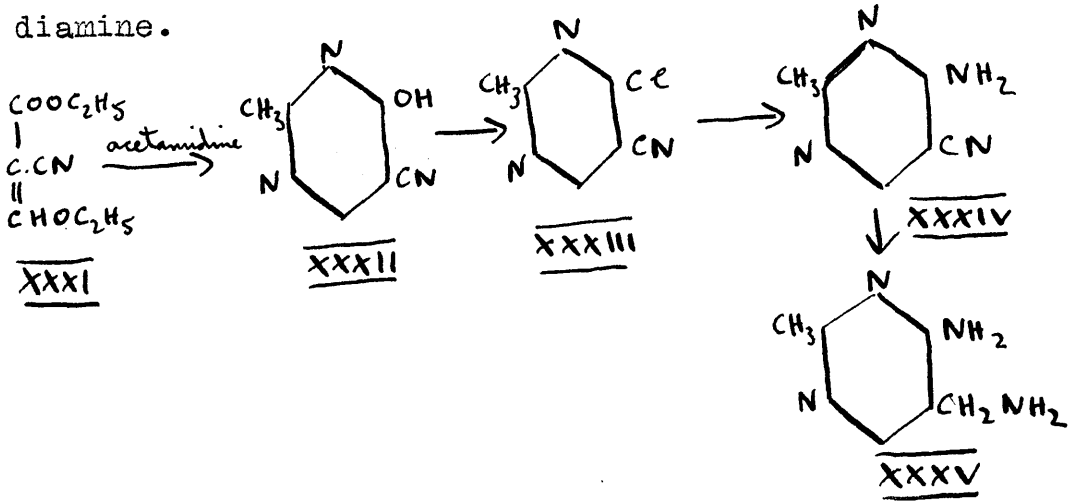
XXIX XXX

Aneurin Chloride

Efforts were at first made to utilise ethyl 4-hydroxy-2-methylpyrimidine-5-acetate (XXIII. p. 40), but all attempts to modify the Curtius or Hofmann degradation with this ester or its derivatives so as to produce the desired 4-amino-5-aminomethyl-2-methylpyrimidine failed, the low resistance of the amino group in position 4 to hydrolytic agents invariably causing production of 4-hydroxy-5-aminomethyl-2-methylpyrimidine; the latter compound, too, could not be chlorinated. On

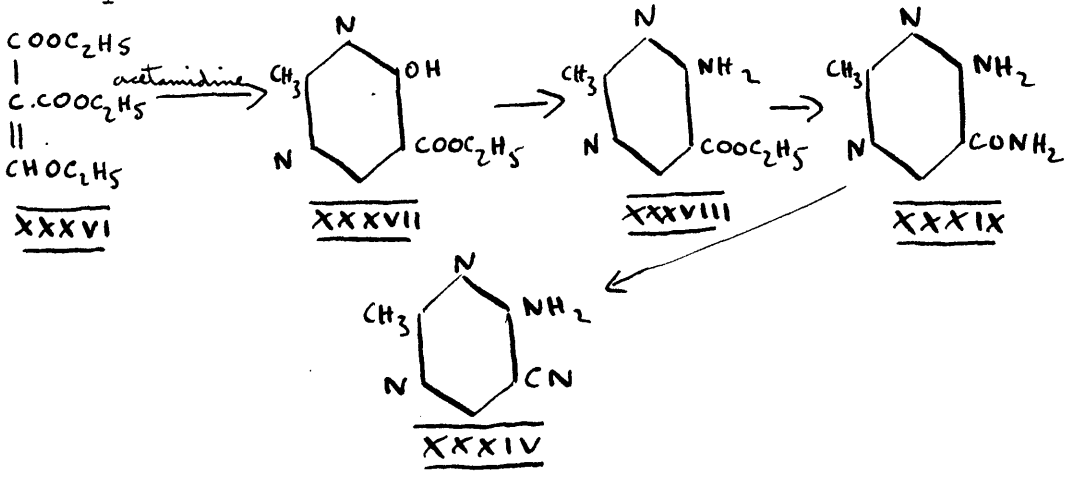
the other hand attempts to aminate 4-chloro-5-chloromethyl-2-methylpyrimidine led to formation of secondary amines; consequently the use of compounds of this series had finally to be abandoned.

Condensation of acetamidine with ethyl α-ethoxymethylene-α-cyanoacetate (XXXI) in absolute alcoholic solution gave an intermediate compound, probably ethyl α-cyano-β-acetamidino-acrylate, which on heating with alkali yielded 4-hydroxy-5-cyano-2-methylpyrimidine XXXII. Refluxing with phosphoryl chloride afforded 4-chloro-5-cyano--2-methylpyrimidine (XXXIII), which could be aminated to give 4-amino-5-cyano-2-methylpyrimidine (XXXIV); the latter compound gave on catalytic hydrogenation 4-amino-5-aminomethyl-2-methylpyrimidine (XXV), isolated as its hydrochloride. The compound (XXXIV) has been prepared in a different way by Grewe (1936) who also describes its reduction to the diamine.



XXXI XXXII XXXIII XXXIV XXXV

An alternative route for the synthesis of (XXXIV), which, though slightly longer, uses a cheaper starting material and is perhaps more reliable than the above, is the following. Ethyl ethoxymethylenemalonate (XXXVI) condensed readily with acetamidine in presence of sodium ethoxide to give ehtyl 4-hydroxy-2-methylpyrimidine-5-carboxylate (XXXVII), which, after successive chlorination with phosphoryl chloride and heating with alcoholic ammonia under pressure, yielded ethyl 4-amino-2-methylpyrimidine-5-carboxylate (XXXVIII). After conversion of the latter into the corresponding amide (XXXIX) with concentrated aqueous ammonia, the product was dehydrated to give the nitrile (XXXIV), which could then be reduced as above mentioned. From the diamine (XXXV), 4-amino-5-thioformamidomethyl-2-methylpyrimidine (XXIX) was readily obtained by treatment in aqueous solution at room temperature with potassium dithioformate.



XXXVI XXXVII XXXVIII XXXIX XXXIV

The way now seemed clear for the synthesis of aneurin. As a result of the experience gained in the synthesis of 3-pyrimidylthiazolium salts (p.35) it was at first endeavoured to condense the thioformamido compound (XXIX) in the form of its sodium derivative, with methyl α-chloro-γ-hydroxy-propyl ketone (XXX; R=H), but although the condensation was tried under various conditions, only traces of aneurin could be obtained, the main product being the hydrochloride of the diamine (XXV). The reason for these failures may have been lain in the instability of the sodium derivative. Compounds (XXIX) and (XXX; R=H) did not yield aneurin when heated together in dioxan solution, but when a mixture of the two substances alone was heated at 140° reaction occurred with considerable darkening and resinification. From the product a substance was isolated, in poor yield, which had the properties of aneurin. This partial failure was ascribed to the already mentioned low reactivity of the halogen ketone, which probably exists largely in the cyclic oxide from (cf. Buchman 1936). To avoid this trouble the thioformamido compound (XXIX) was heated with methyl α-chloro-γ-acetoxypropyl ketone (XXX; R=$OCOCH_3$) at 115°-

120° for a few minutes; smooth reaction occurred with production of a brownish yellow mass, which crystallised on trituration with hot absolute alcohol containing hydrogen chloride. After recrystallisation from alcohol a product was obtained m.p. 233-234° having all the properties of aneurin chloride obtained from natural sources. The acetyl group of the halogen ketone is eliminated at some stage in the process; the most likely explanation is that the acetyl group in the initial reaction product hydrolyses off during the heating with alcohol containing hydrogen chloride.

The synthetic material could not be distinguished from the natural by the formaldehyde-azo test (Kinnersley and Peters 1934) or the thiochrome test (p. 18) and it showed, within the limits of experimental error, a similar biological activity (380,000 I.U. per g.; natural vitamin 400,000 I.U. per g.), as measured by the electrocardiagraphic method on rats (Birch and Harris 1934). Natural aneurin chloride isolated from rice polishings usually shows a melting point 249-250° when pure,

but a low-melting form has been reported by Kinnersley O'Brien and Peters (1935a) who give m.p. 230° ± 2°; Williams and Cline (1936) state that their synthetic chloride had m.p. 233-234°. The synthetic product here described apparently corresponds to the latter, but it is not considered that the difference in melting point is due to stereoisomerism as was tentatively suggested by Williams and Cline; it seems more probable that the existence of two forms of equal biological potency is due to dimorphism, a phenomenon which has been noted by Kinnersley O'Brien and Peters (1935a) in the case of the sulphate and by Windaus and his collaborators (1933) in the case of the picrolonate. Mixed with natural aneurin chloride of m.p. 249° the synthetic product had m.p. 243-246°, and by seeding a solution with a crystal of natural vitamin a product m.p. 245-247° was at once obtained. Since the completion of this work Williams and Cline (1937) have also reported interconversion of the two forms. In accordance with this view both natural and synthetic products gave identical picrolonates. Furtner, by oxidation of the synthetic vitamin with alkaline potassium ferricyanide a product

was obtained m.p. 224-225°, identical in every respect with thiochrome prepared either from natural aneurin or by synthesis.

The syntheses of aneurin and thiochrome which have been described confirm the structures assigned to these compounds and establish beyond doubt the chemical constitution of the antineuritic vitamin (vitamin B_1). Much work remains to be done before its mode of action in living organisms is fully understood, and further clinical trials will be necessary to assess its value in the treatment of nervous diseases. Hitherto such work has undoubtedly been hampered by scarcity of pure vitamin B_1 from natural sources, but this difficulty is now removed, the synthetic vitamin being readily accessible.

Isolation of Aneurin from Rice Polishings. The procedure employed followed closely that described by Williams, Waterman and Keresztesy (J. Amer.Chem. Soc., 1934, 56, 1187). It was, however, found that the helianthine precipitation used by these authors could be omitted without affecting either the yield or purity of the final product.

Thermal Decomposition of Aneurin. The vitamin chloride (19.4mg) was dissolved in a little methyl alcohol and the solution rubbed with silver oxide (14.1mg. of a preparation containing 96% Ag_2O; 13.2mg. corresponded to 2 equivalents) until the solution was free of Cl^- ions. After filtering, the solution was evaporated to dryness and the residue heated in vacuo (0.1mm) to 180^0-190^0. A mixture of crystals and resin distilled out. This mixture was collected and again subjected to vacuum distillation in a vertical tube; the distillate formed three layers on the walls of the tube: (A) a small upper layer of liquid; (B) an intermediate zone of crystals; (C) a lower layer of yellowish resin.

Fractions (A) and (C) were non-fluorescent and could not be crystallised.

Fraction (B) was again distilled and finally purified by allowing to stand with absolute ether for several hours which dissolved out resinous impurities leaving the product as a mass of colourless needles m.p. 155-160°. The substance appeared to be basic in character, contained no sulphur and its aqueous or alcoholic solutions showed strong blue fluorescence. The fluorescence was not destroyed by addition of acid. (Found: C,56.2; H,5.5; N,29.7. $C_9H_{10}ON_4$ requires C,56.9; H,5.3; N,29.5). Yield; 1.5mg.

The experiment was repeated under varying conditions using both silver oxide and silver carbonate to liberate the vitamin base but no better results could be obtained; the yield too was variable the amount of product from 20mg. vitamin ranged from 1 to 1.5mg. Further work on this substance was accordingly abandoned.

Thiochrome from Aneurin. To a solution of the vitamin chloride (20mg.) in methyl alcohol (1-2cc), methyl alcoholic potassium hydroxide (2cc. of 15%) and aqueous potassium ferricyanide (1cc. of 33%)

were added. After addition of butyl alcohol (10cc.) and sufficient water to bring inorganic salts into solution the mixture was shaken vigorously for 2 minutes and the blue-fluorescent butyl alcohol layer tapped off; the aqueous layer was extracted with further quantities of butyl alcohol until the last extract showed no fluorescence. The combined butyl alcoholic extracts were washed with a little water and dried over sodium sulphate to remove the last traces of potassium ferricyanide. On shaking the dried extracts with dilute hydrochloric acid (pH 2-3) the blue fluorescence disappeared and a greenish-yellow substance passed into the aqueous acid layer. The greenish-yellow acid solution was separated and evaporated to dryness under reduced pressure (bath temperature 40°), and the crystalline residue dissolved in a little concentrated aqueous potassium hydroxide (25%) and extracted repeatedly with chloroform till the extracts showed no fluorescence. The combined chloroform extracts which had a vivid blue fluorescence were dried over sodium sulphate and finally for a short time over potassium carbonate. The dried extract was now concentrated under reduced pressure to a volume of

about 4cc. and set aside. In a short time sulphur yellow flaky crystals of thiochrome separated which after recrystallisation from chloroform had m.p. 225-226° with decomposition. Yield, 9mg. i.e. 60%. (Found: C,54.6; H,5.5; N,21.6; S,12.3. $C_{12}H_{14}ON_4S$ requires C,54.9; H,5.3; N,21.4; S,12.2).

The substance had all the properties described for thiochrome by Kuhn and his collaborators (Z.physiol.Chem., 1935, 234, 196). The comparison with thiochrome isolated directly from yeast was carried out by Prof. Kuhn who reported: Both substances had m.p. 226-227° (Berl-block, shortened thermometer) and a mixed m.p. showed no depression. The absorption spectrum and fluorescence -pH curve were identical for both specimens.

Thiochrome can be prepared in a similar fashion from partially purified rice concentrates; the yield of crystalline product is however much lower than that obtained when pure vitamin is used as starting material, for yellow, oily substances are formed at the same time which are very difficult to separate from the thiochrome. Weakly alkaline solutions of the vitamin oxidised with air, oxygen, hydrogen peroxide or potassium permanganate, and

also acid solutions oxidised with selenium dioxide give products containing thiochrome but these methods are not to be recommended where isolation of the crystalline pigment is desired.

In order to use the development of blue fluorescence on oxidation as a test for the presence of aneurin in vitamin concentrates it is only necessary to shake an aqueous extract of the material with butyl alcohol, separate the aqueous layer and after making it alkaline to add potassium ferricyanide solution. Extraction of the mixture with butyl alcohol yields a blue fluorescent butyl alcoholic layer the fluorescence being visible in daylight, save when the concentration of vitamin is very low in which case it will be most readily detected in ultra-violet light. This test may be recommended for qualitative work.

Properties of Thiochrome. Thiochrome is readily soluble in methyl alcohol, moderately so in water and ethyl alcohol, and sparingly soluble in acetone, chloroform and ether. It sublimes unchanged in a high vacuum (0.1mm) at 210-215°. After heating for 30 minutes on the water bath with 20% sodium hydroxide, thiochrome, unlike aneurin, gives a

solution free of sulphide-ions (negative test with sodium nitroprusside). Tested biologically by the heart-rate method in rats (Birch and Harris, Biochem. J., 1934, 28, 602) thiochrome was inactive in doses of 5γ, 10γ, and 20γ, and the formaldehyde-azo-reaction of Kinnersley and Peters (Biochem.J., 1934, 28, 667) was negative. A negative result in this test was also given by the leuco-compound which could be obtained in solution on reducing with sodium hydrosulphite or with hydrogen in presence of a platinised silica catalyst; leuco-thiochrome is readily re-oxidised to give the original pigment.

Thiochrome Hydrochloride. To thiochrome (8mg.) dissolved in a minimum of methyl alcohol four volumes of acetone were added followed by a few drops of ethereal hydrogen chloride. The hydrochloride came down at once as a bulky precipitate which could be recrystallised from a mixture of methyl alcohol and acetone. It formed almost colourless needles which developed a yellow colour on drying in a desiccator (Found: Cl,17.0; 17.8. $C_{12}H_{14}ON_4S$ HCl requires Cl,11.9. $C_{12}H_{14}ON_4S$. 2HCl requires Cl,21.2). On heating the hydrochloride slow decomposition set in from 200°

onwards, and at 217-221° it melted to a reddish brown viscous liquid.

Catalytic hydrogenation of the hydrochloride (2 mg.) in dilute methyl alcoholic hydrogen chloride using a palladium catalyst gave a very hygroscopic colourless substance, which showed no fluorescence in neutral or alkaline solution and could not be re-oxidised to thiochrome.

Deamination Experiments with Aneurin Thiochrome and 2:6-dihydroxy-4:5-diamino-pyrimidine. Aneurin chloride was heated at 100° with concentrated hydrochloric acid in a sealed tube during 4 hours. The solution was concentrated, cooled, made alkaline with baryta, and the liberated ammonia driven by a stream of air into standard sulphuric acid and estimated by titration.

Thus with 5.6mg. chloride in 2c.c. concentrated hydrochloric acid

Found: NH_3, 0.28mg. Calculated for 1mol.NH_3, 0.27mg.

The formaldehyde-azo-test was negative when applied to a solution of the deaminated vitamin, which also failed to give any fluorescent product on oxidation with potassium ferricyanide.

From thiochrome (5.18mg.) under precisely

similar conditions no ammonia was split off; indeed, most of the thiochrome was recovered unchanged (m.p. and mixed m.p.).

2:6-dihydroxy-4:5-diaminopyrimidine sulphate (Traube, Ber., 1900, 33, 1382, 3043). (20.4mg.) similarly treated gave .29mg. NH_3 (calculated for loss of 1 mol. NH_3, .3mg.).

Synthesis of Quaternary Thiazolium Salts.

3-Benzyl-2:4-dimethylthiazolium bromide. A mixture of benzyl bromide and 2:4-dimethylthiazole (Hantzsch A. 1889, 250, 264) in equimolecular proportion was heated on a water bath during 15 minutes. The crystalline mass which was produced was washed thoroughly with ether and recrystallised by dissolution in absolute alcohol and adding sufficient absolute ether to cause a faint turbidity. The bromide separated in colourless needles m.p.173° (Found: Br, 5.0. $C_{12}H_{14}NS$ Br requires Br, 4.9). The yield was quantitative.

2:3:4-Trimethylthiazolium Chloride. The N-methyl-thioacetamide required for this experiment was prepared by heating a mixture of N-methyl-acetamide and phosphorus pentasulphide using benzene as a

diluent; it was obtained in 20% yield and had m.p. 59°.

Chloroacetone (5cc.) was warmed to 80° on a water bath and powdered N-methyl-thioacetamide (5g.) was added in small portions at a time. At each addition a violent reaction occurred and by the end of the operation the contents of the flask formed a solid mass. The product was stirred with dry ether to remove unchanged chloroacetone, dissolved in a little methyl alcohol and ether added till a slight turbidity appeared. On standing 2:3:4-trimethylthiazolium chloride separated in colourless exceedingly hygroscopic needles, m.p. 235° with decomposition. (Found in dried material Cl, 21.0; $C_6H_{10}NSCl$ requires Cl, 21.7). The yield was practically quantitative and the product was identical with a specimen of chloride prepared by the action of silver chloride on 2:4-dimethylthiazole methiodide (prepared by direct addition of methyl iodide to 2:4-dimethylthiazole.)

3-Phenyl-2:4-dimethylthiazolium Salts. A mixture of thioacetamilide (5g.) and chloroacetone (5.5cc.) was heated on a boiling water bath, in a flask

fitted with a calcium chloride tube to prevent any access of moisture. After a few minutes a sudden and violent reaction took place and the contents of the flask acquired a thick syrupy consistency. After cooling, the brownish reaction product was dissolved in some 30cc. water, boiled with animal charcoal and filtered; on adding excess of 20% aqueous perchloric acid, 3-phenyl-2:4-dimethylthiazolium perchlorate separated in colourless needles. Recrystallised from a mixture of acetone and ether it had m.p. 180°. Yield, quantitative. (Found: C,45.1; H,4.5; N,4.5. $C_{11}H_{12}O_4NSCl$ requires C,45.6; H,4.1; N,4.8).

The picrate formed small yellow needles m.p. 115°. The chloride was obtained as colourless needles by dissolving the picrate in 5% methyl alcoholic hydrogen chloride and precipitating with ether. It was extremely hygroscopic and had m.p. 184° with decomposition, when heated in a sealed tube. Generally speaking the chlorides of thiazolium salts are hygroscopic and other salts e.g. perchlorates are often preferable for isolation purposes. The iodide had the properties described by Clarke and Gurin (J.Amer.Chem.Soc., 1935, 57, 1876).

S-Acetonyl-thioacetanilide Hydrochloride. Thioacetanilide (5g.) was added to chloroacetone (6g.) at 15-20°, and the solution left to stand at this temperature. After some three hours, colourless crystals suddenly began to separate, and, within a further half hour, the contents of the flask set to a crystalline mass. The crystals were washed first with acetone and then with ether to remove chloroacetone and recrystallised from methyl alcohol by addition of ether. The hydrochloride was thus obtained in colourless needles m.p. 112°; the yield was quantitative. (Found: C,53.9; H,5.8; N,5.9; S,13.0; Cl,14.3. $C_{11}H_{14}ONSCl$ requires C,54.2; H,5.8; N,5.8; S,13.1; Cl,14.6.).

The free base was obtained as an unstable oil, which decomposed when attempts were made to distil it, even under reduced pressure. Its perchlorate had m.p. 130°. From the hydrochloride a semicarbazone could be obtained m.p. 250° with decomposition. On heating at 90-100° for a time the hydrochloride was slowly converted into 3-phenyl-2:4-dimethylthiazolium chloride (above).

Hydrolysis of S-acetonyl-thioacetanilide. On boiling with water or dilute hydrochloric acid the above hydrochloride underwent decomposition with the separation of oily droplets. The mixture produced in this way was submitted to steam distillation; the distillate had an unpleasant odour reminiscent of mercaptan, and it gave, with mercuric chloride, a white precipitate m.p. 85° which contained sulphur.

The distillation residue was extracted once or twice with ether. From the ethereal extracts a crystalline substance was isolated; it was identified as acetanilide (mixed m.p. 114-115°). The aqueous layer from the ether extraction was made alkaline and again extracted with ether. The ether extract was shown to contain aniline which was isolated and characterised as its acetyl derivative.

Another portion of the original aqueous mixture was treated with semicarbazide hydrochloride and sodium acetate. A mono-semicarbazone separated, which crystallised from methyl alcohol in colourless prisms m.p. 213° with decomposition. (Found: N,19.4; S,28.7. $C_7H_{13}ON_3S_2$ requires N, 19.2; S, 29.2). The semicarbazone answered the tests for compounds

containing a free -SH group (red colouration on addition of sodium nitrite to an acetic acid solution; white precipitate with mercuric chloride).

3-σ-Tolyl-2:4-dimethylthiazolium salts. The thioacet-σ-toluidide required for these experiments was prepared by the action of phosphorus pentasulphide on acet-σ-toluidide; it had m.p. 68°.

The condensation of thioacet-σ-toluidide and chloroacetone was carried out in the manner above described in the case of thioacetanilide. The 3-σ-tolyl 2:4-dimethylthiazolium chloride produced being very hygroscopic it was converted into the perchlorate m.p. 172° with decomposition, and analysed as such. (Found: C,47.7;H,5.0;N,4.5. $C_{12}H_{14}O_4NSCl$ requires C,47.4; H,4.6; N,4.6). The corresponding picrate melted at 150° and the iodide at 217-218°, melting being accompanied by decomposition in both cases.

The corresponding intermediate product S-acetonyl-thioacet-σ-toluidide was obtained in similar fashion to the lower homologue described above. The hydrochloride had m.p. 125° (Found: C,55.9; H,6.5; N,5.6. $C_{12}H_{16}NSCl$ requires C,55.9; H,6.2; N,5.4.) It was rather more resistant to hydrolysis than S-acetonyl-thioacetanilide hydrochloride, but after a boiling for a time with water or dilute mineral acid it gave σ-toluidine, acet-σ-toluidide and the same sulphur as was described above.

3- (σ-Nitrophenyl)-2:4-dimethylthiazolium Salts.

Preparation of σ-nitro-thioacetanilide: σ-Nitro-acetanilide (1 mol.) and phosphorus pentasulphide (3.5 mols.) are mixed intimately in a mortar, and the mixture heated in 1g. portions in test tubes on a water-bath (the use of larger quantities at a time is dangerous). The warming should not last more than 3 minutes when the mass sinters together; if continued further the reaction mixture ignites spontaneously. The test tubes are cooled crushed in a mortar and the mixture of glass and reaction products extracted with alcohol. The alcohol solution is now saturated with solid caustic soda allowed to stand for a time, then diluted with water filtered, and the product precipitated by saturating the solution with carbon dioxide. The precipitate is redissolved in 2% sodium hydroxide solution, filtered from impurities, and the σ-nitro-thio-acetanilide again precipitated with carbon dioxide. It crystallises from dilute acetone in yellow prisms m.p. 114° (Found: C,49.0; H,4.3; N,14.0. $C_8H_8O_2N_2S$ requires C,49.0; H,4.1; N,14.3).

The thiazolium salts of this group were prepared in the manner described for the 3-phenyl-

-salts. 3-(σ-nitrophenyl) -2:4-dimethylthiazolium perchlorate crystallised from water in colourless leaflets m.p. 205°. (Found: C,40.1; H,3.5; N,8.4; S,9.3; Cl,10.4. $C_{11}H_{11}O_6N_2SCl$ requires C,39.3; H,3.6; N,8.3; S,9.5; Cl,10.6).

C-Alkyl Estimations (by Dr. H. Roth) on Aneurin, Thiochrome and other Substances. (Oxidation with chromic acid)

a) 3-Phenyl-2:4-dimethylthiazolium iodide. 7.457, 6.841mg. substance: 3.09, 2.85c.c. n/100 NaOH. Found: $C_{11}H_{12}NSI$ gives 1.31, 1.32 equivalents acetic acid.

b) 5-Methyluracil. 6.848, 7.190mg. substance: 4.26, 4.34c.c. n/100 NaOH. Found: $C_5H_6O_2N_2$ gives 0.78, 0.76 equivalents acetic acid.

c) Pyrimidine-sulphonic acid from sulphute clearage of aneurin. 5.766, 4.981mg. substance: 1.78, 1.51c.c. n/100 NaOH. Found: $C_6H_9O_3N_3S$ gives 0.63, 0.62 equivalents acetic acid.

d) 3-Methyl-thiazolobenziminazole. 8.273, 7.180mg. substance: 4.50, 3.85c.c. n/100 NaOH. Found: $C_{10}H_8N_2S$ gives 1.02, 1.00 equivalents acetic acid.

e) Thiochrome. 5.687mg. substance: 3.45c.c. n/100

NaOH. Found $C_{12}H_{14}ON_4S$ gives 1.59 equivalents acetic acid.

f) Aneurin Chloride. 7.120, 7.328mg. substance: 2.83, 2.96cc. n/100 NaOH. Found: $C_{12}H_{18}ON_4SCl_2$ gives 1.34, 1.36 equivalents acetic acid.

3-Methyl-(thiazolo-2^1:3^1:2:1)-benziminazole (VII). A mixture of -phenylenediamine (2g.) and thiourea (1.4g.) was heated at 180° during 1 hour. Ammonia was evolved and the reaction mixture, initially liquid, set to a solid reddish crystalline mass. After cooling and recrystallising from hot alcohol thiobenziminazoline was obtained in almost colourless crystals m.p. 295-300° (Yield, 1.3g.).

The above thiobenziminazoline (1g.) was heated for a few minutes with chloroacetone (0.6g.) at 100 -110° for 3-5 minutes. Sudden reaction occurred and the contents of the flask melted and then solidified again. After cooling, the product was dissolved in water, the solution washed with ether to get rid of any unchanged chloroacetone, and then made alkaline. The precipitate of 3-methyl-thiazolobenziminazole was recrystallised from dilute alcohol; small, colourless prisms m.p. 164-165°

(Found: C,63.9; H,4.3; N,14.9; S,17.3. $C_{10}H_8N_2S$ requires C,63.8; H,4.3; N,14.9; S,17.0). Yield, 1g. In alcoholic solution, the substance showed a feeble blue fluorescence when viewed in ultraviolet light.

Methyl α-Chloro-γ-hydroxypropyl ketone and its Derivatives.

Ethyl α-2-Acetoxyethylacetoacetate. To a suspension of dry sodium ethyl acetoacetate (152g.) in dry benzene (700c.c.) β-bromoethyl acetate (167g.) was added at a temperature of 15-20°. The mixture was heated on the water bath until the solution reacted faintly alkaline (6-10 hours), then cooled, poured into ice water and extracted with ether. After removal of the ether the residual oil was distilled under reduced pressure, the fraction boiling at 138-142° (14mm) being collected (Yield, 25%). Haller and March (loc.cit.) give b.p. 147-150° (13mm).

Ethyl α- Chloro-α-2-acetoxyethylacetoacetate. Sulphuryl chloride (82g.) was added gradually with stirring to the above ester (123g.), the temperature being kept at 0°. When all had been added, (ca. 1 hour) the solution was kept at 0° for a further hour, then diluted with ether (250c.c.) and refluxed for a

short time to remove sulphur dioxide and hydrogen chloride. The ether was removed, and the residual oil repeatedly fractionated in vacuo. The main fraction b.p. 120-121° (2mm) was collected (Yield 86%). (Found: C,47.9; H,6.0; Cl,13.3. $C_{10}H_{15}O_5Cl$ requires C,47.9; H,6.0 Cl,14.1.).

Methyl α-Chloro-γ-acetoxypropyl Ketone (XIII; R=$COCH_3$). The above ester was heated under reflux for 6 hours with a mixture of dilute sulphuric acid (20c.c. of 15%) and glacial acetic acid (20c.c.). The solution was cooled, poured into water, and extracted with ether. After removal of the ether and acetic acid a colourless liquid was obtained, which, after several fractionations, boiled at 90-93° (2mm) (Yield, 40%). (Found: C,47.1; H,6.2; Cl, 19.8. $C_7H_{11}O_3Cl$ requires C, 47.0; H, 6.2; Cl, 19.9).

Methyl α-Chloro-γ-hydroxypropyl Ketone(XIII; R = H).

The chloro-ester (above) was heated under reflux during 4 hours with a mixture of dilute sulphuric acid (35c.c. of 35%) and alcohol (70c.c.), then poured into water and the mixture extracted with ether. On removal of ether from the dried extract, an oil was left which after repeated fractionation

gave a colourless liquid b.p. 85-92° (16 mm) (Yield, 20%) (Found: Cl,25.4. $C_5H_9O_2Cl$ requires Cl, 26.0).

4-Methyl-5-β-hydroxyethylthiazole. An ethereal solution of thioformamide was prepared by shaking together finely powdered phosphorus pentasulphide (12g), formamide (20g) and absolute ether (200c.c.) for ca. 20 hours (Gabriel, Ber., 1916, 49, 1145.); the clear ether layer was decanted and used as a stock solution of thioformamide.

The chloroketone (XIII; R=H) (250mg) was added to ethereal thioformamide solution (10c.c.), and the mixture allowed to stand when colourless crystals of the thiazole hydrochloride slowly separated. After 5 hours the ether was distilled off, and the residue heated for 1 hour at 100°, cooled, and dissolved in dilute hydrochloric acid. After extraction with ether to remove any unchanged ketone, the solution was made strongly alkaline and again extracted with ether. The extract on evaporation yielded 4-methyl-5-β-hydroxyethylthiazole as an almost colourless oil b.p. 250-255° (capillary method of Emich). The base was not purified further;

treatment with ethereal picric acid gave a picrate crystallising from alcohol in yellow needles m.p. 162-163°. (Found: S,8.2. $C_{12}H_{12}O_8N_4S$ requires S,8.6). A mixed m.p. with 4-methyl-5-β-hydroxyethylthiazole picrate (m.p. 162°)prepared from aneurin showed no depression.

Ethyl α-2-Phenoxyethylacetoacetate. This ester was prepared from phenoxyethyl bromide and sodium ethyl acetoacetate in alcoholic solution (cf. Boyd Barrett and Robinson, loc.cit.). It had b.p. 148° (4mm). (Found: C,68.1; H,7.3. $C_{14}H_{18}O_4$ requires C,67.2; H,7.2).

Ethyl α-Chloro-α-2-phenoxyethylacetoacetate. The above ester (10g) was chlorinated with sulphuryl chloride (6g) in the manner described under the corresponding acetoxy compound. The product was a colourless liquid b.p. 135-140° (5mm). (Yield, 70%) (Found: C,59.3; H,6.1; Cl,11.9. $C_{14}H_{17}O_4Cl$ requires C,59.1; H,6.0; Cl,12.4).

Methyl α-Chloro-γ-phenoxypropyl Ketone (XIII; R=C_6H_5). The above chloro-ester (7g) was hydrolysed by refluxing for 4 hours with a mixture of dilute sulphuric acid (14c.c. of 15%) and glacial acetic acid (14c.c.). After repeated distillation

the main fraction of the product boiled at 168-172° (12mm). (Found: C,62.0; H,6.0; Cl,12.2. $C_{11}H_{13}O_2Cl$ requires C,62.1; H,6.1; Cl,16.7). The low chlorine content may be due to partial decomposition during distillation; that it is mainly the desired ketone is shown by its condensation with thioacetamide.

2:4-Dimethyl-5-β-phenoxyethylthiazole. The above chloroketone (200mg) reacted rapidly with thioacetamide (60mg) when the mixture was warmed for a few minutes over a free flame. On working up, the free base was obtained as a colourless thick oil. It gave a picrate crystallising from alcohol in yellow needles m.p. 122°. (Found: N,12.1; S,6.8. $C_{19}H_{18}O_8N_4S$ requires N,12.1; S,6.9).

Thioacylation of Pyrimidines.

6-Methyl-5-thioacetylaminouracil (XIV). 6Methyl-5-aminouracil (1g) (Behrend, Annalen, 1885, 231, 250.) dissolved in dioxan (50c.c.) was heated on the water bath with dithioacetic acid (0.9g) (Pohl,Ber., 1907, 40, 1304) during 4 hours. The mixture was cooled and diluted with petroleum ether. The yellowish precipitate crystallised from hot water in colourless

needles m.p. 265-267° (Found: C,42.4; H,4.8; N,21.1. $C_7H_9O_2N_3S$ requires C, 42.2; H,4.6; N,21.2) Yield, quantitative.

6-Methyl-5-thioformylaminouracil. 6-methyl-5-aminouracil (1g) in dioxane (50c.c.) was heated under reflux with dithioformic acid (0.7g) (Levi, Atti R. Accad.Lincei, 1923, (5), 32, I, 569). The crude thioformyl derivative precipitated with petroleum ether was difficult to purify. After recrystallisation from water it had m.p. 260-262° (Found: N,21.0; $C_6H_7O_2N_3S$ requires N, 22.7; $C_6H_7O_2N_3S.\ H_2O$ requires N,20.7).

3-(2'4'-dihydroxy-6'-methylpyrimidyl-5')-4-methylthiazolium chloride. The above thioformyl compound (1 mol) mixed with chloroacetone (4-5 mols) was heated carefully over a free flame. Vigorous reaction occurred, and after 10-15 minutes the mixture was cooled, and the product precipitated as a gum by addition of ether. It crystallised from a mixture of alcohol and acetone in colourless needles m.p. 306° with decomposition. (Found: C,40.9; H,4.6; N,15.6; Cl,13.6. $C_9H_{11}O_2N_3SCl$ requires C,41.4; H 4.3; N,16.1; Cl,13.6).

4-Amino-5-thioformamido-6-methylpyrimidine XV; R=H; R_2=CH_3). To 6-methyl-4:5-diaminopyrimidine (1.5g) Gabriel and Colman, Ber., 1907, 34, 1254) dissolved in water (10c.c.) is added potassium dithioformate (2g); after a short time traces of crystalline material m.p. 300° separate. The solution is filtered and placed in a shallow dish, which is allowed to stand over sulphuric acid in a desiccator filled with carbon dioxide. After standing overnight the crystalline precipitate is collected; the filtrate may be treated with a further quantity of potassium dithioformate and the process repeated until the yield is nearly quantitative. The thioformyl-compound crystallises from water in colourless needles. (Found: C,43.0; H,5.2; S,18.6. $C_6H_8N_4S$ requires C,42.9; H,4.8; S,19.0). On heating it melts sharply at 168°, with evolution of hydrogen sulphide; the melt resolidifies, and on further heating melts at 230°. Gabriel, (Ber., 1907, 34, 1247) gives m.p. 235° for 4-methylpurine. Conversion to 4-methylpurine occurs slowly at temperatures from 100° onwards. The substance is very soluble in alcohol, less so in methyl alcohol, acetone and water, and insoluble in ether.

2-Amino-4-hydroxy-6-ethylpyrimidine. A mixture of ethyl propionylacetate (13-3g) (Willstätter and Clarke, Ber., 1914, 47, 298) guanidine carbonate (8g) and absolute alcohol (25c.c.) was heated under reflux for 4 hours, cooled, and the product filtered off and recrystallised from hot water. Colourless prisms m.p. 247-248° (Yield, 7g) (Found: C,51.6; H,6.2; N,29.6 $C_6H_9ON_3$ requires C,51.8; H,6.2; N,30.2). It could not be thioformylated with potassium dithioformate.

When the above compound (1g) was heated with concentrated hydrochloric acid (6c.c.) for 20 hours at 160°, 4-ethyluracil m.p. 205° was obtained (Yield, 60%).

2-Amino-4-chloro-6-ethylpyrimidine. A mixture of the above compound (3.5g) and phosphoryl chloride (10c.c.) was heated under reflux for 2 hours. The refluxing brownish solution was poured on ice, made alkaline with ammonia, and the precipitated chloro-compound collected. It crystallised from alcohol in colourless needles m.p. 120-121°. (Yield, 60%) (Found: C,45.2; H,4.9; N,26.1. $C_6H_8N_3Cl$ requires C,45.7; H,5.1; N,26.7).

2:4-Diamino-6-ethylpyrimidine. The above chloro-compound (0.6g) was heated with saturated alcoholic ammonia (20c.c.) in a sealed tube at 180° during 6 hours. The alcohol was removed, the residue dissolved in a little water and solid potassium hydroxide added. The precipitated diamine was collected and re-crystallised from ethyl acetate containing a little petroleum ether. Colourless needles m.p. 160-161° (Yield, 80%) (Found: N,40.0. $C_6H_{10}N_4$ requires N,40.6). The substance could not be thioformylatedwith potassium dithioformate.

4-Amino-5-thioformamido-6-ethylpyrimidine XV; R_1=H; R_2=C_2H_5). 6-Ethyl-4:5-diaminopyrimidine was prepared from 6-ethyluracil using, with slight modifications, the method of Robinson and Tomlinson (J.1935, 1283). The following method of isolating the diamine is simpler, and gives much improved yields; The reaction mixture obtained on reduction of 2-chloro-6-ethyl-4:5-diamino-pyrimidine is filtered, concentrated to remove alcohol, diluted somewhat with water and solid potassium hydroxide is added. The precipitated diamine is filtered off

and crystallises from ethyl acetate in large yellowish prisms m.p. 164-165°: Robinson and Tomlinson (loc.cit.) give m.p. 159-161°. A further quantity may be obtained by extracting the alkaline mother liquor with ethyl acetate (Total yield, 80% or more.).

The diamine (100mg), thioformylated in aqueous solution with potassium dithioformate in the manner described above, gave a product crystallising from water in colourless needles m.p. 178° with evolution of hydrogen sulphide (Yield theoretical) (Found: C,45.5; H,6.0; S,17.1. $C_7H_{10}N_4S$ requires C,46.1; H,5.5; S,17.6).

2:4-Diamino-5-thioformamido-6-methylpyrimidine XV; R_1=NH_2; R_2=CH_3). 2:4:5-Triamino-6-methylpyrimidine (Gabriel and Colman, loc.cit.) on treatment with potassium dithioformate as above gave colourless needles (from water) m.p. 255° with evolution of hydrogen sulphide. (Found: S,17.2. $C_6H_9N_5S$ requires S,17.5).

3-(2':4'-diamino-6'-methylpyrimidyl-5')-4-methyl-thiazolium chloride hydrochloride. To a solution of the above thioformyl compound (1 mol) in acetone was added chloroacetone (2 mols) and the mixture

left for 3 days at room temperature, diluted with an equal volume of alcohol, and the mixture refluxed for a further 4 hours. During the heating colourless needles separated and, after cooling, these were collected and recrystallised from a mixture of alcohol and acetone containing hydrogen chloride. Colourless needles m.p. 315° with decomposition; the crystals contain water of crystallisation, which is only expelled with difficulty. (Found: C,31.2; H,5.4; N,19.9; S,9.0; Cl,20.4%. $C_9H_{13}N_5SCl_2 \cdot {}_3H_2O$ requires C,31.0; H,5.5; N,20.1; S,9.2; Cl,20.4). The corresponding picrate has m.p. 255°. On shaking with alkaline potassium ferricyanide a substance is produced which though non-fluorescent in visible light is blue fluorescent in ultra-violet light; the fluorescence disappears, on making acid but reappears on making alkaline again.

Synthesis of 3-Pyrimidylthiazolium Salts.

3-(6'-Ethyl-4'-aminopyrimidyl-5')-4-methyl-5-β-hydroxyethyl-thiazolium chloride hydrochloride (IV).

To a mixture of 4-amino-5-thioformamido-6-ethylpyrimidine (108.5mg = 1 mol) (see above) and

absolute alcohol (10c.c.), was added a solution of sodium ethoxide in alcohol (1c.c. containing 13.7mg = 1 atom Na). To the clear solution formed methyl α-chloro-γ-hydroxypropyl ketone (0.1c.c. i.e. excess) was added, and the mixture left overnight at room temperature. After filtering from sodium chloride alcoholic hydrogen chloride (0.3c.c. containing 27.7mg. = 1 mol HCl) was added and the solution heated under reflux for 4 hours. At the end of this time a further quantity of alcoholic hydrogen chloride (0.3c.c. 1 mol HCl) was added, heating continued for 1 hour, and the solution cooled and excess of acetone added to precipitate the quarternary salt, which crystallised from standing in the ice chest for a few hours. The hygroscopic product crystallised from an alcohol-acetone mixture in bundles of small colourless needles containing water or crystallisation. On heating water is expelled at about 100-110°, and on further heating the salt melts at 220° with decomposition. (Found: C,41.1; H,6.1; S,8.5; Cl,20.5. $C_{12}H_{18}ON_4SCl_2 \cdot H_2O$ requires C,40.6; H,5.6; S,9.0; Cl,20.0).

Oxidation with alkaline potassium ferricyanide gave solutions which, though non-fluorescent in

in visible light, had blue fluorescence in ultra violet light. The formaldehyde-azo-test is positive and indistinguishable from that given by natural aneurin. Tested by the electrocardiagraphic method 1.2 mg contained < I I.U. The inactivity of the substance was confirmed by Professor R.A. Peters who kindly examined it, and to whom I wish to express my thanks.

3-(6'-Ethyl-4'-aminopyrimidyl-5')-4-methylthiazolium chloride hydrochloride. 4-Amino-5-thioformamido-6-ethylpyrimidine (108.5mg) was converted into its sodium salt, and condensed with chloroacetone (0.1c.c.) in a manner similar to that described above, the total period of heating being in this case only 3 hours. The product crystallised from alcohol-acetone in hygrocopic colourless needles m.p. 252-253° with decomposition. (Found: C,40.6; H,5.1; S,10.5; Cl,23.6. $C_{10}H_{14}N_4SCl_2$ requires C,40.9; H,4.8; S,10.9; Cl,24.2).

The substance reacted negative in the formaldehyde-azo-test, and tested by the electro-cardiagraphic method 2.8mg. contained<1 I.U. Oxidation with alkaline potassium ferricyanide gave a solution which had weak blue fluorescence in

ultra-violet light.

3-(6'-Methyl-4'-aminopyrimidyl-5')-4 methyl-5-β-hydroxyethyl-thiazolium chloride hydrochloride. 4-Amino-5-thioformamido-6-methylpyrimidine (100mg) was converted to its sodium salt and condensed with methyl α-chloro-γ-hydroxypropyl ketone (0.1c.c.) in the manner above described, the total period of heating being 5 hours. The product crystallised from alcohol-ethyl acetate in colourless needles which on heating lost water of crystallisation at 100-110° and melted with decomposition at 250° (Found: C,38.8; H,5.4; S,8.5; Cl,21.0. $C_{11}H_{16}ON_4SCl_2 H_2O$ requires C,38.7; H,5.4; S,9.4; Cl,20.8). The substance gives a positive formaldehyde-azo-test and oxidation with alkaline potassium ferricyanide gives a solution which is blue fluorescent in ultra-violet light. Tested by the electrocardiagraphic method 2.8mg. contained $<$1 I.U.

3-(6'-Methyl-4'-aminopyrimidyl-5')-4-methylthiazolium chloride hydrochloride. 4-Amino-5-thioformamido-6-methylpyrimidine (100mg), condensed in the form of the sodium salt with chloro-acetone (0.1c.c.), the period of heating being 3 hours, gave a product

crystallising from alcohol-acetone in needles m.p. 254-255° with decomposition. Owing to its extremely hygroscopic character it was difficult to analyse. (Found: C,33.9; H,5.7. $C_9H_{12}N_4SCl_2 \cdot 2H_2O$ requires C,34.2; H,5.1). The substance did not give the formaldehyde-azo-test and tested biologically by the electrocardiagraphic method 5mg. contained <1 I.U. Oxidation with alkaline potassium ferricyanide gave a solution blue-fluorescent in ultra-violet light.

2:6-Dihydroxy-8-thiopurine. This substance has been described by Fischer (loc.cit) who prepared it by heating bromoxanthine with potassium hydrogen sulphide. It was prepared in the following way: 2:6-dihydroxy-4:5-diaminopyrimidine (1 mol) (Traube, Ber., 1900, 33, 3382) was heated with thiourea (4 mols) at 240-250° for 1 hour. The melt was cooled and extracted repeatedly with boiling water; the extract, on cooling, deposited a nearly colourless powder having the properties recorded by Fischer (loc.cit.) (Found: in material dried at 150° high vac.: N,30.8; $C_5H_4O_2N_4S$ requires N,30.4).

4'-Methyl-2:6-dihydroxy-(thiazolo-2':3':8:7)-purine. 2:6-Dihydroxy-8-thiopurine (120mg) was boiled with chloroacetone (200mg) for 20 minutes then cooled and

diluted with ether. The solid residue was recrystallised by dissolving in hot dilute ammonia and making weakly acid with acetic acid; on cooling the product separated as a white micro-crystalline powder which did not melt below 250°. (Found: C,42.7; H,2.8; N,25.0; S,14.7 $C_8H_6O_2N_4S$ requires C,43.2; H,2.7; N,25.2; S,14.4) The substance is soluble in ammonia, caustic alkalies and hydrochloric acid and insoluble in dilute acetic acid or cold water. A solution in concentrated ammonia gives no immediate precipitate with silver nitrate (distinction from 2:6-dihydroxy-8-thiopurine). Its ammoniacal solution fluoresces light blue in ultra-violet light, the fluorescence disappearing on making the solution acid.

4-Ethyl-8-thiopurine. 4-Ethyl-5:6-diaminopyrimidine (100mg) was heated with thiourea (150mg) to 170-180° for 1 hour by which time evolution of ammonia had ceased. The melt was cooled, triturated with water, and the insoluble residue dissolved in hot dilute ammonia. After treatment with charcoal and boiling off the ammonia, the solution on cooling deposited yellowish needles m.p. 300°. (Found: C,46.9; H,4.4. $C_7H_8N_4S$ requires C,46.7; H, 4.4).

Experiments on the Condensation of 4-Methyl-and-4-Ethyl-8-thiopurines with α-halogenated -ketones.

The general method used was heating the sodium derivative of the thiopurine with the appropriate halogenated ketone in alcoholic solution for 12 hours. The ketones used were chloroacetone and methyl-α-chloro-γ-hydroxypropyl ketone; in every case solutions were obtained, which, when neutral or alkaline, showed blue fluorescence in ultra-violet light, but no fluorescence in visible light could be detected. As the products were difficult to isolate in a pure state the experiments were not pursued further, it being clear that no substances similar to thiochrome were obtainable in this way.

Synthesis of Thiochrome.

Ethyl-4-Hydroxy-2-methylpyrimidine-5-acetate(XXIII).

To acetamidine hydrochloride (94.5g), dissolved in a cold solution of sodium (23g) in absolute alcohol (600c.c.), was added freshly distilled ethyl formylsuccinate (202g) (Wislicenus, A., 1908, 363, 347). After standing for 2 hours at room temperature, the mixture was heated under reflux for a further 2 hours. Ethyl acetate (ca 250c.c.) was added, the

mixture again heated to boiling, filtered from sodium chloride and allowed to cool. The product separated as fine colourless needles m.p. 178°, after recrystallisation from ethyl acetate or alcohol. (Found: C,55.4; H,6.1; N,14.9; $C_9H_{12}O_3N_2$ requires C,55.1; H,6.1; N,14.3) (Yield: 54%).

4-Hydroxy-2-methylpyrimidine-5-acethydrazide. On heating a mixture of the above ester (100g) with hydrazine hydrate (135c.c. of 50%) on the water bath for 2 hours the ester dissolved and separation of the hydrazide occurred. It crystallised from alcohol, in which it is sparingly soluble, in heavy colourless prisms m.p. 246° (Found: C,46.5; H,5.7; N,30.7. $C_7H_{10}O_2N_4$ requires C,46.2; H,5.5; N,30.8). (Yield: 80-85%).

The hydrazide may also be obtained in approximately the same yield by heating the ester with 70% hydrazine hydrate solution for a short time, or by warming for 5 hours in alcoholic solution with hydrazine hydrate.

4-Hydroxy-5-urethanomethyl-2-methylpyrimidine. The above hydrazide (20g) was suspended in absolute alcohol (300c.c.) containing hydrogen chloride (6g). To the cold mixture was added amyl nitrite (19.3g)

and the whole warmed to 50-60° until evolution of nitrogen ceased (about 1 hour). During the heating the hydrazide slowly dissolved and a jelly-like substance separated. After cooling, ether was added to precipitate the remainder of the product. The jelly obtained was filtered, and the residue dried in a desiccator; this product, the urethane hydrochloride had m.p. 209° (Yield, 98%.)

The urethane prepared from the hydrochloride by treatment with alcoholic ammonia crystallises from ethyl acetate in colourless needles m.p. 173°. (Found: C,51.3; H,6.2; N,19.9. $C_9H_{13}O_3N_3$ requires C,51.2; H,6.2; N,19.9).

4-Hydroxy-5-aminomethyl-2-methylpyrimidine (XXIV).- The urethane hydrochloride (5g) was heated with concentrated hydrochloric acid (50c.c.) in a sealed tube at 100° during 2 hours. The clear solution was evaporated to small bulk in vacuo, and ether added; the hydrochloride of the desired base separated in colourless needles. Recrystallised from absolute alcohol, it had m.p. 278-282° (Found: c,41.6; H,6.2; Cl,20.9. $C_6H_9ON_3$ HCl requires C,41.0; H,5.7; Cl,20.2). (Yield, quantitative.) Further purification could not be effected by crystallisation

The corresponding hydrobromide m.p. 270° can readily be prepared, in quantitative yield, by heating the urethane with strong hydrobromic acid (60%) on the water bath for 3 hours. The free base could not be crystallised, but it yielded stable thioformyl and acetyl derivatives.

4-Hydroxy-5-thioformamidomethyl-2-methylpyrimidine. To a solution of the amine hydrochloride in water were added potassium carbonate (1 equiv.), and excess of potassium dithioformate. In a few minutes the thioformyl derivative separated; it crystallised from water in colourless platelets m.p. 199-200°. (Found: C,45.7; H,5.4; S,17.4. $C_7H_9ON_3S$ requires C,45.9; H, 4.9; S,17.4).

4-Hydroxy-5-acetamidomethyl-2-methylpyrimidine. A mixture of amine hydrochloride (350mg) fused sodium acetate (350mg) and acetic anhydride (5c.c.) was heated under reflux for 30 minutes, evaporated to dryness in vacuo, and the residue extracted with chloroform, filtering from inorganic material. After removal of the chloroform the residue crystallised from dioxane in colourless prisms m.p. 219-220°. (Found: N,23.0. $C_8H_{11}O_2N_3$ requires N,23.3).

4-Hydroxy-5-hydroxymethyl-2-methylpyrimidine (XXV).

After a series of experiments using various methods of deamination the following was adopted as being the most reliable. To the hydrochloride of the amine (XXIV) (5g) dissolved in dilute hydrochloric acid (ca 60c.c. of 5%), is added, drop by drop, a concentrated aqueous solution of sodium nitrite (15g). The mixture is now heated in an open flask on the water bath for 7 hours, and the brownish, slightly alkaline solution then submitted to continuous extraction with ethyl acetate in a liquid Soxhlet apparatus. The crude product, which separates from ethyl acetate solution in reddish crusts, is collected and extracted with a large quantity of boiling dioxan which leaves behind a quantity of insoluble by-products; these have not been further examined, but appear to explode when heated in the dry state. The dioxan solution on concentration deposits 4-hydroxy-5-hydroxymethyl-2-methyl-pyrimidine in colourless needles; a further small amount may be obtained by precipitation of the mother liquor with petroleum ether, and treatment of the precipitate with dioxan as above described. After recrystallisation from dioxane

the substance has m.p. 215-216°. (Found: N,20.2. $C_6H_8O_2N_2$ requires N,20.0) (Yield, 1g).

4-Chloro-5-chloromethyl-2-methylpyrimidine (XXI).- The above hydroxy-methyl compound (1g) was heated with phosphoryl chloride (4c.c.) at 115-120° during 20 minutes, when it slowly dissolved to a brownish solution. After removal of the phosphoryl chloride in vacuo, the thick residue was treated with ice-water, the mixture made alkaline with potassium carbonate and extracted 4 times with ether; on removal of the ether, the residue set to a mass of crystals. Recrystallised from a small quantity of petroleum ether (b.p. 40-60°) the product formed long prisms m.p. 54°. (Found: Cl,39.7. $C_6H_6N_2Cl_2$ requires Cl,40.1) (Yield 0.65g).

2-Amino-4-methyl-5-β-hydroxethylthiazole (XXII).- A mixture of methyl α-chloro-γ-hydroxypropyl ketone (3g) and powdered thiourea (17g) was heated to 100°; within a few minutes a violent reaction occurred which quickly subsided. After a further 5 minutes the mixture was cooled, dissolved in water, and any unchanged halogenated ketone removed by extraction with ether. After making strongly alkaline the

the thiazole base was extracted with a large amount of ether. The extract, dried over sodium sulphate and evaporated, left a residue which distilled as a pale yellow oil at 172-175°/2mm. (Yield, 2.5g). On keeping for several weeks the oil set to a hard crystalline mass m.p. 85-90°. The base was not further purified; treatment with etheral picric acid yielded a picrate crystallising from alcohol in pale yellow needles m.p. 213° (Found: N,17.5; S,7.8. $C_{12}H_{13}O_8N_5S$ requires N,18.1; S,8.2)

Thiochrome (II). - A mixture of 4-chloro-5-chloromethyl-2-methyl-pyrimidine (XXI) (580mg) and 2-amino-4-methyl-5-β-hydroxyethyl-thiazole (XXII) (470mg) was heated on an oil bath to 110°. The initially clear liquid suddenly began to become cloudy, and after some 15 minutes was opaque and viscous. The brown melt was cooled, and extracted with ether to remove any unchanged starting material, then dissolved in water (ca 15c.c.) and the solution made alkaline with cold sodium hydroxide solution. After filtering from a cream coloured amorphous precipitate, the yellowish solution, which showed strong blue fluorescence, was extracted with butyl alcohol until the extracts were no longer

fluorescent. The combined butyl alcohol extracts were now shaken three times with dilute hydrochloric acid (1%) when the fluorescence disappeared. The greenish yellow aqueous acid extracts were combined, and evaporated to dryness in vacuo at 30-40°, and the residue made strongly alkaline by addition of a small amount of 12% methyl alcoholic potassium hydroxide. The mixture was shaken three times with chloroform (total volume 750c.c.) adding a few drops of water to bring inorganic matter into solution, and facilitate separation. The intensely blue fluorescent chloroform extracts were combined, dried rapidly over potassium carbonate and evaporated to small bulk (ca 8c.c.) in vacuo. On cooling, thiochrome separated in pale yellow flakes. Recrystallised from chloroform it had m.p. 225-226° (uncorr.) (Found: C,55.3; H,5.7; N,21.0. $C_{12}H_{14}ON_4S$ requires C,54.9; H,5.3; N,21.4.)

Comparison of Synthetic Thiochrome and Thiochrome from Aneurin. Both substances had the same m.p. 225-226° (uncorr.) and a mixed m.p. showed no depression. The crystalline form in both cases was identical and no divergence could be detected in their fluorescent properties. For further evidence

of identity I am indebted to Dr. A.E. Gillam of Manchester University who kindly compared the absorption of the two substances. Natural and synthetic thiochrome showed virtually identical absorption maxima at 368 and 369 mμ respectively. The spectroscopic evidence therefore indicates that the two substances are qualitatively and, in so far as could be ascertained with the available material, quantitatively identical.

2:4-Dichloro-5-chloromethyl-6-methylpyrimidine. - On heating a mixture of 2:6-dihydroxy-5-hydroxymethyl-6-methylpyrimidine (6g) (Kircher, A., 1911, 385, 293.) and phosphoryl chloride (15c.c.) under reflux during 30-40 minutes, the pyrimidine dissolved to give a deep brown solution. After removing excess of phosphoryl chloride in vacuo the thick residue was triturated with ice water made alkaline with potassium carbonate and extracted with ether. The ether extract on evaporation left a brownish resin which was dissolved as far as possible in petroleum ether (b.p. 40-60°) filtered from amorphous impurities and the filtrate again evaporated. The residue crystallised from a small volume of petroleumether in heavy colourless prisms m.p. 38-39° (Found: Cl,50.4.

$C_6H_5N_2Cl_3$ requires Cl,50.4). (Yield, 3g).

3:7-Dimethyl-9-chlorothiochromine (XXVI; R=H). A mixture of the above trichloro-compound (1.3g) and 2-amino-4-methylthiazole (0.7g) (Traumann, A., 1888,249, 38.) was heated to 110° for ca 15 minutes, when the initially, clear liquid mixture became brown and viscous. After removing any unchanged starting material from the product with ether, it was dissolved in a little water, made strongly alkaline, and filtered from a cream coloured amorphous precipitate. The yellowish, blue-fluorescent filtrate was extracted with butyl alcohol and the fluorescent substance isolated by a process exactly analogous to that described above for thiochrome. Recrystallised from chloroform the product formed pale yellow, woolly needles m.p. 291-292° with decomposition. (Found: C,47.3; H,3.8; N,22.1; S,12.5; Cl,14.1. $C_{10}H_9N_4SCl$ requires C,47.5; H,3.6 N,22.2; S,12.6; Cl,14.1).

The compound is soluble in water and alcohol, sparingly so in chloroform, and practically insoluble in ether and acetone. In neutral or alkaline solution it has an intense blue fluorescence similar to, but slightly stronger than, that of

thiochrome; as with the latter substance, addition of acid causes the blue fluorescence to disappear the acid solution being greenish yellow.

2-β-Hydroxyethyl-3:7-dimethyl-9-chlorothiochromine (XXVI; R=CH_2CH_2OH).

2:4-Dichloro-5-chloromethyl-6-methylpyrimidine (1g) and 2-amino-4-methyl-5-β-hydroxyethylthiazole (650mg) were heated together at 110° during 15 minutes, and the brown resin which was produced was worked up exactly as described above for thiochrome. The product crystallised from chloroform in pale yellow platelets m.p. 260-261° with decomposition. (Found: S,10.4; $C_{12}H_{13}ON_4SCl$ requires S,10.8).

The substance was closely similar to thiochrome in its solubilities and the blue fluorescence of its neutral or alkaline solutions was very similar to that shown by the latter compound under the same conditions; addition of acid caused disappearance of blue-fluorescence and formation of a greenish yellow solution.

Synthesis of Aneurin.

4-Hydroxy-5-cyano-2-methylpyrimidine(XXXII.) To an ice-cold solution of sodium (10.2g) in absolute alcohol (300cc), was added acetamidine hydrochloride

(41.4g), the mixture shaken for a few minutes and quickly filtered from precipitated sodium chloride. To the cooled filtrate was added ethyl ethoxymethylene-cyanacetate (75g) (De Bollemont, 1899, C.r. 128, 1340. Bull.Soc.Chim., (31, 25, 20) in portions, with shaking. As the ester went into solution a yellow colour developed and almost immediately a crystalline substance began to separate. After standing overnight at 0^{o} the precipitate was collected; it crystallised from ethyl acetate in colourless needles m.p. 108-110^{o} (Found: C,52.7; H,6.3; N,23.1 $C_8H_{11}O_2N_3$ requires C,53.0; H,6.1; N,23.2). This product may be ethyl α-cyano-β-acetamidino-acrylate. Yield 37g.

The above intermediate product (36g.) was heated on the water bath for 5 minutes with a solution of sodium hydroxide (9g.) in water (360cc.). The yellow solution was cooled, acidified with acetic acid and concentrated in vacuo, to about half the original volume. On standing, 4-hydroxy-5-cyano-2-methylpyrimidine separated, it crystallised from water in fine colourless needles or rods m.p. 233-235^{o}. (Found: C,53.3; H,4.0; N.30.8. $C_6H_5ON_3$ requires C,53.3; H,3.7; N,31.1) Yield, 9g.

Efforts were made to cause direct production of the pyrimidine so as to avoid, if possible, the losses involved in the ring closure of the intermediate ester with sodium hydroxide; for this purpose condensations were made at various temperatures using varying amounts of sodium ethoxide, but without satisfactory results.

4-Chloro-5-cyano-2-methylpyrimidine (XXXIII). When 4-Hydroxy-5-cyano-2-methylpyrimidine (5g.) was heated under reflux with phosphoryl chloride (15cc) during 30 minutes, most of the material dissolved up to give a dark brown solution. After removing the $POCl_3$ in vacuo the mixture was poured into ice water, neutralised with potassium carbonate, and extracted with ether. After drying the extract over sodium sulphate and removing the solvent, the chloro-product remained as a reddish yellow resin; in this condition it was pure enough for amination purposes. Recrystallised from light petroleum it formed long colourless rods m.p. 63-64° (Found: Cl, 22.6. $C_6H_4N_3Cl$ requires Cl, 23.1). Yield, 60-70%

4-Amino-5-cyano-2-methylpyrimidine (XXXIV) The above chloro-product (2g) was heated with absolute alcoholic ammonia (6c.c. saturated at 0°) in a sealed

tube at 100° during 4 hours. After removal of the alcohol and ammonia in vacuo the residue was boiled with ca 100cc. chloroform, filtered from ammonium chloride and evaporated. On recrystallising from methyl alcohol the product formed colourless needles m.p. 249° with partial sublimation Grewe (loc.cit.) gives m.p. 249° (Found: C,54.0; H,4.8. Calc. for $C_6H_6N_4$: C,53.7; H,4.5). Yield 40%

Ethyl 4-Hydroxy-2-methylpyrimidine-5-carboxylate (XXXVII). To a solution of sodium (12.8g) in absolute alcohol (500cc) at 0° were added acetamidine hydrochloride. (26.3g) and ethyl ethoxymethylene-malonate (60g.). (Claisen, Ber., 1893, 26, 2731). After standing for 1 hour at room temperature the mixture was heated on a water bath under reflux for a further hour. After removing most of the alcohol in vacuo the residue was diluted with water, and unchanged ester removed by extraction with ether. The aqueous solution was now acidified with acetic acid and the pyrimidine extracted with ethyl acetate. The residue obtained on evaporating the dried ethyl acetate solution crystallised from acetone in long woolly needles m.p. 191°. (Found: C,52.8; H,5.9. $C_8H_{10}O_3N_2$ requires C,52.7; H,5.5). Yield 60%

Ethyl 4-Amino-2-methylpyrimidine-5-carboxylate. (XXXVIII). A mixture of the above ester (96g.) and phosphoryl chloride (250cc) was heated under reflux for 30 minutes. The red solution so formed was evaporated in vacuo to remove phosphoryl chloride, the resinous residue treated with a little ice water, made alkaline with potassium carbonate, and extracted with chloroform. The dried chloroform solution on evaporation left the crude chloro-ester as a reddish oil, which without further purification was heated in an autoclave at 100° during 3 hours, with 10 times its volume of absolute alcoholic ammonia (4N).

After cooling, the alcohol and excess ammonia were removed under reduced pressure and the residue recrystallised several times from water. The product formed long colourless needles m.p. 120°. (Found: N,23.2. $C_8H_{11}O_2N_3$ requires N,23.2) Yield, 65%

4-Amino-2-methylpyrimidine-5-carboxylic acid amide (XXXIX). The above finely powdered amino-ester (50g.) was shaken at room temperature with concentrated aqueous ammonia (320cc; S.G. ·880) during 36 hours. The needle shaped crystals of

starting material disappeared gradually, although no apparent dissolution was observed. After filtering from ammonia, the solid material was recrystallised from absolute alcohol. Small prisms m.p. 264-265° (Found: N,36.7: $C_6H_8ON_4$ requires N,36.8). Yield, 65%. A further small quantity can be obtained by concentrating the ammoniacel mother liquors.

4-Amino-5-cyano-2-methylpyrimidine (XXXIV). The above amide (2g) was heated under reflux with phosphoryl chloride (15cc) during 2-3 hours, the mixture then poured on ice, made alkaline with potassium carbonate, and extracted with chloroform. After drying over sodium sulphate the chloroform was removed; the residue crystallised from methyl alcohol in needles m.p. 249° with partial sublimation. A,mixed m.p. with another sample of 4-amino-5-cyano-2-methyl pyrimidine showed no depression. Yield, 50%

When large quantities of material were used in this preparation the yield of product diminished considerably; this may be due to the insolubility of the amide in phosphorylchloride, and phosphorus pentachloride may be preferable on the large scale.

4-Amino-5-aminomethyl-2-methylpyrimidine hydrochloride.

The above amino-nitrile in acetic acid solution was subjected to catalytic hydrogenation in presence of palladised charcoal (cf. Grewe, Z.physiol.Chem., 1936, 243, 89). The product had m.p. 264-265°. The same result was achieved using a platinum oxide catalyst though the reduction was slower.

4-Amino-5-thioformamidomethyl-2-methyl pyrimidine (XXIX). An aqueous solution of the above hydrochloride was neutralised with potassium bicarbonate and a slight excess (ca. 1.2mol) potassium dithioformate added. After a short time the thioformyl derivative separated. It crystallised from alcohol in colourless platelets m.p. 187°. with decomposition (Found: C,46.1;H5.5. $C_7H_{10}N_4S$ requires C, 46.1; H,5.5).

Aneurin Chloride. A mixture of 4-amino-5-thioformamido-methyl-2-methylpyrimidine (500mg) and methyl α-chloro-γ-acetoxypropylketone (600mg.)was heated in a paraffin bath during 15 minutes. The mixture first became liquid and then became brownish and viscous, a thiazole-like odour becoming noticeable. The mass was cooled and triturated

repeatedly with dry ether when it fell to a yellowish brown powder. This was collected and heated with ca 3cc absolute alcohol containing a little hydrogen chloride; after a few minutes the product began to crystallise, without having completely dissolved. After cooling it was collected and separated from a small amount of sparingly soluble 4-amino-5-aminomethyl-2-methylpyrimidine hydrochloride by fractional crystallisation from absolute alcohol. The product had m.p. 233-234° unchanged by recrystallisation. (Found: C, 40.5; H, 6.0; N,15.5; S,8.5; Cl, 20.1. $C_{12}H_{18}ON_4SCl_2 \cdot H_2O$ requires C,40.6; H,5.6; N,15.8; S,9.0; Cl, 20.0).

The same product could be obtained, though in smaller yield, by using methyl α-chloro-γ-hydroxypropylketone in place of its acetate in the above reaction, the condensation in this case being carried out at 140°.

Comparison of Natural and Synthetic Aneurin Chloride.

Examined qualitatively by the formaldehyde-azo-test and the thiochrome test no difference in the behaviour of the two products could be detected. Tested biologically by the electrocardiagraphic method the synthetic material showed no activity of

380,000 I.U. per g. and the natural 400,000 I.U. per g.

Treatment with cold aqueous picrolonic acid caused, with each sample, separation of yellow needle like crystals of a picrolonate m.p. and mixed m.p. 164-165°; recrystallisation of this product from water in the ordinary way gave in each case a mixture of needles and prisms with a rather indefinite m.p. ca 170-180° not depressed on mixing. On heating this material for 5 minutes with a small amount of water - insufficient to dissolve it completely - and filtering hot, the residue was found to consist of prisms m.p. 228-229° with decomposition. A mixed m.p. showed no depression.

These results correspond exactly with the data for the dimorphous aneurin picrolonate described by Windaus Tschesche Laqueur and Schultz (Z.physiol. Chem., 1932, 204, 123).

On oxidation with potassium ferricyanide in alkaline solution the synthetic material gave a product m.p. 225-226° identical with thiochrome in all its properties; a mixed m.p. with a specimen of thiochrome showed no depression.

The only apparent difference in the two

chlorides lay in the m.p. for the specimen from natural sources had m.p. 249-250°, the synthetic being 233-234°. A mixture of the two had m.p. 243-246°. On seeding a solution of the synthetic product with a crystal of the natural, a product separated m.p. 245-247°, while a solution of natural vitamin seeded with the synthetic gave crystals m.p. 241-244°. In all cases the crystals appeared to be colourless platelets. In view of these facts and the formation of the dimorphous picrolonate I am of the opinion that aneurin chloride is itself dimorphous.

Barger, G., Bergel, F. & Todd, A.R. (1935) Nature, 136, 259; Ber. 68, 2257.

Bergel, F. & Todd, A.R. (1936) Nature, 138, 76, 406.

Bernal, J.D. & Crowfoot,D (1933) Nature, 131, 911.

Birch, T.W. & Harris, L.J. (1934) Biochem.J., 28, 602.

Birch, T.W. & Harris,L.J. (1935) Nature, 135, 654.

Boyd Barrett, H. & Robinson, R. (1932) J.Chem.Soc. 318.

Buchman, E.R. (1936) J.Amer.Chem.Soc., 58, 1803.

Buchman, E.R. & Williams, R.R. (1935) ibid., 57, 1751.

Carter, C.W. & Drury, A.N. (1929) J.Physiol.Proceedings, 68, i.

Chick, H. & Roscoe,M.H. (1929) Biochem.J., 23, 498.

Clarke, H.T. & Gurin, S. (1935) J.Amer.Chem.Soc. 57, 1876.

Cline, J.K., Williams, R.R., Ruchle, A.E. & Waterman, R.E. (1937) J.Amer.Chem.Soc. 57,1876.

Eijkmann, C. (1890) Geneesk.Tijdschr.Ned. Ind. 30 295; 1896, 36, 214; 1898, 38, 275.

Fischer, E. (1898) Ber., 31, 431.

Funk, C. (1911) J.Physiol., 43, 395; 1912, 45, 75, 481.

Funk, C. (1912) J.State.Med. 20, 341.

Gabriel, S. (1901) Ber., 34, 1254.

Gabriel, S. & Colman, J. (1901) Ber., 34, 1246.

Grewe, R. (1936) Z.physiol.Chem., 242, 89.

Grijns, G. (1901) Geneesk.Tijdschr.Ned. Ind. 41, 3.

Haller, A. & March, F. (1905) Bull.soc.chim., 33, 618; Compt.rend.1908,139, 100.

Hofmann, A.W. (1878) Ber., 11, 339.

Hopkins, F.G. (1912) J.Physiol., 44, 425.

Jansen, B.C.P. (1935) Nature, 135, 267.

Jansen, B.C.P. (1936) Rec.trav.chim.Pays-bas. 55, 1046.

Jansen, B.C.P. & Donath, W.F. (1926) Meded.kon.Akad.Wetensch. Amst.35, No.7; Meded. Dienst.Volksgez.Ned.Ind. 1927, I, 190.

Johns, C.O. (1908) Amer.chem.J., 41, 58.

Kinnersley, H.W., Peters, R.A. & Reader, V. (1928) Biochem.J., 22, 276; ibid. 24, 1820.

Kinnersley, H.W. & Peters, R.A. (1934) ibid., 28, 667.

Kinnersley, H.W., O'Brien, J.R. & Peters, R.A. (1935a) ibid., 29, 701.

Kinnersley, H.W., O'Brien, J.R. & Peters, R.A. (1935b) ibid., 29, 2369.

Kuhn, R., Wagner-Jauregg, T., van Klaveren, F.W. & Vetter, H. (1935) Z.physiol.Chem., 234,196.

Kuhn, R. & Vetter, H. (1935) Ber., 68, 2383.

McCollum, E.V. & Davis, M. (1913) J.Biol.Chem., 15, 167.

McCollum, E.V. & Davis, M. (1915) J.Biol.Chem., 21, 179; 23, 181.

McCollum, E.V. & Kennedy, C. (1916) ibid., 24, 491.

Makino, K. & Imai, T.I. (1936) Z.physiol.Chem., 239, I.

Moggridge, R.C.G. & Ogston, A.G. (1935) Biochem.J., 29, 866.

Ochiai, (1936) Ber., 69, 1650.

Ogston, A.G. & Peters, R.A. (1936) Biochem.J., 30, 736.

Ohdake, S. (1932) Bull.agr.chem.Soc.Japan, 8, Nos.1-3, 7-9.

Osborne, T.B. & Mendel, L.B. (1913) J.Biol.Chem., 15, 311.

Passmore, R., Peters, R.A. & Sinclair, H.M. (1933) Biochem.J., 27, 842.

Pawlewski, B. (1935) Nature, 135, 107.

Peters, R.A. & Thompson, R.H.S. (1934) J.Physiol. 81, 22P.

Ritchie Russell,W. (1936) Lancet, 230, 727; Edinburgh Med.J., 43, 315.

Seidell, A. (1921) Ind.Eng.Chem., 13, 1111; U.S. Pub.Health Rep, 1922, 37, 801.

Schopfer, W.H. & Jung, A. (1935) Verh.der freien Verein. Schweiz.Physiologie. 8 Sitzg., Geneva, June 1935, p.6.

Smith, M.E. & Hendrick, E.G. (1926) Pub.Health Rep.Wash. 41, 201.

Suzuki, V., Shinamura, T. & Ohdake, S. (1912) Biochem.Zeitschr., 43, 89.

Takaki, K. (1885) Sei-I-Kwai, August 1885; April 1886; ibid, 6, 73. Cf. also Lancet, 1906,i,1361, 1451, 1520.

Todd, A.R., Bergel, F. & Karimullah. (1936) Ber., 69, 217; J.Chem. Soc., p. 1557.

Todd, A.R., Bergel, F. & Jacob, A. (1936) J.Chem.Soc., p. 1555.

Todd, A.R., Bergel, F., Fraenkel-Conrat, H. & Jacob, A. (1936) ibid., p. 1601.

Todd, A.R., Bergel, F., Karimullah & Keller, R. (1937) J.Chem.Soc., p. 361.

Todd, A.R. & Bergel, F. (1937) ibid., p. 364.

van Veen, A.G. (1930) Rec.trav.chim.Pays-Bas, 49, 1178; 50,200,208. 610; 1932, 51, 265, 273. Z.physiol.Chem., 1932, 208, 125.

Williams, R.J. (1919) J.Biol.Chem., 38, 465.

Williams, R.R. (1935) J.Amer.Chem.Soc., 57, 229. Details of this work are given in the following papers: Williams, R.R., Waterman, R.E., Keresztesy, J.C. & Buchman, E.R., ibid, 57, 536; Williams, R.R., Buchman, E.R. & Ruehle, A.E., ibid., 57, 1095; Buchman, E.R., Williams, R.R. & Keresztesy, J.C. ibid., 57, 1849.

Williams, R.R. (1936) J.Amer.Chem.Soc., 58, 1063.

Williams, R.R. & Cline, J.K. (1936) ibid., 58, 1504.

Williams, R.R.& Cline, J.K. (1937) ibid., 59, 216.

Williams, R.R. & Ruehle, A.E. (1935) ibid., 57, 1856.

Williams, R.R., Waterman, R.E. & Keresztesy, J.C. (1934) J.Amer.Chem.Soc., 56, 1187.

Williams, R.R., Waterman, R.E. & Keresztesy, J.C. (1935) Science, 81, 535.

Windaus, A., Tschesche, R., Ruhkopf, H., Laquer, F. & Schultz, I. (1931) Nachr.Ges.Wiss.Göttingen (Math.Phys.Klasse),p.207; Z.physiol.Chem., 1932, 204, 123.

Windaus, A., Tschesche, R. & Ruhkopf, H. (1932) Nachr.Ges.Wiss.Göttingen 342.

Windaus, A., Tschesche, R. & Grewe, R. (1934) Z.physiol.Chem., 228, 27.

Windaus, A., Tschesche, R. & Grewe, R. (1935) ibid., 237, 98.

Wintersteiner, O., Williams, R.R. & Ruehle, A.E. (1935) J.Amer.Chem.Soc. 57, 517.

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A Crystalline Fluorescent Dehydrogenation Product from Vitamin B_1

THE production of fluorescent solutions on oxidation of vitamin B_1 (antineurin) has been indicated in these columns by Peters[1], but no crystalline fluorescent product has hitherto been reported.

An alkaline solution of potassium ferricyanide transforms the vitamin hydrochloride ($C_{12}H_{18}ON_4SCl_2$) into a pale yellow, sulphur-containing compound (crystals m.p. 221°, from chloroform) having, in neutral or alkaline solution, an intense blue fluorescence; it possesses all the recorded properties of the 'thiochrome' ($C_{12}H_{14}ON_4S$) of Kuhn and his colleagues[2], including a similar absorption spectrum. This result is to us the more interesting, as thermal decomposition of the vitamin also yields a blue fluorescent compound[3], $C_9H_{10}ON_4$, which may have a related constitution.

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July 31.

[1] NATURE, **135**, 107; 1935.
[2] R. Kuhn, Th. Wagner-Jauregg, F. W. van Klaveren and H Vetter, *Z. physiol. Chem.*, **234**, 196; 1935.
[3] G. Barger, B. C. P. Jansen, and A. R. Todd, *Chem and Ind.*, **54**, 596; 1935.

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Über das Thiochrom aus Vitamin B_1 (Antineurin)

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SONDERABDRUCK AUS:
BERICHTE DER DEUTSCHEN CHEMISCHEN GESELLSCHAFT

VERLAG CHEMIE G. M. B. H.
BERLIN

443. G. Barger, F. Bergel und A. R. Todd*): Über das Thiochrom aus Vitamin B_1 (Antineurin).

[Aus d. Medizin.-chem. Institut d. Universität Edinburgh.]

(Eingegangen am 4. November 1935.)

Vor einiger Zeit haben R. Kuhn, Th. Wagner-Jauregg, F. W. van Klaveren und H. Vetter[1]) über die Isolierung eines gelben Farbstoffes aus Hefe berichtet, der in Lösung eine intensive blaue Fluorescenz zeigt. Sie stellten dafür die Bruttoformel $C_{12}H_{14}ON_4S$ auf und schlugen den Namen „Thiochrom" vor. Es wurde die Vermutung ausgesprochen, daß in dieser Substanz ein Dehydro-vitamin B_1 vorliegen könnte, da seine Bruttoformel eine große Ähnlichkeit mit der des Vitamins ($C_{12}H_{18}ON_4SCl_2$) aufweist und die Hefe verhältnismäßig reich an diesem Vitamin ist. Ein solcher Zusammenhang schien umsomehr wahrscheinlich, als fluorescierende Substanzen bereits unter den oxydativen (in Lösung)[2]) und thermischen[3]) Abbauprodukten des Vitamins beobachtet worden waren.

Da, wie bekannt, es bisher nicht gelungen war, das Molekül des Vitamins unter milden oxydativen Bedingungen in krystallisierte fluorescierende Derivate überzuführen, nahmen wir die Untersuchungen in dieser Richtung auf, in der Hoffnung, wenigstens Substanzen zu finden, die Ähnlichkeit mit dem schwer zugänglichen thermischen Abbauprodukt besitzen würden.

Wie schon an anderer Stelle[4]) kurz berichtet, ist es gelungen, das Vitamin B_1 (= Antineurin, nach Jansens Vorschlag) mit einer Ausbeute von 33—40% in alkalischer Lösung bei 15—20° mit Kaliumferricyanid in eine Substanz von der Formel $C_{12}H_{14}ON_4S$ zu verwandeln, die sich mit Thiochrom als identisch erwies. Auch an dieser Stelle möchten wir Hrn. Prof. R. Kuhn, Heidelberg, unseren besten Dank dafür aussprechen, daß er freundlicherweise die angenommene Identität unseres Produktes mit seinem Thiochrom aus Hefe durch direkten Vergleich bestätigte (vergl. experimentell. Teil).

Zur Darstellung von Thiochrom ist es nicht unbedingt nötig, von reinem krystallisierten Antineurin auszugehen. Bereits mehr oder weniger vorgereinigte Konzentrate aus Reis-Häutchen können auf die gleiche Weise wie das reine Antineurin zum Farbstoff verarbeitet werden. Doch zeigen die Ausbeuten dieser Versuche eine große Diskrepanz zu den möglichen, die sich aus dem durch biologischen Test bestimmten Gehalt an Antineurin errechnen lassen. Das Auftreten von blauer Fluorescenz bei der Oxydation von Konzentraten mit Kaliumferricyanid kann als orientierender Test für Antineurin besonders im ultravioletten Licht verwendet werden; doch geben erst verhältnismäßig hochkonzentrierte Extrakte, wie das auch bei Peters' Formaldehyd-Azotest der Fall ist, reproduzierbare Resultate. Auch andere Oxydationsmittel, wie Wasserstoffsuperoxyd, Selendioxyd, Kaliumpermanganat, sind imstande, Antineurin zum Thiochrom zu oxydieren, allerdings mit unbefriedigenden Ergebnissen. Blau fluorescierende Lösungen, die in ihren Eigenschaften mit Lösungen von reinem Thiochrom identisch sind, können bereits durch autoxydative Einwirkung von Luft oder Sauerstoff

*) Beit Memorial Research Fellow.

[1]) Ztschr. physiol. Chem. **234**, 196 [1935].

[2]) R. A. Peters, Nature **135**, 107 [1935].

[3]) G. Barger, B. C. P. Jansen u. A. R. Todd, Chem. and Ind. **54**, 596 [1935].

[4]) G. Barger, F. Bergel u. A. R. Todd, Nature **136**, 259 [1935].

auf die Lösungen der freien Vitaminbase entstehen, was auch das Vorkommen des Farbstoffes in der Hefe erklären könnte.

Die Existenz eines quartären Stickstoffatoms im Antineurin scheint ziemlich gesichert. Weisen doch seine allgemeinen Eigenschaften, die Sulfit-Spaltung von Williams[5]), seine thermische Zersetzung[6]) und die Resultate der elektrometrischen Titration[7]) darauf hin, trotzdem letztere von den verschiedenen Autoren auf verschiedene Weise ausgedeutet werden. Unter dieser Voraussetzung sollte die Bruttoformel des Vitamin-Hydrochlorids $[C_{12}H_{17}ON_4S]Cl$, HCl, und die der freien Base, $C_{12}H_{18}O_2N_4S$ lauten. Deshalb ist es unmöglich, die Bildung von Thiochrom als eine einfache Dehydrierungs-Reaktion zu betrachten. Unterscheidet sich doch seine Formel von der des Vitamins um H_4O, d. i. H_2O+H_2. Diese Überlegung findet ihre Stütze in der Tatsache, daß das Thiochrom im biologischen Versuch (Herzfrequenz-Methode) sich als vollkommen inaktiv erweist[8]) und bei der katalytischen Hydrierung nicht in Antineurin zurückverwandelt wird.

Unähnlich dem Vitamin, sublimiert Thiochrom unverändert im Hochvakuum bei 210—215°. Dies legt, neben seiner Löslichkeit in organischen Lösungsmitteln, die Vermutung nahe, daß es kein quartäres Stickstoffatom mehr besitzt. Sein Hydrochlorid hat die ungewöhnliche Eigenschaft, sich zuerst in farblosen Krystallen abzuscheiden, um dann beim Filtrieren unter Entfernung der letzten Spuren des Lösungsmittels deutlich gelb zu werden. Eine Erklärung hierfür zu geben, ist schwierig. Eine Wasser-Anlagerung liegt nicht vor, da die gelbe Farbe auch in der Trockenpistole nicht verschwindet. Die Färbung hat vielleicht mit der Tatsache zu tun, daß die Analysen-Werte des Thiochrom-Hydrochlorids für Chlor zwischen einem und zwei Atomen liegen. Es muß also ein teilweiser Verlust an HCl bereits an der Luft eingetreten sein, was auf eine äußerst schwache basische Gruppe neben einer normalen schließen läßt.

Im Antineurin selbst ist nach allen vorliegenden Ergebnissen[9]) neben dem quartären Stickstoff noch eine an einem Pyrimidinring gebundene NH_2-Gruppe vorhanden. Diese Aminogruppe wird quantitativ durch konz. Salzsäure bei 100° als Ammoniak abgespalten. Unter gleichen Bedingungen gibt Thiochrom kein Ammoniak ab, sondern wird zum größten Teil unverändert wiedergewonnen. Außerdem läßt sich das desaminierte Vitamin, das in Lösung erhalten wurde und wahrscheinlich verwandt mit dem von Williams beschriebenen Chloro-oxy-vitamin[10]) ist, durch Kaliumferricyanid nicht in blau fluorescierende Produkte verwandeln. Daraus ergibt sich, daß diese Aminogruppe des Vitamins sicher an der Bildung des Thio-

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[5]) R. R. Williams, R. E. Waterman, J. C. Keresztesy u. E. R. Buchman, Journ. Amer. chem. Soc. **57**, 536 [1935]. [6]) a. a. O.

[7]) T. W. Birch u. L. J. Harris, Nature **135**, 654 [1935]; R. C. G. Moggridge u. A. G. Ogston, Biochem. Journ. **29**, 866 [1935]; R. R. Williams u. A. E. Ruehle, Journ. Amer. chem. Soc. **57**, 1856 [1935].

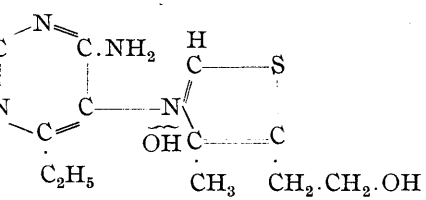
[8]) Nach einer gerade erschienenen, etwas spekulativen Mitteilung von H. W. Kinnersley, J. R. O'Brien u. R. A. Peters, Biochem. Journ. **29**, 2369 [1935], soll eines der nicht isolierten „Quinochrome" noch Vitamin-Wirkung zeigen, was wohl auf einer Überschätzung der Permanganat-Oxydation (in Gegenwart von Alkohol!) beruhen dürfte.

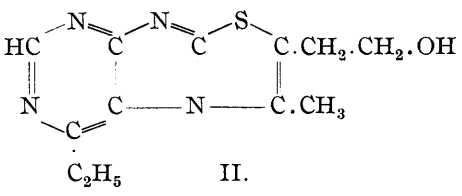
[9]) vergl. R. R. Williams, E. R. Buchman u. A. E. Ruehle, Journ. Amer. chem. Soc. **57**, 1093 [1935].

[10]) E. R. Buchman u. R. R. Williams, Journ. Amer. chem. Soc. **57**, 1751 [1935].

chroms beteiligt ist. In diesem Zusammenhange mag erwähnt werden, daß auch Peters' Formaldehyd-Azotest von der Existenz dieser Gruppe abhängt, da er beim desaminierten Vitamin und dem Thiochrom völlig negativ ausfällt.

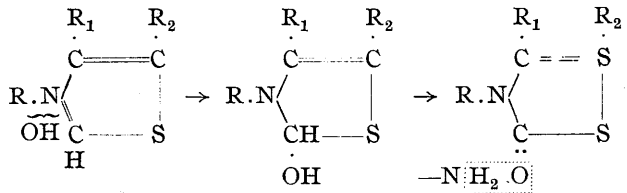
Die blaue Fluorescenz des Thiochroms ist ähnlich der des 6.7-Dimethyl-alloxazins[11]). Es ist daher nicht ohne weiteres von der Hand zu weisen, daß Thiochrom ein Pyrimidazin-Derivat ist, um so mehr als Antineurin nach Williams von einem *o*-Diamino-pyrimidin abgeleitet werden kann. Wie bekannt, schlug Williams vor einiger Zeit[12]) für das Vitamin die Konstitutionsformel I vor. Bei näherer Betrachtung ist es jedoch schwierig, sich





II.

daraus die Bildung eines sechsgliedrigen Azinringes mit Hilfe von Kaliumferricyanid vorzustellen, in Anbetracht der Tatsache, daß der Schwefel im Thiochrom-Molekül erhalten bleibt. Es besteht keine große Wahrscheinlichkeit, daß die Methylgruppe des Thiazols einen solchen Ringschluß bewerkstelligt. In Analogie zu Pyridin-Derivaten scheint eher die Oxydation des Vitamins in Form der Pseudobase plausibel, wobei darauffolgend die gebildete Ketogruppe mit der freien Aminogruppe sich kondensieren könnte:



Benützt man Williams Vitamin-Formel I, so sollte die entstandene Verbindung die Struktur II haben. Daraus ergäbe sich auch eine zwanglose Erklärung dafür, daß der Schwefel im Thiochrom zum Unterschied vom Vitamin sich gegen Alkali stabil verhält. Es läge ein kondensiertes Thiazolin-Ringsystem vor, in dem der Schwefel analog dem Sauerstoff im Furan-Cumaron und dem Stickstoff im Pyrrol-Indol eine gewisse Unangreifbarkeit erworben hat. Ob ein solches System Fluorescenz aufweist, kann nur die Synthese ergeben.

Versuche in der Richtung, derartige Verbindungen, aber auch solche mit sechsgliedrigen Azinringen aufzubauen, werden gerade unternommen. Im Verlauf dieser Synthesen entdeckten wir[13]), daß *N*-Alkyl- und *N*-Aryl-thioamide sich verhältnismäßig leicht mit α-Chlor-ketonen umsetzen

[11]) R. Kuhn u. Mitarbeiter, a. a. O.
[12]) R. R. Williams, Journ. Amer. chem. Soc. **57**, 229 [1935].
[13]) Mitbearbeitet von Dr. Karimullah.

und dabei direkt quartäre Thiazoliumsalze liefern. Wir erwähnen dies in diesem Zusammenhange, weil H. T. Clarke und S. Gurin[14]) kürzlich die Darstellung von *N*-Phenyl-thiazoliumsalzen auf demselben Wege beschrieben haben. Unsere Resultate bestätigen und erweitern ihre Beobachtungen. Die Einzelheiten unserer Ergebnisse in dieser Richtung sollen in Kürze an anderer Stelle veröffentlicht werden.

Wir haben Hrn. Prof. B. C. P. Jansen, Amsterdam, für die Anregung zum Studium des Vitamins und die freundliche Überlassung eines Teiles des für unsere Versuche benötigten, krystallisierten Antineurins und dem Medical Research Council für die bewilligten Mittel bestens zu danken.

Beschreibung der Versuche.

Thiochrom aus Antineurin.

Zu einer Lösung von 20 mg Antineurin-Hydrochlorid in 1—2 ccm Methylalkohol werden 2 ccm 15-proz. methylalkohol. Kalilauge und 1 ccm 33-proz. wäßrige Kaliumferricyanid-Lösung hinzugefügt. Dann gibt man 10 ccm Butylalkohol, sowie die für die Lösung der anorganischen Salze nötige Menge Wasser hinzu, schüttelt die Mischung 2 Min. heftig und trennt die blau fluorescierende Butylalkohol-Schicht ab. Diese Extraktion wird mit weiteren Mengen Butylalkohol solange wiederholt, bis der letzte Extrakt keine Fluorescenz mehr zeigt. Die vereinigten butylalkoholischen Auszüge werden mit ein wenig Wasser gewaschen und über Natriumsulfat getrocknet, um die letzten Spuren von Kaliumferricyanid zu entfernen. Schüttelt man die getrockneten Extrakte mit verd. Salzsäure (p_H=2—3), so verschwindet die blaue Fluorenscenz, und eine grünlich-gelbe Substanz geht in die Säure über. Die grün-gelbe saure Lösung wird abgetrennt und im Vakuum (Bad 40°) zur Trockne eingedampft. Der krystallinische Rückstand wird in wenig konz. Kalilauge (25-proz.) gelöst und wiederholt mit Chloroform extrahiert, bis die letzten Auszüge keine Fluorescenz mehr zeigen. Die vereinigten Chloroform-Extrakte, die leuchtend blau fluorescieren, werden über Natriumsulfat und anschließend kurz über Kaliumcarbonat getrocknet. Schließlich wird im Vakuum auf etwa 3—4 ccm eingeengt und in den Eisschrank gestellt. Nach einiger Zeit scheiden sich die schwefelgelben Krystalle des Thiochroms ab, die nach dem Umlösen aus Chloroform den Schmp. 221° (unkorr.) zeigen; Ausbeute 5—6 mg = 33—40% d. Th.

2.664 mg Sbst. (bei 80—100°, 0.1 mm getrockn.): 5.33 mg CO_2, 1.32 mg H_2O. — 1.571 mg Sbst.: 0.292 ccm N (20°, 757 mm). — 2.480 mg Sbst.: 2.220 mg $BaSO_4$.

$C_{12}H_{14}ON_4S$. Ber. C 54.9, H 5.3, N 21.4, S 12.2.
Gef. „ 54.6, „ 5.5, „ 21.6, „ 12.3.

Die Substanz besitzt alle Eigenschaften, die für das Thiochrom von Kuhn und Mitarbeitern[15]) angegeben werden. Der Vergleich, den Prof. Kuhn mit seinem Thiochrom aus Hefe durchgeführt hat, ergab folgende Resultate: Beide Substanzen zeigten den Schmp. 226—227° (Berl-Block, abgekürztes Thermometer); der Misch-Schmp. ergab keine Depression; Absorptionsspektrum[16]) und Fluorescenz-p_H-Kurve waren identisch.

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[14]) Journ. Amer. chem. Soc. **57**, 1876 [1935]. [15]) a. a. O.

[16]) Dr. E. B. Ludlam hatte schon vorher die große Ähnlichkeit der Absorptionsspektra festgestellt, wofür wir ihm bestens danken.

Thiochrom wird auf ähnliche Weise aus vorgereinigten Antineurin-Konzentraten aus Reis-Kleie dargestellt; die Ausbeute an krystallisiertem Material ist aber sehr viel geringer, als wenn man von reinem Vitamin ausgeht; denn es entstehen nebenbei ölige Substanzen von gelber Farbe, die nicht mehr zur Krystallisation gebracht werden können. Schwach alkalische Lösungen des Vitamins, die mit Luft, Sauerstoff, Wasserstoffsuperoxyd und Kaliumpermanganat oder schwach saure, die mit Selendioxyd behandelt werden, ergeben ebenfalls thiochrom-haltige Lösungen, die aber vorläufig zu wenig günstigen Isolierungs-Ergebnissen führten. Um mit Hilfe des Auftretens der blauen Fluorescenz Konzentrate auf Antineurin zu prüfen, hat man nur nötig, den jeweiligen Vitamin-Extrakt mit Butylalkohol auszuschütteln, die abgetrennte wäßrige Lösung alkalisch zu machen und mit Kaliumferricyanid zu versetzen. Eine weitere Extraktion mit Butylalkohol bringt im Falle eines an Thiochrom reichen Konzentrats die blaue Fluorescenz im Butylalkohol schon am Tageslicht zur Erscheinung; im Falle an Thiochrom ärmerer Konzentrate wird die Fluorescenz im ultravioletten Licht sichtbar. Diese Testmethode hat nur qualitativen Wert.

Eigenschaften des Thiochroms.

Thiochrom ist gut löslich in Methylalkohol, ziemlich gut in Wasser, mäßig in Äthylalkohol und ziemlich schwer löslich in Aceton, Chloroform und Äther. Es sublimiert im Hochvakuum unverändert bei einer Temperatur von 210—215°. Nach $^1/_2$-stdg. Erhitzen in 20-proz. Natronlauge zeigt Thiochrom zum Unterschied vom Antineurin keine Nitroprussidnatrium-Reaktion auf S''. Im biologischen Test nach der Herzfrequenz-Methode an Ratten[17]) läßt das Thiochrom in Dosen von 5 γ, 10 γ und 20 γ keine physiologische Aktivität erkennen. Der Formaldehyd-Azotest nach R. A. Peters[18]) ist vollkommen negativ im Falle des Thiochroms und seiner Leukoverbindung, die in Lösung mit Hilfe von Natriumhydrosulfit oder durch katalytische Reduktion (Platin-Kieselgur-Katalysator) erhalten werden kann. Das Leukothiochrom wird sehr leicht zum ursprünglichen Thiochrom zurückoxydiert.

Thiochrom-Hydrochlorid: Zu 8 mg Thiochrom, das in möglichst wenig Methylalkohol gelöst ist, werden das 4-fache Volumen an Aceton und einige Tropfen ätherischer Salzsäure hinzugefügt. Das Hydrochlorid, welches sich zuerst voluminös abscheidet, wird aus einer Mischung von Methylalkohol-Aceton umkrystallisiert. Es bildet fast farblose Nädelchen, die sich selbst beim Trocknen im Exsiccator und in der Trockenpistole deutlich gelb färben.

2.580 mg Sbst.: 1.775 mg AgCl. — 3.471 mg Sbst.: 2.495 mg AgCl.

$C_{12}H_{14}ON_4S, HCl$. Ber. Cl 11.9.

$C_{12}H_{14}ON_4S, 2HCl$. Ber. Cl 21.2.

Gef. „ 17.02, 17.78.

Beim Erhitzen des Hydrochlorids in einem Schmelzpunkts-Röhrchen tritt oberhalb 200° langsame Zersetzung auf, wobei die Substanz bei 217—221° zu einer viscosen, rötlichen Flüssigkeit zusammenschmilzt.

Bei der katalytischen Hydrierung von 2 mg Hydrochlorid in verd. methylalkoholisch-salzsaurer Lösung mit Palladium-Katalysator wird nach

[17]) T. W. Birch u. L. J. Harris, Biochem. Journ. **28**, 602 [1934].

[18]) H. W. Kinnersley u. R. A. Peters, Biochem. Journ. **28**, 667 [1934].

einiger Zeit beim Abdampfen des Lösungsmittels im Vakuum eine sehr hygroskopische, farblose Substanz erhalten, die weder in alkalischer Lösung fluoresciert, noch leicht zum Thiochrom zurückoxydierbar ist. Die weitere Untersuchung dieser Substanz steht noch aus.

Desaminierungsversuche mit Antineurin und Thiochrom.

Antineurin-Hydrochlorid wird mit konz. Salzsäure 4 Stdn. in einem Einschlußrohr auf 100° erhitzt. Die Lösung wird eingeengt, abgekühlt, mit Baryt alkalisch gemacht, das gebildete Ammoniak mittels eines Luftstroms in vorgelegte Schwefelsäure übergetrieben und in üblicher Weise titriert.

5.6 mg Antineurin-Hydrochlorid in 2 ccm konz. Salzsäure.
Ber. für 1 Mol NH_3 0.27 mg. Gef. NH_3 0.28 mg.

Der Formaldehyd-Azotest einer Lösung des desaminierten Vitamins ist negativ. Desgleichen führt die Oxydation einer solchen Lösung mit Kaliumferricyanid zu keinem fluorescierenden Produkt.

Aus 5.18 mg Thiochrom, unter genau den gleichen Bedingungen mit Salzsäure behandelt, konnte kein abgespaltenes Ammoniak erhalten werden. Aus der Lösung ließ sich auf dem üblichen Wege Thiochrom wiedergewinnen, das keine Schmelzpunkts-Depression mit dem Ausgangsmaterial zeigte.

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A. R. TODD, F. BERGEL und KARIMULLAH

Über Aneurin, II. Mitteil.: Über die Synthese von *N*-Aryl-thiazoliumsalzen; über Einzelheiten in der Konstitution des Aneurins und Thiochroms

*

SONDERABDRUCK AUS:
BERICHTE DER DEUTSCHEN CHEMISCHEN GESELLSCHAFT

VERLAG CHEMIE G. M. B. H.
BERLIN

38. A. R. Todd[1]), F. Bergel und Karimullah: Über Aneurin[1a]), II. Mitteil.: Über die Synthese von *N*-Aryl-thiazoliumsalzen; über Einzelheiten in der Konstitution des Aneurins und Thiochroms.

[Aus d. Medizin.-chem. Institut d. Universität Edinburgh.]

(Eingegangen am 21. Dezember 1935.)

a) Über die Synthese von *N*-Aryl-thiazoliumsalzen: Wir haben bereits im ersten Teil[2]) unserer Abhandlungen über die Chemie des Vitamins B_1 (Aneurin) angedeutet, daß kürzlich H. T. Clarke und S. Gurin[3]) in ihrer Veröffentlichung über die Synthese des Thiazol-Spaltstücks aus Aneurin u. a. eine Methode zur Darstellung von *N*-Phenyl-thiazoliumsalzen erwähnen, die wir unabhängig von ihnen ebenfalls gefunden haben, wobei unsere Resultate die der amerikanischen Autoren um einiges erweitern. Da diese Methode für die synthetische Seite des Vitamin-Problems von einiger Bedeutung sein dürfte, möchten wir an der Hand von Modell-Beispielen die Einzelheiten bekanntgeben.

N-Alkyl-thiazoliumsalze, auch die Benzylverbindung, lassen sich leicht durch direkte Einwirkung von Alkylhalogeniden auf Thiazol-

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[1]) Beit Memorial Research Fellow.

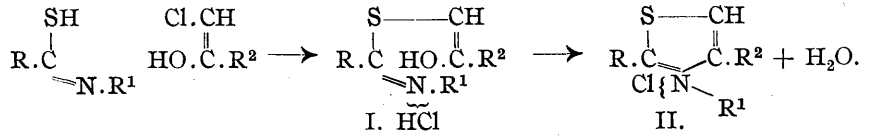
[1a]) Durch einen bedauerlichen Irrtum wurde in der vorhergehenden Veröffentlichung der Name „Antineurin" für das Vitamin B_1 verwendet. Inzwischen hat uns Hr. Prof. Jansen freundlichst darauf aufmerksam gemacht, daß sein Vorschlag „Aneurin" lautete. Um Mißverständnisse zu vermeiden, soll von nun an dieser Name für das Vitamin B_1 gebräuchlich werden.

[2]) B. **68**, 2257 [1935].

[3]) Journ. Amer. chem. Soc. **57**, 1876 [1935].

Derivate darstellen. Alle Bestrebungen jedoch, die analogen *N*-Aryl-Verbindungen auf gleichem Wege aus halogenierten Benzol-Derivaten zu erhalten, schlugen fehl. Dieselben negativen Ergebnisse zeigten in 5-Stellung halogenierte Pyrimidine.

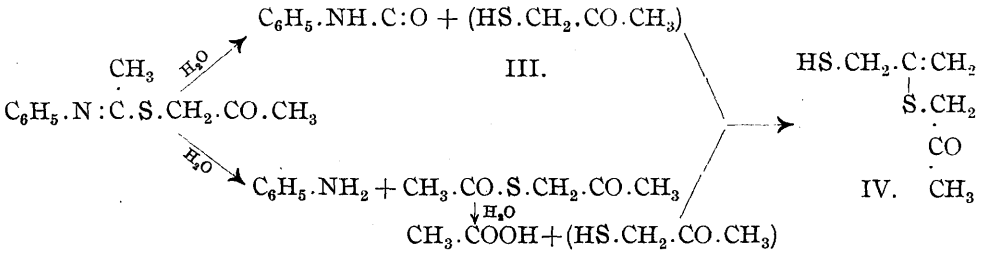
Es wurde als nächstes versucht, die wohlbekannte Hantzschsche Synthese von Thiazolen so zu modifizieren, daß sie zur Darstellung quartärer Thiazoliumsalze führen mußte. Es schien von vornherein nicht unmöglich, daß der Ersatz unsubstituierter Thio-amide in der Hantzschschen Synthese durch Thio-amide von der allgemeinen Formel R.CS.NH.R^1 nach folgendem Schema zur Bildung der gewünschten Thiazoliumsalze führen könnte:



Diese Voraussetzung stellte sich als richtig heraus; die Reaktion ist ganz allgemein anwendbar.

In einem Vorversuch wurden *N*-Methyl-thioacetamid und Chloraceton zusammengebracht, wobei bereits in der Kälte, rascher in der Wärme, in beinahe quantitativer Ausbeute *N*-Methyl-2.4-dimethyl-thiazoliumchlorid (II, R = R^1 = R^2 = CH_3) gebildet wurde. Es wurde in das Jodid verwandelt und mit einem auf dem üblichen direkten Weg dargestellten Produkt identifiziert. Bei diesem Versuche konnte kein Zwischenprodukt, das der Formel I entsprochen hätte, gefaßt werden.

Verwendete man Thio-acetanilid und erhitzte es mit Chlor-aceton auf dem Wasserbade, mit oder ohne Alkohol als Lösungsmittel, so entstand direkt *N*-Phenyl-2.4-dimethyl-thiazoliumchlorid (II, R = R^2 = CH_3; R^1 = C_6H_5). Ließ man dagegen die Reaktion bei 15—20° verlaufen, so erhielt man eine unbeständige krystalline Substanz, die aber durch kurzes Erhitzen in das entsprechende Thiazoliumsalz verwandelt werden konnte. Die Vermutung, daß in diesem Produkt das Hydrochlorid des *S*-Acetonyl-thioacetanilids (I, R = R^2 = CH_3; R^1 = C_6H_5) vorliegt, wurde durch die Analyse und das Verhalten bei der hydrolytischen Spaltung bestätigt. In Wasser oder verd. Mineralsäuren unterliegt nämlich die Substanz, langsam in der Kälte, rascher beim Erhitzen, einer Hydrolyse, die unter Bildung von Anilin, Acetanilid und einer schwefel-haltigen Verbindung, die durch Kondensation eines hypothetischen Thiol-acetons (III) entstanden sein mußte, vor sich geht. Da letztere ein Semicarbazon bildet, mit $HgCl_2$ einen farblosen Niederschlag gibt und Reaktionen auf eine SH-Gruppe liefert, ist sie vielleicht, der Analyse nach, entsprechend Formel IV aufzufassen. Das Auftreten von Acetanilid unter den Endprodukten der Spaltung zeigt, daß der Zerfall interessanterweise nicht nur zwischen N und C, sondern auch zwischen C und S erfolgt:

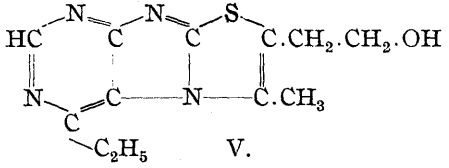


Kondensation von Chlor-aceton mit Thioacet-*o*-toluidid und *o*-Nitro-thioacetanilid führt zu *N*-*o*-Tolyl- bzw. *N*-*o*-Nitrophenyl-2.4-dimethyl-thiazoliumchlorid. Auch hierbei konnten die entsprechenden Zwischenprodukte gefaßt werden.

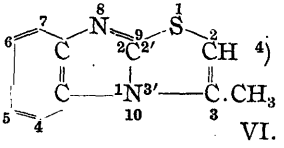
Die quartären Thiazoliumchloride konnten sämtlich isoliert werden, doch ist es angesichts ihrer großen Hygroskopizität besser, zur Isolierung die Jodide und besonders die Perchlorate zu verwenden.

Nähere Angaben über die Darstellung der entsprechenden Pyrimidin-Derivate erfolgen später. Die Synthese der als Ausgangsmaterial nötigen, bisher unbekannten 5-Thioacyl-amino-pyrimidine ist mit gewissen Schwierigkeiten verbunden.

b) Über Einzelheiten in der Konstitution des Aneurins und Thiochroms: In unserer ersten Veröffentlichung[2]) haben wir vor kurzem Formel V für das Thiochrom vorgeschlagen. Seit der Einsendung



V.



VI.

dieser Arbeit haben A. Windaus, R. Tschesche und R. Grewe[5]) und in einer anderen Veröffentlichung R. Kuhn und H. Vetter[6]) eine ähnliche Formulierung vertreten. Sie unterscheidet sich nur dadurch von der unsrigen, daß im Pyrimidin-Teil in 2- und 4-Stellung Methylgruppen angenommen werden, während unser Ringsystem in 4-Stellung nur eine Äthylgruppe (wie sie auch in der Williamsschen Formulierung vorgesehen ist) trägt. Während Windaus und Mitarbeiter ihre theoretische Auffassung vom Thiochrom von der noch nicht endgültig geklärten Konstitution der von ihnen durch Salpetersäure bezw. Bariumpermanganat erhaltenen Pyrimidin-Spaltstücke (a. a. O.) herleiten, stützt Kuhn seine Auffassung auf die Ergebnisse seiner *C*-Methyl-Bestimmungen[7]).

Zur Klärung der Natur und Zahl der Alkylgruppen im Aneurin und Thiochrom haben wir schon vor einiger Zeit Bestimmungen der *C*-ständigen Alkylgruppen von einer Reihe von Substanzen, die in folgender Tabelle vereinigt sind, durchführen lassen[8]):

[4]) Bezifferung gemäß den Vorschlägen in Band III d. Literatur-Register d. Organ. Chemie. [5]) Ztschr. physiol. Chem. **237**, 98 [1935]. [6]) B. **68**, 2375 [1935].
[7]) B. **68**, 2383 [1935]. [8]) Bestimmungen von Hrn. Dr. H. Roth (Heidelberg).

Name der Verbindung	Durchschnitt der Äquivalente Säure, bezog. auf Essigsäure
N-Phenyl-2.4-dimethyl-thiazoliumjodid	1.31
5-Methyl-uracil	0.77
Pyrimidin-sulfonsäure aus Aneurin nach Williams	0.62
3-Methyl-[thiazolo-*2'. 3'*: *2. 1*-benzimidazol]	1.0
Thiochrom	1.6
Aneurin-Chlorhydrat	1.35

Zieht man angesichts dieser Tabelle in Betracht, daß für eine Alkylgruppe, und dies kann unserer Meinung nach auch eine Äthylgruppe sein, der Äquivalentwert zwischen 0.62 und 1 schwankt, so ergibt sich daraus, daß 1) Williams Pyrimidin-Spaltstück nur eine Alkylgruppe, d. h. eine Äthylgruppe, besitzt, 2) Thiochrom und Aneurin die gleiche Zahl von Alkylgruppen, nämlich zwei, haben, 3) der höhere Wert beim Thiochrom gegenüber dem Aneurin (Kuhn[9]) errechnet überraschenderweise 1.75 Äquivalente) gedeckt ist, durch die Tatsache, daß die von uns dargestellte Verbindung 3-Methyl-[thiazolo-benzimidazol] (siehe weiter unten) das *C*-Methyl quantitativ abspaltet; es ist in seiner Konstitution analog der vorgeschlagenen Thiochrom-Formel; dagegen gibt das, wie das Aneurin, quartäre Thiazoliumsalz verminderte Ausbeuten.

Uns scheint nach diesen Überlegungen die Formulierung nach Williams mit einem Äthyl-pyrimidin die bevorzugte zu sein.

Das soeben erwähnte Thiazolo-benzimidazol wurde im Verlauf unserer synthetischen Versuche in der Thiochrom-Reihe aus Phenylendiamin und Thio-harnstoff bei nachheriger Kondensation des gebildeten Thio-benzimidazolins mit Chlor-aceton, dargestellt. Damit ergibt sich ein Weg zur Totalsynthese des Thiochroms selbst. Während das Benzprodukt nur schwach blau im ultravioletten Licht fluoresciert, wird diese Eigenschaft bei den entsprechenden Pyrimidin-Derivaten, über die zusammenfassend zu berichten sein wird, immer deutlicher.

Für finanzielle Unterstützung durch die Rockefeller-Stiftung möchten wir auch an dieser Stelle unseren Dank aussprechen.

Beschreibung der Versuche.

N-Benzyl-2.4-dimethyl-thiazoliumbromid.

Äquimolekulare Mengen von Benzylbromid und 2.4-Dimethyl-thiazol wurden 15 Min. auf dem Wasserbade erwärmt. Der entstandene Krystallbrei wurde mit Äther gewaschen und aus Alkohol-Äther umkrystallisiert. Schmp. 173°.

3.683 mg Sbst.: 0.156 ccm N (10°, 747 mm).

$C_{12}H_{14}NSBr$. Ber. N 4.9. Gef. N 5.0.

N-Methyl-2.4-dimethyl-thiazoliumchlorid.

Das für die Umsetzung nötige *N*-Methyl-thioacetamid wurde aus *N*-Methyl-acetamid durch Erhitzen mit P_2S_5 in Benzol dargestellt (Schmp. 59°, Ausbeute 20% d. Th.). 5 ccm Chlor-aceton wurden auf dem Wasser-

[9]) a. a. O.

bade auf 80° erwärmt und 5 g *N*-Methyl-thioacetamid in kleinen Portionen hinzugefügt. Es erfolgte jedesmal eine heftige Reaktion, wobei am Ende die Reaktionsmasse fest wurde. Sie wurde in wenig Methylalkohol gelöst und mit Äther bis zur beginnenden Trübung versetzt. Beim Stehen krystallisierte das Chlorid aus. Farblose, sehr hygroskopische Nadeln, Schmp. 235° unt. Zers. Ausbeute fast quantitativ. Die Substanz erwies sich als identisch mit dem aus 2.4-Dimethyl-thiazol-Methyljodid mit Silberchlorid dargestellten Cl-Salz.

$C_6H_{10}NSCl$. Ber. Cl 21.7. Gef. Cl 21.0.

N-Phenyl-2.4-dimethyl-thiazoliumsalze.

Eine Mischung von 5 g Thio-acetanilid und 5.5 ccm Chlor-aceton wurde auf dem Wasserbade unter Ausschluß von Feuchtigkeit erhitzt. Nach einigen Minuten trat eine heftige Reaktion ein, und das Gemisch verwandelte sich in einen zähen Sirup. Dieser wurde in 25—30 ccm Wasser gelöst, mit Tierkohle behandelt und filtriert. Auf Zusatz einer 20-proz. Lösung von Überchlorsäure fiel das *N*-Phenyl-2.4 dimethyl-thiazoliumperchlorat in farblosen Nadeln aus. Aus Aceton-Äther umkrystallisiert, zeigte es einen Schmp. von 180°. Ausbeute quantitativ.

$C_{11}H_{12}O_4NSCl$. Ber. C 45.6, H 4.1, N 4.8.
Gef. „ 45.1, „ 4.5, „ 4.5.

Das Pikrat bildete gelbe Nädelchen, die bei 115° schmolzen. Das Chlorid wurde in farblosen Nadeln erhalten, wenn man das Pikrat in 5-proz. methylalkohol. Salzsäure löste und mit Äther fällte. Es ist außerordentlich hygroskopisch und schmilzt im geschlossenen Röhrchen bei 184° unt. Zers. Das Jodid hatte die von Clarke und Gurin[3]) angegebenen Eigenschaften.

S-Acetonyl-thioacetanilid-Hydrochlorid.

Zu 6 ccm Chlor-aceton wurden 5 g Thio-acetanilid bei 15—20° hinzugefügt und das Gemisch sich selbst überlassen. Nach etwa 3 Stdn. begannen sich plötzlich Krystalle auszuscheiden, und nach weiteren 30 Min. war der Kolben-Inhalt eine feste Masse geworden. Das Produkt wurde zuerst mit Aceton und dann mit Äther zur Entfernung von unverändertem Chloraceton gewaschen und aus Methylalkohol-Äther umkrystallisiert. Das so gewonnene *S*-Acetonyl-thioacetanilid-Hydrochlorid bildete farblose Nädelchen und schmolz bei 112°. Ausbeute fast quantitativ.

Die freie Base wurde als unbeständiges Öl, das sich selbst bei Vakuum-Destillation zersetzte, erhalten. Das entsprechende Perchlorat schmolz bei 130°. Außerdem ließ sich ein Semicarbazon aus dem Hydrochlorid darstellen, das bei 230° unt. Zers. schmolz. Beim Erwärmen auf dem Wasserbade ging das Chlorid langsam in das *N*-Phenyl-2.4-dimethyl-thiazoliumchlorid über.

$C_{11}H_{14}ONSCl$. Ber. C 54.2, H 5.8, N 5.8, S 13.1, Cl 14.6.
Gef. „ 53.9, „ 5.8, „ 5.9,„ 13.0, „ 14.3.

Hydrolyse des *S*-Acetonyl-thioacetanilids: Das oben erwähnte Chlorid zersetzte sich beim Kochen mit Wasser oder verd. Salzsäure unter Bildung von öligen Tröpfchen. Das Reaktionsgemisch wurde einer Destillation mit Wasserdampf unterworfen. Das Destillat hatte einen mercaptan-ähnlichen, unangenehmen Geruch und gab mit $HgCl_2$ eine weiße Fällung (Schmp. 85°), die Schwefel enthielt. Beim Erwärmen der ursprünglichen wäßrigen Lösung

mit Semicarbazid-Chlorhydrat und Natriumacetat fiel ein Semicarbazon aus, das aus Methylalkohol in farblosen Prismen krystallisierte. Schmp. 213° unt. Zers. Das Semicarbazon gab positive Reaktionen auf freies SH; so entstand Rotfärbung mit einem Körnchen Natriumnitrit in Eisessig, und Quecksilberchlorid verursachte eine weiße Fällung.

3.874 mg Sbst.: 8.100 mg $BaSO_4$. — 3.563 mg Sbst.: 0.592 ccm N (13°, 744 mm).

$C_7H_{13}ON_3S_2$. Ber. N 19.2, S 29.2.
Gef. „ 19.4, „ 28.7.

Aus dem Rückstand von der Wasserdampf-Destillation wurde durch Äther-Extraktion eine krystalline Substanz isoliert, die sich als Acetanilid herausstellte (Misch-Schmp. 114—115°). Der wäßrige Rest wurde alkalisch gemacht und mit Äther extrahiert. Der Äther-Extrakt enthielt Anilin (durch sein Acetylderivat identifiziert).

N-*o*-Tolyl-2.4-dimethyl-thiazoliumsalze.

Das für die Umsetzung nötige Thioacet-*o*-toluidid wurde aus Acet-*o*-toluidid mittels P_2S_5 gewonnen; Schmp. 68°. Die Kondensation mit Chloraceton folgte in den Grundzügen den beim Thio-acetanilid beschriebenen Einzelheiten. Das Perchlorat schmilzt bei 172°, das Pikrat bei 150° und das Jodid bei 217—218° unt. Zers.

$C_{12}H_{14}O_4NSCl$ (Perchlorat). Ber. C 47.4, H 4.6, N 4.6.
Gef. „ 47.7, „ 4.97, „ 4.5.

Das entsprechende Zwischenprodukt *S*-Acetonyl-thioacet-*o*-toluidid wurde in gleicher Weise wie das niedrige Homologe dargestellt. Das Hydrochlorid (Schmp. 125°) ist stabiler gegen hydrolytische Einflüsse, gibt aber nach einiger Zeit Toluidin, Acet-*o*-toluidid und dieselbe schwefelhaltige Komponente, die oben beschrieben ist.

3.271 mg Sbst.: 6.710 mg CO_2, 1.910 mg H_2O. — 3.938 mg Sbst.: 0.102 ccm N (18.5°, 744 mm).

$C_{12}H_{16}ONSCl$. Ber. C 55.9, H 6.2, N 5.4.
Gef. „ 55.95, „ 6.5, „ 5.6.

N-[*o*-Nitro-phenyl]-2.4-dimethyl-thiazoliumsalze.

Das für die Umsetzung nötige *o*-Nitro-thioacetanilid wurde wie folgt dargestellt: 1 Mol. *o*-Nitro-acetanilid wurde mit 3.5 Mol. Phosphorpentasulfid in einem Mörser innig gemischt und in Portionen von etwa 1 g in einem Reagensglas im Wasserbade erhitzt. Das Erwärmen soll nicht länger als 3 Min. dauern, bis gerade die Schmelze zusammensintert. Andernfalls fängt das Gemisch Feuer. Die Reagensgläser werden in einem Mörser zerkleinert und die Masse aus Glas und Reaktionsprodukt mit Alkohol ausgezogen. Die Alkohol-Lösung wurde mit festem Ätznatron gesättigt, stehen gelassen und nach einiger Zeit mit Wasser verdünnt. Hierauf wurde filtriert und mit gasförmigem CO_2 gefällt. Das entstandene Produkt wurde mit 2-proz. Natronlauge erneut zur Lösung gebracht, wobei unverändertes Ausgangsmaterial zurückblieb. Wiederum mit CO_2 gefällt, ließ sich das *o*-Nitro-thioacetanilid aus Aceton-Wasser umkrystallisieren. Gelbe Prismen, Schmp. 114°.

$C_8H_8O_2N_2S$. Ber. C 49.0, H 4.1, N 14.3.
Gef. „ 49.0, „ 4.3, „ 14.0.

Die Thiazoliumsalze dieser Reihe wurden auf dem bereits beschriebenen Wege bereitet. Perchlorat, Schmp. 205°, krystallisierbar aus Wasser, farblose Blättchen.

3.730 mg Sbst.: 5.49 mg CO_2, 1.17 mg H_2O. — 3.432 mg Sbst.: 0.250 ccm N (18°, 744 mm). — 4.511 mg Sbst.: 3.050 mg $BaSO_4$. — 3.851 mg Sbst.: 1.620 mg AgCl.

$C_{11}H_{11}O_6N_2SCl$. Ber. C 39.3, H 3.6, N 8.3, S 9.5, Cl 10.6.
Gef. „ 40.1, „ 3.5, „ 8.4, „ 9.3, „ 10.4.

C-Alkyl-Bestimmungen.

Bei der Oxydation mit Chromsäure nach R. Kuhn und H. Roth wurde gefunden:

a) 7.457 mg, 6.841 mg *N*-Phenyl-2.4-dimethyl-thiazoliumjodid: 3.09, 2.85 ccm $n/_{100}$-NaOH.
$C_{11}H_{12}NSJ$. Gef. Äquiv. Essigsäure 1.31, 1.32.

b) 6.848, 7.190 mg 5-Methyl-uracil: 4.26, 4.34 ccm $n/_{100}$-NaOH.
$C_5H_6O_2N_2$. Gef. Äquiv. Essigsäure 0.78, 0.76.

c) 5.766, 4.981 mg Pyrimidin-sulfonsäure aus Aneurin: 1.78, 1.51 ccm $n/_{100}$-NaOH.
$C_6H_9O_3N_3S$. Gef. Äquiv. Essigsäure 0.63, 0.62.

d) 8.273, 7.180 mg 3-Methyl-[thiazolo-benzimidazol]: 4.50, 3.85 ccm $n/_{100}$-NaOH.
$C_{10}H_8N_2S$. Gef. Äquiv. Essigsäure 1.02, 1.00.

e) 5.687 mg Thiochrom: 3.45 ccm $n/_{100}$-NaOH.
$C_{12}H_{14}ON_4S$. Gef. Äquiv. Essigsäure 1.59.

f) 7.120, 7.328 mg Aneurin-Chlorhydrat: 2.83, 2.96 ccm $n/_{100}$-NaOH.
$C_{12}H_{18}ON_4SCl_2$. Gef. Äquiv. Essigsäure 1.34, 1.36.

3-Methyl-[thiazolo-*2'.3'*:*2.1*-benzimidazol] (VI).

Ein Gemisch von 2 g *o*-Phenylendiamin und 1.4 g Thio-harnstoff wurde 1 Stde. auf 180° erhitzt. Unter starker Ammoniak-Entwicklung setzte sich das anfangs flüssige Gemenge zu einem Krystallbrei um, der etwas rötlich gefärbt war. Aus heißem Alkohol umgelöst, ergab sich Thio-benzimidazolin vom Schmp. 295—300°. Ausbeute 1.3 g.

1 g von letzterem wurde mit 0.6 g Chlor-aceton 3—5 Min. erwärmt. Es trat plötzliche Reaktion ein, unter Schmelzen des ganzen Gemisches und Wiederfestwerden. Das Produkt wurde in Wasser gelöst, mit Äther gewaschen und alkalisch gemacht. Der Niederschlag wurde aus verd. Alkohol umkrystallisiert; kleine, farblose Prismen, Schmp. 164—165°. Ausbeute 1 g. In alkohol. Lösung fluoresciert die Substanz im ultravioletten Licht schwach blau.

3.320 mg Sbst.: 7.780 mg CO_2, 1.290 mg H_2O. — 4.045 mg Sbst.: 0.516 ccm N (15°, 748 mm). — 4.422 mg Sbst.: 5.570 mg $BaSO_4$.

$C_{10}H_8N_2S$. Ber. C 63.8, H 4.3, N 14.9, S 17.0.
Gef. „ 63.9, „ 4.35, „ 14.9, „ 17.3.

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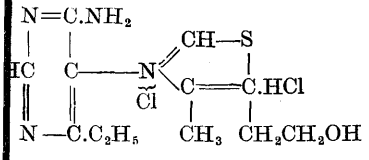
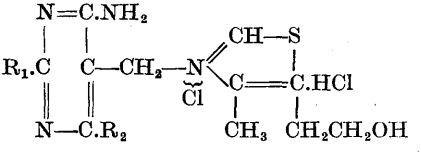
The Structure of Aneurin and Thiochrome

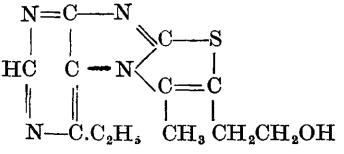
R. R. WILLIAMS has just published[1] a note on the structure of aneurin (vitamin B_1). From a study of degradation products of the vitamin, he concludes that his original formula (I)[2] for aneurin is wrong, and that it should be represented by (II : where $R_1=CH_3$; $R_2=H$) ; this new structure is similar in essentials to that suggested on theoretical grounds by K. Makino and T. I. Imai (II : where $R_1=H$; $R_2=CH_3$)[3].



I



II



III

For thiochrome, which is formed from aneurin by mild oxidation, we suggested on the basis of formula (I) the structure (III)[4]. In the course of subsequent synthetic work we observed that thiazpurines, prepared as models for a thiochrome synthesis, although blue fluorescent in ultra-violet light, never showed fluorescence in daylight. This led us to suspect that (I) might not represent aneurin, and our suspicion was confirmed on completing the synthesis of the compound having structure (I). The synthetic substance, though exhibiting similar colour reactions to aneurin, is not identical with it ; on oxidation with potassium ferricyanide it gives a substance non-fluorescent in daylight, but blue fluorescent in ultra-violet light. The difference in fluorescence between thiochrome and synthetic thiazpurines suggests that the former contains a different ring system. Accordingly, the possibility that the formula of Makino and Imai might represent the vitamin has been explored by synthetic methods. These experiments are not yet complete, but a compound similar in structure to (II) has been prepared, which on oxidation with potassium ferricyanide yields a substance exhibiting

ANEURIN

PART III. METHYL α-CHLORO-γ-HYDROXYPROPYL KETONE AND ITS APPLICATION TO THIAZOLE SYNTHESIS

BY
A. R. TODD,
F. BERGEL,
AND
(Miss) A. JACOB

PART IV. 5-THIOFORMAMIDOPYRIMIDINES

BY
A. R. TODD,
F. BERGEL,
AND
KARIMULLAH

PART V. THE SYNTHESIS OF 3-PYRIMIDYLTHIAZOLIUM SALTS, INCLUDING AN ISOMER OF ANEURIN

BY
A. R. TODD
AND
F. BERGEL

Reprinted from the Journal of the Chemical Society,
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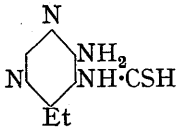
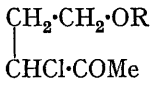
342. *Aneurin. Part III.* Methyl α-Chloro-γ-hydroxypropyl Ketone and its Application to Thiazole Synthesis.*

By A. R. Todd, F. Bergel, and (Miss) A. Jacob.

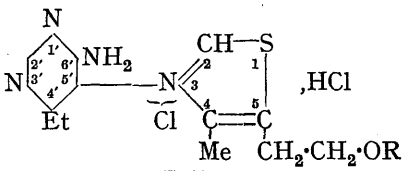
By cleavage of aneurin (vitamin B_1) with an acid solution of sodium sulphite Williams, Waterman, Keresztesy, and Buchman (*J. Amer. Chem. Soc.*, 1935, **57**, 536) obtained an acidic substance $C_6H_9O_3N_3S$, considered to be a pyrimidinesulphonic acid, and a base C_6H_9ONS, which Clarke and Gurin (*ibid.*, p. 1876) showed to be identical with 4-methyl-5-β-hydroxyethylthiazole. Largely on the basis of this work Williams (*ibid.*, p. 229) formulated the vitamin hydrochloride as 3-(6′-amino-4′-ethylpyrimidyl-5′)-4-methyl-5-β-hydroxyethylthiazolium chloride hydrochloride (III; R = H).



(I.)



(II.)



(III.)

It seemed possible that quaternary salts of type (III) might be synthesised by extending the methods employed for 3-arylthiazolium salts (Clarke and Gurin, *loc. cit.*; Todd, Bergel, and Karimullah, *Ber.*, 1936, **69**, 217) to the condensation of 6-amino-5-thioformamido-4-ethylpyrimidine (I) with a suitable α-halogenated ketone (II). In their synthesis of 4-methyl-5-β-hydroxyethylthiazole Clarke and Gurin (*loc. cit.*) condensed methyl α-chloro-γ-ethoxypropyl ketone (II; R = Et) with thioformamide and subsequently de-alkylated the 4-methyl-5-β-ethoxyethylthiazole initially formed, by heating in a sealed tube with concentrated hydrochloric acid. Such treatment is known to deaminate aneurin (Buchman and Williams, *J. Amer. Chem. Soc.*, 1935, **57**, 1751; Barger, Bergel, and Todd, *Ber.*, 1935, **68**, 2257), so the above-mentioned ethoxy-ketone was regarded as useless for our purpose.

We therefore synthesised *methyl α-chloro-γ-hydroxypropyl ketone* (II; R = H) according to the scheme:

$$CH_3{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2Br + CH_3{\cdot}CO{\cdot}CHNa{\cdot}CO_2Et \longrightarrow CH_3{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH(CO_2Et){\cdot}CO{\cdot}CH_3 \quad \text{(IV.)}$$

$$\xrightarrow{SO_2Cl_2} \underset{\text{(V.)}}{CH_3{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CCl(CO_2Et){\cdot}CO{\cdot}CH_3} \xrightarrow{\text{dil. } H_2SO_4} HO{\cdot}CH_2{\cdot}CH_2{\cdot}CHCl{\cdot}CO{\cdot}CH_3$$

The condensation of β-bromoethyl acetate with ethyl sodioacetoacetate at 160° (Haller and March, *Compt. rend.*, 1908, **139**, 100; *Bull. Soc. chim.*, 1905, **33**, 618) is unsatisfactory; better results are obtained by using benzene as a diluent and refluxing the mixture on the water-bath. Chlorination of (IV) with sulphuryl chloride proceeds smoothly, and the desired chloro-ketone is obtained on careful hydrolysis of the product (V).

Methyl α-chloro-γ-hydroxypropyl ketone condensed readily with thioformamide, yielding 4-methyl-5-β-hydroxyethylthiazole, whose *picrate* gave no depression in m. p. when mixed with the specimen (m. p. 162°) prepared from the vitamin.

Efforts were also made to prepare methyl α-halogeno-γ-phenoxypropyl ketones in the hope that the phenoxythiazoles resulting from their condensation with thioamides would yield the corresponding hydroxy-compounds under relatively mild conditions. At first, direct halogenation of methyl γ-phenoxypropyl ketone (Boyd, Barrett, and Robinson, J., 1932, 318) was tried under a variety of conditions, but no homogeneous products could be isolated. The synthesis of *methyl α-chloro-γ-phenoxypropyl ketone* (II; R = Ph) was, however, effected by a method analogous to that employed for the corresponding hydroxy-compound (II; R = H); as is evident from the chlorine content, the substance could

* Part II; *Ber.*, 1936, **69**, 217.

not be purified completely, but condensation with thioacetamide gave in good yield, 2 : 4-dimethyl-5-β-phenoxyethylthiazole, isolated as its *picrate*. Further experiments with phenoxythiazoles were discontinued, as replacement of the phenoxy-group by hydroxyl could not be satisfactorily accomplished.

Experimental.

Ethyl α-2-Acetoxyethylacetoacetate (IV).—To a suspension of dry ethyl sodioacetoacetate (152 g.) in dry benzene (700 c.c.), β-bromoethyl acetate (167 g.) was added at 15—20°. The mixture was heated on the water-bath until the solution reacted faintly alkaline (6—10 hours), then cooled, poured into ice-water, and extracted with ether. After removal of the ether the residual oil was distilled under reduced pressure, the fraction, b. p. 138—142°/12 mm., being collected (yield, 25%). Haller and March (*loc. cit.*) give b. p. 147—150°/13 mm.

Ethyl α-Chloro-α-2-acetoxyethylacetoacetate (V).—Sulphuryl chloride (82 g.) was added during 1 hour with stirring to the ester (IV) (123 g.) at 0°. The solution was kept at 0° for a further hour, then diluted with ether (250 c.c.) and refluxed for a short time to remove sulphur dioxide and hydrogen chloride. The ether was removed, and the residual oil repeatedly fractionated in a vacuum. The main fraction, b. p. 120—121°/2 mm., was collected (yield, 86%) (Found : C, 47·9; H, 6·0; Cl, 13·3. $C_{10}H_{15}O_5Cl$ requires C, 47·9; H, 6·0; Cl, 14·1%).

Methyl α-Chloro-γ-acetoxypropyl Ketone (II; R = $CO{\cdot}CH_3$).—The above ester (V) was heated under reflux for 6 hours with a mixture of dilute sulphuric acid (20 c.c. of 15%) and glacial acetic acid (20 c.c.). The solution was cooled, poured into water, and extracted with ether. After removal of the ether and acetic acid a colourless liquid was obtained which after several fractionations boiled at 90—93/2 mm. (yield, 40%) (Found : C, 47·1; H, 6·2; Cl, 19·8. $C_7H_{11}O_3Cl$ requires C, 47·0; H, 6·2; Cl, 19·9%).

Methyl α-Chloro-γ-hydroxypropyl Ketone (II; R = H).—The chloro-ester (V) was heated under reflux during 4 hours with dilute sulphuric acid (35 c.c. of 35%) and alcohol (70 c.c.), then poured into water, and the mixture extracted with ether. On removal of ether from the dried extract an oil was left which after repeated fractionation gave a colourless liquid, b. p. 85—92°/16 mm. (yield, 20%) (Found : Cl, 25·4. $C_5H_9O_2Cl$ requires Cl, 26·0%).

4-Methyl-5-β-hydroxyethylthiazole.—An ethereal solution of thioformamide was prepared by shaking together finely powdered phosphorus pentasulphide (12 g.), formamide (20 g.), and absolute ether (200 c.c.) for *ca.* 20 hours (Gabriel, *Ber.*, 1916, **49**, 1145); the clear ethereal layer was decanted and used as a stock solution of thioformamide.

When a mixture of the chloro-ketone (II; R = H) (250 mg.) and the thioformamide solution (10 c.c.) was kept, colourless crystals of the thiazole hydrochloride slowly separated. After 5 hours the ether was distilled off, and the residue heated for 1 hour at 100°, cooled, and dissolved in dilute hydrochloric acid. After extraction with ether to remove any unchanged ketone, the solution was made strongly alkaline and again extracted with ether. The extract on evaporation yielded 4-methyl-5-β-hydroxyethylthiazole as an almost colourless oil, b. p. 250—255° (capillary method of Emich). The base was not purified further; treatment with ethereal picric acid gave a *picrate* crystallising from alcohol in yellow needles, m. p. 162—163° (Found : S, 8·2. $C_{12}H_{12}O_8N_4S$ requires S, 8·6%).

Ethyl α-2-Phenoxyethylacetoacetate.—This *ester* was prepared from β-phenoxyethyl bromide and ethyl sodioacetoacetate in alcoholic solution (cf. Boyd, Barrett, and Robinson, *loc. cit.*). It had b. p. 148°/4 mm. (Found : C, 68·1; H, 7·3. $C_{14}H_{18}O_4$ requires C, 67·2; H, 7·2%).

Ethyl α-Chloro-α-2-phenoxyethylacetoacetate.—The above ester (10 g.) was chlorinated with sulphuryl chloride (6 g.) in the manner described under the corresponding acetoxy-compound (V). The *product* was a colourless liquid, b. p. 135—140°/3 mm. (yield, 70%) (Found : C, 59·3; H, 6·1; Cl, 11·9. $C_{14}H_{17}O_4Cl$ requires C, 59·1; H, 6·0; Cl, 12·4%).

Methyl α-Chloro-γ-phenoxypropyl Ketone (II; R = Ph).—The above chloro-ester (7 g.) was hydrolysed by refluxing for 4 hours with a mixture of dilute sulphuric acid (14 c.c. of 15%) and glacial acetic acid (14 c.c.). After repeated distillation the main fraction of the product boiled at 168—172°/12 mm. (Found : C, 62·0; H, 6·0; Cl, 12·2. $C_{11}H_{13}O_2Cl$ requires C, 62·1; H, 6·1; Cl, 16·7%). The low chlorine content may be due to partial decomposition during distillation; that the substance is mainly the desired ketone is shown by its condensation with thioacetamide.

2 : 4-Dimethyl-5-β-phenoxyethylthiazole.—The above chloro-ketone (200 mg.) reacted rapidly with thioacetamide (60 mg.) when the mixture was warmed for a few minutes over a free flame. The free base was finally obtained as a colourless thick oil. It gave a *picrate* crystal-

lising from alcohol in yellow needles, m. p. 122° (Found: N, 12·1; S, 6·8. $C_{19}H_{18}O_8N_4S$ requires N, 12·1; S, 6·9%).

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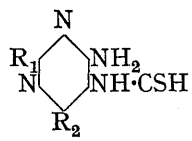
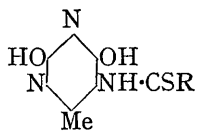
343. *Aneurin. Part IV. 5-Thioformamidopyrimidines.*

By A. R. Todd, F. Bergel, and Karimullah.

For the synthesis of 3-pyrimidylthiazolium salts according to the scheme indicated in the preceding paper it is necessary to synthesise 5-thioformamidopyrimidine derivatives including those of type (I). It is known that an amino-group in position 5 of the pyrimidine nucleus is unique in that it is readily acylated; amino-groups in other positions are not. Thus, formylation of a 5 : 6-diaminopyrimidine leads to the formation of the corresponding 6-amino-5-formamidopyrimidine and not to a diformyl derivative (Gabriel and Colman, *Ber.*, 1901, **34**, 1246; Johns, *Amer. Chem. J.*, 1908, **41**, 58). Thioacylamidopyrimidines are not described in the literature, and we were unable to prepare them by the action of phosphorus pentasulphide on the corresponding acyl derivatives. A similar lack of success was encountered on attempting to replace the 5-amino-group by an *iso*nitrile group with a view to subsequent addition of hydrogen sulphide according to Hofmann (*Ber.*, 1878, **11**, 339).

(I.)  (II.) 

Thioacetic acid reacts readily with primary amines to give the corresponding acetyl derivatives (Pawlewski, *Ber.*, 1898, **31**, 661; 1902, **35**, 110); accordingly we next tried direct thioacylation by heating amines with dithio-acids (R·CS·SH). With dithioacetic acid, this was completely successful and 5-*thioacetamido*-4-*methyluracil* (II; R = Me) was readily obtained from 5-amino-4-methyluracil. Dithioformic acid acts in a similar way,* but the yield is not good and the product is difficult to purify. It was, however, found that the thioformylation can be readily effected by mixing aqueous solutions of 5-amino-pyrimidines and potassium dithioformate; at room temperature in an atmosphere of carbon dioxide the thioformyl derivatives normally separate in almost pure condition, the yield being nearly quantitative. Amino-groups in positions 2, 4 and 6 of the pyrimidine nucleus did not react under these conditions.

In this way 6-*amino*-5-*thioformamido*-4-*methylpyrimidine* (I; $R_1 = H$, $R_2 = Me$), 6-*amino*-5-*thioformamido*-4-*ethylpyrimidine* (I; $R_1 = H$, $R_2 = Et$), and 2 : 6-*diamino*-5-*thioformamido*-4-*methylpyrimidine* (I; $R_1 = NH_2$, $R_2 = Me$) were prepared from the corresponding 5-amino-compounds; they are crystalline substances which evolve hydrogen sulphide above the melting point and yield the corresponding purines. On heating with chloroacetone, they yield the corresponding 3-pyrimidylthiazolium salts.

In the course of this work a considerable number of aminopyrimidines were prepared; most of these are known compounds, but 2-*amino*-6-*hydroxy*-4-*ethylpyrimidine* and 2 : 6-*diamino*-4-*ethylpyrimidine* have not hitherto been described. Neither could be thioformylated with potassium dithioformate.

Experimental.

5-*Thioacetamido*-4-*methyluracil* (II; R = Me).—5-Amino-4-methyluracil (1 g.) (Behrend, *Annalen*, 1885, **231**, 250), dissolved in dioxan (50 c.c.), was heated on the water-bath with di-

* Experiments by Miss A. Jacob.

thioacetic acid (0·9 g.) (Pohl, *Ber.*, 1907, **40**, 1304) during 4 hours. The mixture was cooled and diluted with light petroleum. The yellowish precipitate crystallised from hot water in colourless needles, m. p. 265—267° (Found: C, 42·4; H, 4·8; N, 21·1. $C_7H_9O_2N_3S$ requires C, 42·2; H, 4·6; N, 21·2%). Yield, quantitative.

5-*Thioformamido*-4-*methyluracil* (II; R = H).—5-Amino-4-methyluracil (1 g.) in dioxan (50 c.c.) was heated under reflux with dithioformic acid (0·7 g.) (Levi, *Atti R. Accad. Lincei*, 1923, **32**, I, 569). The crude *thioformyl* derivative precipitated with light petroleum was difficult to purify. After recrystallisation from water it had m. p. 260—262° (Found: N, 21·0. $C_6H_7O_2N_3S$ requires N, 22·7%. $C_6H_7O_2N_3S,H_2O$ requires N, 20·7%).

3-(2′ : 6′-*Dihydroxy*-4′-*methylpyrimidyl*-5′)-4-*methylthiazolium Chloride*.—The above thioformyl compound (1 mol.), mixed with chloroacetone (4—5 mols.), was heated carefully over a free flame. Vigorous reaction occurred, and after 10—15 minutes the mixture was cooled, and the *product* precipitated as a gum by addition of ether. It crystallised from alcohol–acetone in colourless needles, m. p. 306° (decomp.) (Found: C, 40·9; H, 4·6; N, 15·6; Cl, 13·6. $C_9H_{11}O_2N_3ClS$ requires C, 41·4; H, 4·3; N, 16·1; Cl, 13·6%).

6-*Amino*-5-*thioformamido*-4-*methylpyrimidine* (I; R_1 = H, R_2 = Me).—To 5 : 6-diamino-4-methylpyrimidine (1·5 g.) (Gabriel and Colman, *Ber.*, 1901, **34**, 1254), dissolved in water (10 c.c.), potassium dithioformate (2 g.) was added; traces of crystalline material, m. p. above 300°, soon separated. The solution was filtered and kept over sulphuric acid in a desiccator filled with carbon dioxide. After 12 hours the crystalline precipitate was collected (the filtrate may be treated with a further quantity of potassium dithioformate and the process repeated until the yield is nearly quantitative). The *thioformyl* compound crystallised from water in colourless needles (Found: C, 43·0; H, 5·2; S, 18·6. $C_6H_8N_4S$ requires C, 42·9; H, 4·8; S, 19·0%). It melted sharply at 168° with evolution of hydrogen sulphide; the melt resolidified and on further heating melted at 230°. Gabriel (*Ber.*, 1901, **34**, 1247) gives m. p. 235° for 4-methylpurine. Conversion into 4-methylpurine occurs slowly above 100°. The substance is very soluble in alcohol, less so in methyl alcohol, acetone and water, and insoluble in ether.

2-*Amino*-6-*hydroxy*-4-*ethylpyrimidine*.—A mixture of ethyl propionylacetate (13·3 g.) (Willstätter and Clarke, *Ber.*, 1914, **47**, 298), guanidine carbonate (8 g.), and absolute alcohol (25 c.c.) was heated under reflux for 4 hours, cooled, and the *product* filtered off and recrystallised from hot water; it formed colourless prisms (7 g.), m. p. 247—248° (Found: C, 51·6; H, 6·2; N, 29·6. $C_6H_9ON_3$ requires C, 51·8; H, 6·2; N, 30·2%). When it (1 g.) was heated with concentrated hydrochloric acid (6 c.c.) for 20 hours at 160°, 4-ethyluracil, m. p. 205°, was obtained (yield, 60%).

6-*Chloro*-2-*amino*-4-*ethylpyrimidine*.—A mixture of the above compound (3·5 g.) and phosphoryl chloride (10 c.c.) was heated under reflux for 2 hours. The resulting brownish solution was poured on ice and made alkaline with ammonia, and the precipitated *chloro*-compound collected. It crystallised from alcohol in colourless needles, m. p. 120—121° (yield, 60%) (Found: C, 45·2; H, 4·9; N, 26·1. $C_6H_8N_3Cl$ requires C, 45·7; H, 5·1; N, 26·7%).

2 : 6-*Diamino*-4-*ethylpyrimidine*.—The above *chloro*-compound (0·6 g.) was heated with saturated alcoholic ammonia (20 c.c.) in a sealed tube at 180° during 6 hours. The alcohol was removed, the residue dissolved in a little water, and solid potassium hydroxide added. The precipitated *diamine* was collected and recrystallised from ethyl acetate containing a little light petroleum; it formed colourless needles, m. p. 160—161° (yield, 80%) (Found: N, 40·0. $C_6H_{10}N_4$ requires N, 40·6%).

6-*Amino*-5-*thioformamido*-4-*ethylpyrimidine* (I; R_1 = H, R_2 = Et).—5 : 6-Diamino-4-ethylpyrimidine was prepared from 4-ethyluracil by a slight modification of Robinson and Tomlinson's method (J., 1935, 1283). The following process for isolating the diamine is simpler and gives much improved yields: The reaction mixture obtained on reduction of 2-chloro-5 : 6-diamino-4-ethylpyrimidine is filtered, concentrated to remove alcohol, and diluted somewhat with water, and solid potassium hydroxide added. The precipitated diamine crystallises from ethyl acetate in large yellowish prisms, m. p. 164—165°; Robinson and Tomlinson (*loc. cit.*) give m. p. 159—161°. A further quantity may be obtained by extracting the alkaline mother-liquor with ethyl acetate (total yield, 60% or more).

The diamine (100 mg.), thioformylated in aqueous solution with potassium dithioformate in the manner described above, gave a *product* crystallising from water in colourless needles, m. p. 178° with evolution of hydrogen sulphide (yield, theoretical) (Found: C, 45·5; H, 6·0; S, 17·1. $C_7H_{10}N_4S$ requires C, 46·1; H, 5·5; S, 17·6%).

2 : 6-*Diamino*-5-*thioformamido*-4-*methylpyrimidine* (I; R_1 = NH_2, R_2 = Me).—2 : 5 : 6-Triamino-4-methylpyrimidine (Gabriel and Colman, *loc. cit.*) on treatment with potassium

dithioformate as above gave colourless needles (from water), m. p. 255° with evolution of hydrogen sulphide (Found: S, 17·2. $C_6H_9N_5S$ requires S, 17·5%).

3-(2′: 6′-*Diamino-4′-methylpyrimidyl-5′*)-4-*methylthiazolium Chloride Hydrochloride*.—To a solution of the above thioformyl compound (1 mol.) in acetone, chloroacetone (2 mols.) was added. The mixture was left for 3 days at room temperature, then diluted with an equal volume of alcohol, and refluxed for 4 hours. The colourless needles that separated were collected after cooling and recrystallised from alcohol–acetone containing hydrogen chloride; needles, m. p. 315° (decomp.), were obtained containing water of crystallisation, which was only expelled with difficulty (Found: C, 31·2; H, 5·4; N, 19·9; S, 9·0; Cl, 20·4. $C_9H_{13}N_5Cl_2S,3H_2O$ requires C, 31·0; H, 5·5; N, 20·1; S, 9·2; Cl, 20·4%). The corresponding picrate has m. p. 255°. On shaking with alkaline potassium ferricyanide, a substance is produced which, though non-fluorescent in visible light, is blue-fluorescent in ultra-violet light; the fluorescence disappears when the liquid is made acid, but reappears when it is made alkaline again.

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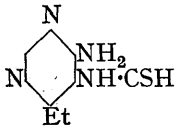
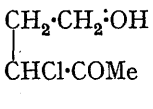
344. *Aneurin. Part V. The Synthesis of 3-Pyrimidylthiazolium Salts, including an Isomer of Aneurin.**

By A. R. Todd and F. Bergel.

Although 3-pyrimidylthiazolium salts can be synthesised by heating 5-thioformamidopyrimidines with chloroacetone (preceding paper), this simple method cannot be applied when chloroacetone is replaced by methyl α-chloro-γ-hydroxypropyl ketone, owing to the low reactivity of the latter substance. The difficulty can, however, be surmounted by using, in place of the free thioformamido-compound, its sodium salt. This condenses readily in absolute-alcoholic solution with α-halogenated ketones and the product, treated with hydrogen chloride, yields the desired 3-pyrimidylthiazolium salt. In this way, the sodium salt of 6-amino-5-thioformamido-4-ethylpyrimidine (I), condensed with methyl α-chloro-γ-hydroxypropyl ketone (II), yielded 3-(6′-*amino*-4′-*ethylpyrimidyl*-5′)-4-*methyl*-5-β-*hydroxyethylthiazolium chloride hydrochloride* (III; $R_1 = H$, $R_2 = Et$).



(I.)



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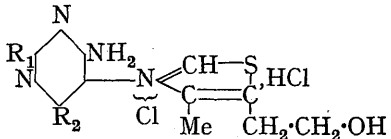


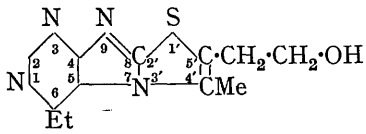
(III.)

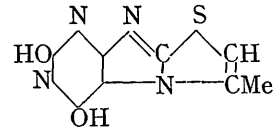
According to the original suggestion of Williams (*J. Amer. Chem. Soc.*, 1935, **57**, 229), (III; $R = H$, $R_2 = Et$) should have been identical with the hydrochloride of aneurin (vitamin B_1). This was not the case; in appearance and general solubilities the synthetic substance resembled the natural vitamin hydrochloride, but it melted much lower (220° as compared with 250°) and when tested on rats by the electrocardiographic method of Birch and Harris (*Biochem. J.*, 1934, **28**, 602) it showed no measurable physiological activity. Several other synthetic 3-pyrimidylthiazolium salts described in the experimental part of the paper were tested biologically with similar negative results, and none of them underwent fission with sodium sulphite in acid solution. The formaldehyde–azo-test of Kinnersley and Peters (*Biochem. J.*, 1934, **28**, 667) is given by (III; $R = H$, $R_2 = Et$) as well as by the actual vitamin. Our observations, however, suggest that a positive result in this test depends in some way on the presence of a β-hydroxyethyl group in position 5 and a hydrogen atom in position 2 of the thiazole nucleus. Thus 3-pyrimidylthiazolium salts without the β-hydroxyethyl group, the oxychlorovitamin of Buchman and Williams (*J. Amer.*

* A preliminary note on the results of this investigation has already been published by us (*Nature*, 1936, **138**, 76).

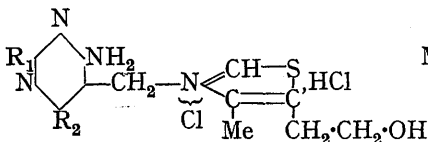
Chem. Soc., 1935, **68**, 1751), and thiochrome (Barger, Bergel, and Todd, *Ber.*, 1935, **68**, 2257) all give negative results.

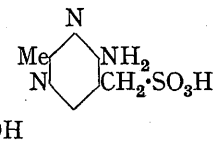
Any possibility that the vitamin might be represented by a closely related structure (III; $R_1 = R_2 = Me$) may be excluded on the following grounds. When synthetic 3-pyrimidylthiazolium salts containing an amino-group in position 6′ are oxidised with alkaline potassium ferricyanide under the conditions used for preparing thiochrome from aneurin, they yield solutions which, though blue-fluorescent in ultra-violet light, show no fluorescence whatever in visible light, in which thiochrome solutions fluoresce strongly. Evidence pointing in the same direction has been obtained in experiments carried out with a view to synthesising thiochrome, for which, on the basis of structure (III; $R = H$, $R_2 = Et$), we proposed the formula (IV) (Barger, Bergel, and Todd, *loc. cit.*).

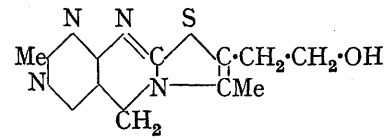
(IV.) 


(V.)

By analogy with 3-methylthiazolobenzimidazole (Todd, Bergel, and Karimullah, *Ber.*, 1936, **69**, 217) thiazolopurines of type (IV) should be capable of synthesis from 8-thiopurines and α-halogenated ketones. By this means Mr. B. A. Hems, B.Sc., prepared from 2 : 6-dihydroxy-8-thiopurine (Fischer, *Ber.*, 1898, **31**, 431) and chloroacetone the *substance* (V). This compound had a feeble though distinct fluorescence in ultra-violet light but none in visible light. In continuation we have condensed 8-thio-6-methylpurine (Gabriel, *Ber.*, 1901, **34**, 1254) and 8-*thio*-6-*ethylpurine* (prepared in a similar fashion from 4 : 5-diamino-6-ethylpyrimidine) with chloroacetone and with methyl α-chloro-γ-hydroxypropyl ketone (II); the products were not purified, but in neutral or alkaline solution they showed feeble blue fluorescence only when viewed in ultra-violet light. The non-fluorescence of thiazolopurines in visible light has recently been noted in addition by Ochiai (*Ber.*, 1936, **69**, 1650). The conclusion that thiochrome is not a thiazolopurine derivative is inevitable. On the available evidence it is clear that aneurin is not a 3-pyrimidylthiazolium salt. The only alternative structure which will accord with the properties of the vitamin is proposed by Makino and Imai (*Z. physiol. Chem.*, 1936, **239**, 1), namely (VI; $R = H$, $R_2 = Me$) or the closely related (VI; $R_1 = Me$, $R_2 = H$) differing only in the position of a methyl group.


(VI.)


(VII.)


(VIII.)

Simultaneously with the completion of this work, Williams (*J. Amer. Chem. Soc.*, 1936, **58**, 1063) announced that the pyrimidinesulphonic acid from the sulphite cleavage of aneurin has the structure (VII), and that aneurin itself is consequently (VI; $R_1 = Me$, $R_2 = H$). A synthesis of (VII) has also been recorded by Grewe (*Z. physiol. Chem.*, 1936, **242**, 89), who, however, does not describe the preparation of 6-amino-2-methyl-5-bromoethylpyrimidine, its immediate precursor; he also states that (VI; $R = Me$, $R_2 = H$) synthesised by the I.G. Farbenindustrie A.G. in their Elberfeld laboratories is identical with the vitamin.

On the basis of the vitamin formula (VI; $R = Me$, $R_2 = H$) thiochrome should have the structure (VIII); this is at present being investigated by synthetic methods.

Experimental.

3-(6′-Amino-4′-ethylpyrimidyl-5′)-4-methyl-5-β-hydroxyethylthiazolium Chloride Hydrochloride (III; $R_1 = H$, $R_2 = Et$).—To a mixture of 6-amino-5-thioformamido-4-ethylpyrimidine (108·5 mg.; 1 mol.) (preceding paper) and absolute alcohol (10 c.c.) was added a solution of

sodium ethoxide in alcohol (1 c.c. containing 13·7 mg.; 1 atom Na). To the clear solution formed, methyl α-chloro-γ-hydroxypropyl ketone (0·1 c.c.; *i.e.*, excess) was added and the mixture left overnight at room temperature. After filtration from sodium chloride, alcoholic hydrogen chloride (0·3 c.c. containing 27·7 mg.; 1 mol. HCl) was added, and the solution heated under reflux for 4 hours. A further quantity of alcoholic hydrogen chloride (0·3 c.c.; 1 mol. HCl) was then added, heating continued for 1 hour, the solution cooled, and excess of acetone added to precipitate the quaternary *salt*, which crystallised in the ice-chest after a few hours. The hygroscopic product crystallised from alcohol–acetone in bundles of small colourless needles containing water of crystallisation. This was expelled at 100—110° and the salt then had m. p. 220° (decomp.) (Found: C, 41·1; H, 6·1; S, 8·5; Cl, 20·5. $C_{12}H_{18}ON_4Cl_2S,H_2O$ requires C, 40·6; H, 5·6; S, 9·0; Cl, 20·0%).

Oxidation with alkaline potassium ferricyanide gave solutions which, though non-fluorescent in visible light, had blue fluorescence in ultra-violet light. The formaldehyde–azo-test was positive and indistinguishable from that given by natural aneurin. Tested by the electrocardiagraphic method, 1·2 mg. contained less than 1 I.U. The inactivity of the substance was confirmed by Professor R. A. Peters, who kindly examined it, and to whom we wish to express our thanks.

3-(6′-*Amino*-4′-*ethylpyrimidyl*-5′)-4-*methylthiazolium Chloride Hydrochloride*.—6-Amino-5-thioformamido-4-ethylpyrimidine (108·5 g.) was converted into its sodium salt and condensed with chloroacetone (0·1 c.c.) in a manner similar to that described above, the total period of heating being in this case only 3 hours. The *product* crystallised from alcohol–acetone in hygroscopic colourless needles, m. p. 252—253° (decomp.) (Found: C, 40·6; H, 5·1; S, 10·5; Cl, 23·6. $C_{10}H_{14}N_4Cl_2S$ requires C, 40·9; H, 4·8; S, 10·9; Cl, 24·2%).

The substance reacted negative in the formaldehyde–azo-test and, tested by the electrocardiagraphic method, 2·8 mg. contained less than 1 I.U. Oxidation with alkaline potassium ferricyanide gave a solution which had weak blue fluorescence in ultra-violet light.

3-(6′-*Amino*-4′-*methylpyrimidyl*-5′)-4-*methyl*-5-β-*hydroxyethylthiazolium Chloride Hydrochloride* (III; R_1 = H, R_2 = Me).—6-Amino-5-thioformamido-4-methylpyrimidine (100 mg.) was converted into its sodium salt and condensed with methyl α-chloro-γ-hydroxypropyl ketone (0·1 c.c.) in the manner above described, the total period of heating being 5 hours. The *product* crystallised from alcohol–ethyl acetate in colourless needles, which lost water of crystallisation at 100—110° and melted and decomposed at 250° (Found: C, 38·8; H, 5·4; S, 8·5; Cl, 21·0. $C_{11}H_{16}ON_4Cl_2S,H_2O$ requires C, 38·7; H, 5·4; S, 9·4; Cl, 20·8%).

The substance gives a positive formaldehyde–azo-test and oxidation with alkaline potassium ferricyanide gives a solution which is blue fluorescent in ultra-violet light. Tested by the electrocardiagraphic method, 2·8 mg. contained less than 1 I.U.

3-(6′-*Amino*-4′-*methylpyrimidyl*-5′)-4-*methylthiazolium Chloride Hydrochloride*.—6-Amino-5-thioformamido-4-methylpyrimidine (100 mg.), condensed in the form of the sodium salt with chloroacetone (0·1 c.c.), the period of heating being 3 hours, gave a *product* crystallising from alcohol–acetone in needles, m. p. 254—255° (decomp.). Owing to its extremely hygroscopic character it was difficult to analyse (Found: C, 33·9; H, 5·7. $C_9H_{12}N_4Cl_2S,2H_2O$ requires C, 34·2; H, 5·1%). The substance did not give the formaldehyde–azo-test and, tested biologically by the electrocardiagraphic method, 5 mg. contained less than 1 I.U. Oxidation with alkaline potassium ferricyanide gave a solution blue-fluorescent in ultra-violet light.

2 : 6-*Dihydroxy*-8-*thiopurine*.—This substance was prepared by Fischer (*loc. cit.*) by heating bromoxanthine with potassium hydrogen sulphide. We obtained it in the following way: 4 : 5-diamino-2 : 6-dihydroxypyrimidine (1 mol.) (Traube, *Ber.*, 1900, **33**, 1382) was heated with thiourea (4 mols.) at 240—250° for 1 hour. The melt was cooled and extracted repeatedly with boiling water; the extract on cooling deposited a nearly colourless powder having the properties recorded by Fischer (*loc. cit.*) (Found in material dried at 150° in a high vacuum: N, 30·8. Calc. for $C_5H_4O_2N_4S$: N, 30·4%).

2 : 6-*Dihydroxy*-4′-*methylthiazolo*-(2′ : 3′ : 8 : 7)*purine* (V).—2 : 6-Dihydroxy-8-thiopurine (120 mg.) was boiled with chloroacetone (200 mg.) for 20 minutes, the mixture then being cooled and diluted with ether. The solid residue was recrystallised from a solution in hot dilute aqueous ammonia made weakly acid with acetic acid; on cooling, the *product* separated as a white micro-crystalline powder which did not melt below 250° (Found: C, 42·7; H, 2·8; N, 25·0; S, 14·7. $C_8H_6O_2N_4S$ requires C, 43·2; H, 2·7; N, 25·2; S, 14·4%). The substance is soluble in aqueous ammonia, caustic alkalis, and hydrochloric acid and insoluble in dilute acetic acid or cold water. A solution in concentrated aqueous ammonia gives no immediate precipitate with silver nitrate (distinction from 2 : 6-dihydroxy-8-thiopurine). Its ammoniacal solution

fluoresces light blue in ultra-violet light, the fluorescence disappearing when the solution is made acid.

8-*Thio*-6-*ethylpurine*.—4 : 5-Diamino-6-ethylpyrimidine (100 mg.) was heated with thiourea (150 mg.) at 170—180° for 1 hour; evolution of ammonia had then ceased. The melt was cooled, and triturated with water, and the insoluble residue dissolved in hot dilute aqueous ammonia. After treatment with charcoal and removal of the ammonia by boiling, the solution was cooled; it deposited yellowish needles, m. p. above 300° (Found: C, 46·9; H, 4·4. $C_7H_8N_4S$ requires C, 46·7; H, 4·4%).

Experiments on the Condensation of 4-*Methyl- and* 4-*Ethyl*-8-*thiopurines with* α-*Halogenated Ketones*.—The general method used was to heat the sodium derivative of the thiopurine with the appropriate halogenated ketone in alcoholic solution for 12 hours. The ketones used were chloroacetone and methyl α-chloro-γ-hydroxypropyl ketone; in every case solutions were obtained which when neutral or alkaline showed blue fluorescence in ultra-violet light, but no fluorescence in visible light could be detected. As the products were difficult to isolate in a pure state, the experiments were not pursued further, it being clear that no substances similar to thiochrome were obtainable in this way.

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ANEURIN. PART VI. A SYNTHESIS OF THIOCHROME AND RELATED COMPOUNDS

BY

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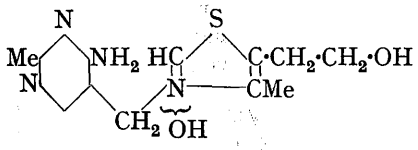
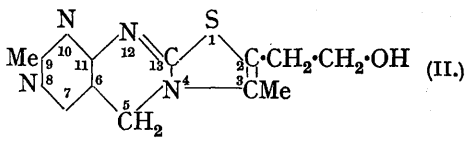
353. *Aneurin. Part VI. A Synthesis of Thiochrome and Related Compounds.**

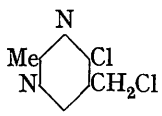
By A. R. Todd, F. Bergel, H. L. Fraenkel-Conrat, and (Miss) A. Jacob.

Kuhn, Wagner-Jauregg, van Klaveren, and Vetter (*Z. physiol. Chem.*, 1935, **234**, 196) isolated from yeast a yellow basic substance, $C_{12}H_{14}ON_4S$, whose neutral or alkaline solutions were characterised by intense blue fluorescence; to it they gave the name thiochrome. The same substance was obtained by Barger, Bergel, and Todd (*Nature*, 1935, **136**, 259; *Ber.*, 1935, **68**, 2257) by oxidation of an alkaline solution of aneurin (I) with potassium ferricyanide. Thiochrome is also formed from aneurin by a number of other oxidising agents (Barger, Bergel, and Todd, *loc. cit.*; Kuhn and Vetter, *Ber.*, 1935, **68**, 2384). Taking into account its mode of formation from the vitamin (I) and its properties, the most probable structure for thiochrome appeared to be (II) (cf. Part V; this vol., p. 1560). Evidence for the accuracy of this view seemed most readily obtainable by synthetic methods.

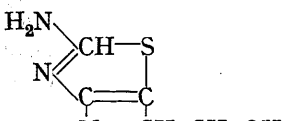
(I.) 



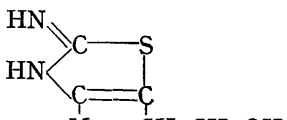
(II.)



(III.)



(IV*a*.)



(IV*b*.)

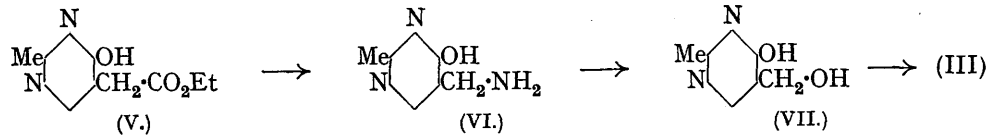
It seemed possible that compounds similar to (II) might be synthesised by condensing 4-chloro-5-chloromethylpyrimidines with 2-aminothiazoles; thiochrome itself would be synthesised in this way from 4-*chloro*-5-*chloromethyl*-2-*methylpyrimidine* (III) and 2-amino-4-methyl-5-β-hydroxyethylthiazole (IV*a* or IV*b*).

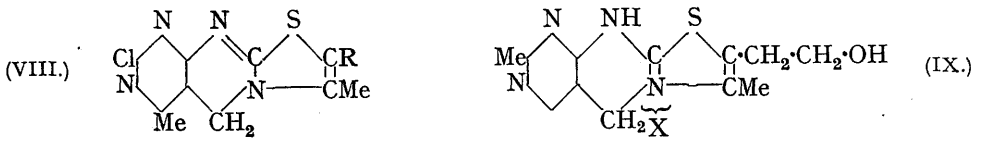
For the synthesis of (III) various methods seemed possible; we adopted the following route, as the earlier intermediates were available in connection with other synthetic investigations. Condensation of acetamidine with ethyl formylsuccinate yielded *ethyl* 4-*hydroxy*-2-*methylpyrimidine*-5-*acetate* (V), from which by Curtius degradation 4-hydroxy-5-aminomethyl-2-methylpyrimidine (VI) was obtained in good yield. Various methods of carrying out this degradation were tried, the most successful being direct conversion of the *hydrazide* into the *urethane* (cf. Jackson and Kenner, J., 1928, 1657) and subsequent hydrolysis with concentrated hydrobromic or hydrochloric acid; a description of other methods of degrading (V) is reserved for a later communication dealing with the synthesis of substances of the aneurin type. The amine (VI) was characterised as its *hydrochloride*, *thioformyl* and *acetyl* derivatives. Replacement of the amino-group by hydroxyl was effected by means of nitrous acid and the resulting 4-*hydroxy*-5-*hydroxymethyl*-2-*methylpyrimidine* (VII), on boiling with phosphoryl chloride, yielded the required chloro-compound (III). 2-Amino-4-methyl-5-β-hydroxyethylthiazole was obtained by condensing methyl α-chloro-γ-hydroxypropyl ketone (Part III; this vol., p. 1555) with thiourea and characterised as its *picrate*.

When a mixture of (III) and (IV) was heated at 110° for a short time, reaction occurred with the formation of a thick resin, which was in the main soluble in water; the aqueous solution, when made alkaline, deposited a considerable amount of an insoluble substance, presumably formed by interaction of the chloromethyl group of the pyrimidine with the

* A preliminary note on the results of this investigation has already been published (*Nature*, 1936, **138**, 406).

2-amino-group of the thiazole. The filtered alkaline solution had the intense blue fluorescence characteristic of thiochrome solutions; from it was isolated a crystalline substance having all the properties of thiochrome prepared from aneurin.





The identity of the synthetic material was established by careful comparison with a specimen of thiochrome prepared from the vitamin; both had the same m. p., the mixed m. p. showed no depression, and no divergences in other properties could be detected. The synthesis proves that thiochrome * has the structure (II) and may also be regarded as a proof of the structure of aneurin (I), a synthesis of which has recently been reported by Williams and Cline (*J. Amer. Chem. Soc.*, 1936, **58**, 1504).

In a similar fashion 9-*chloro*-3 : 7-*dimethylthiochromine* (VIII; R = H) and 9-*chloro*-3 : 7-*dimethyl*-2-β-*hydroxyethylthiochromine* (VIII; R = CH_2·CH_2·OH) were prepared by condensation of 2 : 4-*dichloro*-5-*chloromethyl*-6-*methylpyrimidine* with 2-amino-4-methylthiazole and 2-amino-4-methyl-5-β-hydroxyethylthiazole respectively; both these compounds are similar in properties to thiochrome and exhibit almost identical blue fluorescence in neutral or alkaline solution.

That salts of thiochrome on electrometric titration behave as if they contained a quaternary nitrogen atom has been recorded by Ogston and Peters (*Biochem. J.*, 1936, **30**, 736), although thiochrome itself has the properties of a tertiary base (Barger, Bergel, and Todd, *loc. cit.*). A probable explanation for this anomaly is that thiochrome may be in reality an anhydro-base, and that its salts may have structure (IX), it being assumed that the quaternary base liberated from such salts is very unstable and passes into the anhydro-form by loss of water.

Although the synthetic method described leaves no doubt as to the structure of the final product, it is impossible to state whether the 2-amino-thiazole (IV*a*) reacts as such or in the tautomeric 2-imino-thiazoline form (IV*b*). Owing to the number of side reactions the yield of thiochrome in the final condensation is rather unsatisfactory; experiments designed to obtain a more efficient synthetic method are in progress.

Experimental.

Ethyl 4-*Hydroxy*-2-*methylpyrimidine*-5-*acetate* (V).—To acetamidine hydrochloride (94·5 g.), dissolved in a cold solution of sodium (23 g.) in absolute alcohol (600 c.c.), was added freshly distilled ethyl formylsuccinate (202 g.) (Wislicenus, *Annalen*, 1908, **363**, 347). After standing for 2 hours at room temperature, the mixture was heated under reflux for a further 2 hours. Ethyl acetate (*ca.* 250 c.c.) was added, and the mixture again heated to boiling, filtered from sodium chloride, and allowed to cool. The product separated in fine colourless needles, m. p. 178° after recrystallisation from ethyl acetate or alcohol (Found: C, 55·4; H, 6·1; N, 14·9. $C_9H_{12}O_3N_2$ requires C, 55·1; H, 6·1; N, 14·3%). Yield, 54%.

4-*Hydroxy*-2-*methylpyrimidine*-5-*acethydrazide*.—When the above ester (100 g.) was heated with hydrazine hydrate (135 c.c. of 50%) on the water-bath for 2 hours, it dissolved and separation of the *hydrazide* occurred. This crystallised from alcohol, in which it was sparingly soluble, in heavy colourless prisms, m. p. 246° (Found: C, 46·5; H, 5·7; N, 30·7. $C_7H_{10}O_2N_4$ requires C, 46·2; H, 5·5; N, 30·8%). Yield, 80—85%. The hydrazide may also be obtained

* The name *thiochromine* is proposed for the condensed ring system present in thiochrome (II), which could be then described as 3 : 9-*dimethyl*-2-β-*hydroxyethylthiochromine*.

in approximately the same yield by heating the ester with 70% hydrazine hydrate solution for a short time, or by warming it for 5 hours in alcoholic solution with hydrazine hydrate.

4-Hydroxy-5-urethanomethyl-2-methylpyrimidine.—The above hydrazide (20 g.) was suspended in absolute alcohol (300 c.c.) containing hydrogen chloride (6 g.), amyl nitrite (19·3 g.) added to the cold mixture, and the whole warmed at 50—60° until evolution of nitrogen ceased (about 1 hour). During the heating the hydrazide slowly dissolved and a jelly-like substance separated. After cooling, ether was added to precipitate the remainder of the product. The jelly obtained was filtered, and the residue dried in a desiccator; this product, the urethane hydrochloride, had m. p. 209° (yield, 98%). The *urethane*, prepared from the hydrochloride by treatment with alcoholic ammonia, crystallised from ethyl acetate in colourless needles, m. p. 173° (Found: C, 51·3; H, 6·2; N, 19·9. $C_9H_{13}O_3N_3$ requires C, 51·2; H, 6·2; N, 19·9%).

4-Hydroxy-5-aminomethyl-2-methylpyrimidine (VI).—The urethane hydrochloride (5 g.) was heated with concentrated hydrochloric acid (50 c.c.) in a sealed tube at 100° during 2 hours. The clear solution was evaporated to small bulk in a vacuum, and ether added; the *hydrochloride* of the desired base separated in colourless plates. Recrystallised from absolute alcohol, it had m. p. 278—282° (Found: C, 41·6; H, 6·2; Cl, 20·9. $C_6H_9ON_3$,HCl requires C, 41·0; H, 5·7; Cl, 20·2%) (yield, quantitative). Further purification could not be effected by crystallisation. The corresponding hydrobromide, m. p. 270°, can readily be prepared in quantitative yield by heating the urethane with hydrobromic acid (60%) on the water-bath for 3 hours. The free base could not be crystallised, but it yielded stable thioformyl and acetyl derivatives.

4-Hydroxy-5-thioformamidomethyl-2-methylpyrimidine. To a solution of the amine hydrochloride in water were added potassium carbonate (1 equiv.) and excess of potassium dithioformate. In a few minutes the *thioformyl* derivative separated; it crystallised from water in colourless platelets, m. p. 199—200° (Found: C, 45·7; H, 5·4; S, 17·4. $C_7H_9ON_3S$ requires C, 45·9; H, 4·9; S, 17·4%).

4-Hydroxy-5-acetamidomethyl-2-methylpyrimidine. A mixture of the amine hydrochloride (350 mg.), fused sodium acetate (350 mg.), and acetic anhydride (5 c.c.) was heated under reflux for 30 minutes, the product evaporated to dryness in a vacuum, and the residue extracted with chloroform. The extract was filtered from inorganic material, and the chloroform removed; the residue crystallised from dioxan in colourless prisms, m. p. 219—220° (Found: N, 23·0. $C_8H_{11}O_2N_3$ requires N, 23·2%).

4-Hydroxy-5-hydroxymethyl-2-methylpyrimidine (VII).—A concentrated aqueous solution of sodium nitrite (15 g.) was added drop by drop to the hydrochloride of the amine (VI) (5 g.) dissolved in dilute hydrochloric acid (*ca.* 60 c.c. of 5%), the mixture heated in an open flask on the water-bath for 7 hours, and the brownish alkaline solution continuously extracted with ethyl acetate (Soxhlet). The crude product, which separated from the ethyl acetate solution in reddish crusts, was collected and extracted with a large quantity of boiling dioxan, which left insoluble by-products (these have not been further examined, but explode when heated in the dry state). The dioxan solution on concentration deposited *4-hydroxy-5-hydroxymethyl-2-methylpyrimidine* in colourless needles; a further small amount was obtained by precipitation of the ethyl acetate mother-liquor with light petroleum and treatment of the precipitate with dioxan as above described. After recrystallisation from dioxan the substance (1 g.) had m. p. 215—216° (Found: N, 20·2. $C_6H_8O_2N_2$ requires N, 20·0%).

4-Chloro-5-chloromethyl-2-methylpyrimidine (III).—The above hydroxymethyl compound (1 g.) was heated with phosphoryl chloride (4 c.c.) at 115—120° during 20 minutes; it slowly dissolved to a brownish solution. After removal of the phosphoryl chloride in a vacuum the thick residue was treated with ice-water, and the mixture made alkaline with potassium carbonate and extracted four times with ether. After removal of the ether the residue set to a mass of crystals. Recrystallised from a small quantity of light petroleum (b. p. 40—60°), the *product* formed long prisms (0·65 g.), m. p. 54° (Found: Cl, 39·7. $C_6H_6N_2Cl_2$ requires Cl, 40·1%).

2-Amino-4-methyl-5-β-hydroxyethylthiazole (IV).—A mixture of methyl α-chloro-γ-hydroxypropyl ketone (3 g.) and powdered thiourea (1·7 g.) was heated to 100°; within a few minutes a violent reaction occurred which quickly subsided. After a further 5 minutes the mixture was cooled, dissolved in water, and any unchanged halogenated ketone removed by extraction with ether. From the aqueous solution, made strongly alkaline, the thiazole base was extracted with a large amount of ether. The extract, dried over sodium sulphate and evaporated, left a residue, which distilled as a pale yellow oil (2·5 g.) at 172—175°/2 mm. After several weeks

the oil set to a hard crystalline mass, m. p. 85—90°. The base was not further purified; treatment with ethereal picric acid yielded a *picrate* crystallising from alcohol in pale yellow needles, m. p. 213° (Found: N, 17·5; S, 7·8. $C_{12}H_{13}O_8N_5S$ requires N, 18·1; S, 8·2%).

Thiochrome (II).—A mixture of 4-chloro-5-chloromethyl-2-methylpyrimidine (III) (580 mg.) and 2-amino-4-methyl-5-β-hydroxyethylthiazole (IV) (470 mg.) was heated to 110°, the initially clear liquid becoming opaque and viscous after some 15 minutes. The brown melt was cooled, extracted with ether to remove any unchanged initial material, and dissolved in water (*ca.* 15 c.c.), and the solution made alkaline with cold aqueous sodium hydroxide. After filtering from a cream-coloured amorphous precipitate, the yellowish solution, which showed strong blue fluorescence, was extracted with butyl alcohol until the extracts were no longer fluorescent. The combined butyl alcohol extracts were shaken three times with dilute hydrochloric acid (1%), the fluorescence disappearing. The greenish-yellow aqueous acid extracts were combined and evaporated to dryness in a vacuum at 30—40°, and the residue made strongly alkaline by addition of a small amount of 12% methyl-alcoholic potassium hydroxide. The mixture was shaken three times with chloroform (total vol. 750 c.c.), a few drops of water being added to bring inorganic matter into solution and facilitate separation. The intensely blue fluorescent chloroform extracts were combined, dried rapidly over potassium carbonate, and evaporated to small bulk (*ca.* 8 c.c.) in a vacuum. On cooling, thiochrome separated in pale yellow flakes; Recrystallised from chloroform, it had m. p. 225—226° (uncorr.) (Found: C, 55·3; H, 5·7 N, 21·0. Calc. for $C_{12}H_{14}ON_4S$: C, 54·9; H, 5·3; N, 21·4%).

Comparison of Synthetic Thiochrome and Thiochrome from Aneurin.—Both substances had the same m. p. 225—226° (uncorr.) and a mixed m. p. showed no depression. The crystalline form in both cases was identical and no divergence could be detected in their fluorescent properties. For further evidence of identity we are indebted to Dr. A. E. Gillam of Manchester University, who kindly compared the absorption of the two substances. Natural and synthetic thiochrome showed virtually identical absorption maxima at 3680 and 3690 A. respectively. The spectroscopic evidence therefore indicates that the two substances are qualitatively and, in so far as could be ascertained with the available material, quantitatively identical.

2 : 4-*Dichloro*-5-*chloromethyl*-6-*methylpyrimidine*.*—2 : 6 - Dihydroxy - 5 - hydroxymethyl - 6 - methylpyrimidine (6 g.) (Kircher, *Annalen*, 1911, **385**, 293), heated with phosphoryl chloride (15 c.c.) under reflux during 30—40 minutes, dissolved to give a deep brown solution. After removal of the excess of phosphoryl chloride in a vacuum the thick residue was triturated with ice-water, made alkaline with potassium carbonate, and extracted with ether. The extract on evaporation left a brownish resin, which was dissolved as far as possible in light petroleum (b. p. 40—60°) and filtered from amorphous impurities, and the filtrate again evaporated. The residue crystallised from a small volume of light petroleum in heavy colourless prisms (3 g.), m. p. 38—39° (Found: Cl, 50·4. $C_6H_5N_2Cl_3$ requires Cl, 50·4%).

9-*Chloro*-3 : 7-*dimethylthiochromine* (VIII; R = H).—A mixture of the above trichloro-compound (1·3 g.) and 2-amino-4-methylthiazole (0·7 g.) (Traumann, *Annalen*, 1888, **249**, 38) was heated at 110° for *ca.* 15 minutes, the initially clear liquid becoming brown and viscous. After removal of any unchanged initial material by means of ether, the product was dissolved in a little water, made strongly alkaline, and filtered from a cream-coloured amorphous precipitate. The yellowish, blue-fluorescent filtrate was extracted with butyl alcohol, and the fluorescent substance isolated by a process exactly analogous to that described above for thiochrome. Recrystallised from chloroform, the *product* formed pale yellow, woolly needles, m. p. 291—292° (decomp.) (Found: C, 47·3; H, 3·8; N, 22·1; S, 12·5; Cl, 14·1. $C_{10}H_9N_4ClS$ requires C, 47·5; H, 3·6; N, 22·2; S, 12·6; Cl, 14·1%). The compound is soluble in water and alcohol, sparingly so in chloroform, and practically insoluble in ether and acetone. In neutral or alkaline solution it has an intense blue fluorescence similar to but slightly stronger than that of thiochrome; as with the latter substance, addition of acid causes the blue fluorescence to disappear, the acid solution being greenish-yellow.

9-*Chloro*-3 : 7-*dimethyl*-2-β-*hydroxyethylthiochromine* (VIII; R = $CH_2{\cdot}CH_2{\cdot}OH$).—2 : 4-Dichloro-5-chloromethyl-6-methylpyrimidine (1 g.) and 2-amino-4-methyl-5-β-hydroxyethylthiazole (650 mg.) were heated together at 110° during 15 minutes and the brown resin produced was worked up exactly as described above for thiochrome. The *product* crystallised from chloroform in pale yellow platelets, m. p. 260—261° (decomp.) (Found: S, 10·4. $C_{12}H_{13}ON_4ClS$ requires S, 10·8%). The substance was closely similar to thiochrome in its solubilities, and the blue fluorescence of its neutral or alkaline solutions was very similar to that shown by the latter

* This compound was first obtained by Dr. R. Keller in the course of other work.

compound under the same conditions; addition of acid caused disappearance of the blue fluorescence and formation of a greenish-yellow solution.

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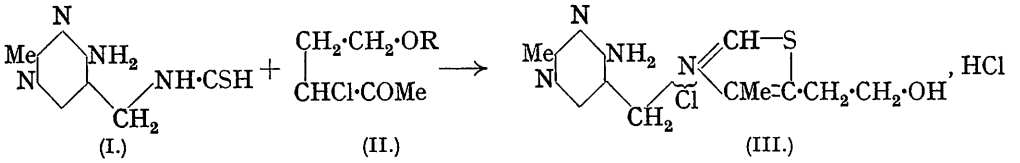
73. *Aneurin. Part VII. A Synthesis of Aneurin.*

By A. R. Todd and F. Bergel.

Williams and Cline (*J. Amer. Chem. Soc.*, 1936, **58**, 1504) have reported a synthesis of aneurin (III) and indicated briefly the route they employed. No details have yet been published, nor are details available of the synthesis carried out by the I. G. Farbenindustrie A. G. (cf. Grewe, *Z. physiol. Chem.*, 1936, **242**, 89). Both appear to be carried out by the addition of 4-amino-5-bromomethyl-2-methylpyrimidine to 5-β-hydroxyethyl-4-methylthiazole.

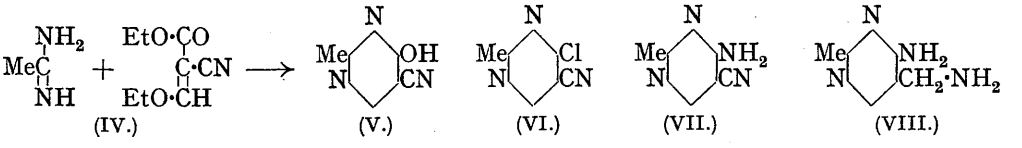
We have synthesised the vitamin by a method which is an extension of that already described for 3-pyrimidylthiazolium salts (Part V; J., 1936, 1559) and depends on the condensation of 4-*amino-5-thioformamidomethyl-2-methylpyrimidine* (I) with methyl α-chloro-γ-hydroxypropyl ketone (II; R = H) or one of its derivatives.



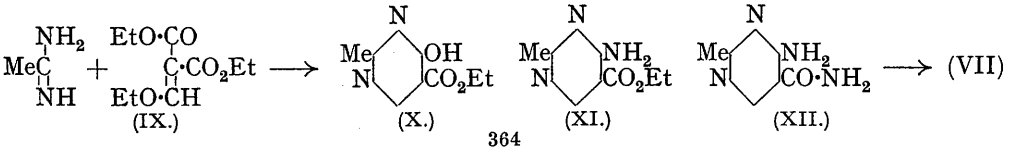
(I.) (II.) (III.)

For the synthesis of (I) we at first endeavoured to utilise as a starting material ethyl 4-hydroxy-2-methylpyrimidine-5-acetate (cf. Part VI; J., 1936, 1601), but all attempts to modify the Curtius or Hofmann degradation with this ester and its derivatives so as to produce the desired 4-amino-5-aminomethyl-2-methylpyrimidine failed, the low resistance of the amino-group in position 4 to hydrolytic agents invariably causing production of 4-hydroxy-5-aminomethyl-2-methylpyrimidine; the latter compound, too, could not be chlorinated. On the other hand, attempts to aminate 4-chloro-5-chloromethyl-2-methylpyrimidine led to formation of secondary amines; consequently the use of compounds of this series was abandoned.

Condensation of acetamidine with ethyl α-ethoxymethylene-α-cyanoacetate (IV) in absolute alcoholic solution gave an intermediate compound, probably ethyl α-cyano-β-acetamidinoacrylate, which on heating with alkali yielded 4-*hydroxy-5-cyano-2-methylpyrimidine* (V). Refluxing with phosphoryl chloride afforded 4-*chloro-5-cyano-2-methylpyrimidine* (VI), which could be aminated to give 4-amino-5-cyano-2-methylpyrimidine (VII); (VII) gave on catalytic hydrogenation 4-amino-5-aminomethyl-2-methylpyrimidine (VIII), isolated as its hydrochloride. The compound (VII) has been prepared in a different way by Grewe (*loc. cit.*), who also describes its reduction to the diamine.



(IV.) (V.) (VI.) (VII.) (VIII.)



(IX.) (X.) (XI.) (XII.)

An alternative route for the synthesis of (VII), which, though slightly longer, uses a cheaper starting material and is perhaps more reliable than the above, is the following. Ethyl ethoxymethylenemalonate (IX) condensed readily with acetamidine in presence of sodium ethoxide to give *ethyl 4-hydroxy-2-methylpyrimidine-5-carboxylate* (X), which, after successive chlorination with phosphoryl chloride and heating with alcoholic ammonia under pressure, yielded *ethyl 4-amino-2-methylpyrimidine-5-carboxylate* (XI). After conversion of (XI) into the corresponding *amide* (XII) with concentrated aqueous ammonia, the product was dehydrated to give the nitrile (VII), which could then be reduced as above mentioned. From (VIII), 4-amino-5-thioformamidomethyl-2-methylpyrimidine (I) was readily obtained by treatment with aqueous potassium dithioformate (cf. preceding paper).

The way now seemed clear for the synthesis of aneurin. As a result of our experience in the synthesis of 3-pyrimidylthiazolium salts (Part V, *loc. cit.*) we first endeavoured to condense (I) in the form of its sodium derivative with methyl α-chloro-γ-hydroxypropyl ketone (II; R = H) (Part III; J., 1936, 1555; Buchman, *J. Amer. Chem. Soc.*, 1936, **58**, 1803), but although various conditions were tried, only traces of aneurin could be obtained, the main product being the hydrochloride of (VIII). The reason for these failures may have lain in the instability of the sodium derivative. Compounds (I) and (II; R = H) did not yield aneurin when heated together in dioxan solution, but when a mixture of the two compounds alone was heated at 140° reaction occurred with considerable darkening and resinification. From the product a substance was isolated in poor yield which had the properties of aneurin. The low reactivity of the hydroxy-ketone (II; R = H), which probably exists mainly in the cyclic oxide form (cf. Buchman, *loc. cit.*), has already been mentioned (Part V, *loc. cit.*). Accordingly we heated a mixture of (I) and methyl α-chloro-γ-acetoxypropyl ketone (II; R = Ac) (Part III, *loc. cit.*) at 115—120° for a few minutes; smooth reaction occurred with production of a brownish-yellow mass, which crystallised on trituration with hot absolute alcohol containing a trace of hydrogen chloride. After recrystallisation from alcohol a product was obtained, m. p. 233—234°, having all the properties of aneurin chloride obtained from natural sources. The acetyl group in the ketone (II; R = Ac) is apparently eliminated in the reaction; this might be most readily explained by assuming that this compound also exists largely in the cyclic oxide form.

The synthetic material could not be distinguished from the natural vitamin by the formaldehyde-azo-test (Kinnersley and Peters, *Biochem. J.*, 1934, **28**, 667) or the thiochrome test (Part I; *Ber.*, 1935, **68**, 2257) and it showed a similar biological activity (380,000 I. U. per g.; natural vitamin, 400,000 I. U. per g.) as measured by the electrocardiagraphic method on rats (Birch and Harris, *Biochem. J.*, 1934, **28**, 602). Natural aneurin chloride isolated from rice polishings usually shows a m. p. 249—250° when pure, but a low-melting form has been reported by Kinnersley, O'Brien, and Peters (*Biochem. J.*, 1935, **29**, 701), who give m. p. 230° ± 2°; Williams and Cline (*loc. cit.*) state that their synthetic chloride has m. p. 232—234°. Our synthetic product apparently corresponds to the latter, but we do not consider the difference in m. p. is due to stereoisomerism as was tentatively suggested by Williams and Cline; it seems more probable that the existence of two forms of equal biological potency is due to dimorphism, a phenomenon which has been noticed by Kinnersley, O'Brien, and Peters (*loc. cit.*) in the case of the sulphate. Mixed with natural aneurin of m. p. 249°, the synthetic material had m. p. 243—246°. In accordance with this view we have compared the picrolonates prepared from the natural and the synthetic vitamin (see p. 367). Further, by oxidation of the synthetic vitamin with potassium ferricyanide in alkaline solution we have obtained a product, m. p. 225—226°, identical in every respect with thiochrome prepared either from natural aneurin or synthetically (Part VI, *loc. cit.*).

The synthesis of analogues of aneurin with a view to the determination of the necessary substituents for biological activity in 3-pyrimidinomethyl-thiazolium salts will form the subject of a later communication.

Experimental.

4-Hydroxy-5-cyano-2-methylpyrimidine (V).—To an ice-cold solution of sodium (10·2 g.) in absolute alcohol (300 c.c.) was added acetamidine hydrochloride (41·4 g.); the mixture was shaken for a few minutes and quickly filtered from precipitated sodium chloride. To the cooled

filtrate was added ethyl α-ethoxymethylene-α-cyanoacetate (75 g.) (de Bollemont, *Compt. rend.*, 1899, **128**, 1340; *Bull. Soc. chim.*, 1901, **25**, 20) in portions with shaking. As the ester went into solution a yellow colour developed and almost immediately a crystalline substance began to separate. After standing overnight at 0°, the precipitate was collected; it crystallised from ethyl acetate in colourless needles (37 g.), m. p. 108—110° (Found: C, 52·7; H, 6·3; N, 23·1. $C_8H_{11}O_2N_3$ requires C, 53·0; H, 6·1; N, 23·2%). This product may be ethyl α-cyano-β-acetamidinoacrylate.

The above intermediate product (36 g.) was heated on the water-bath for 5 minutes with a solution of sodium hydroxide (9 g.) in water (360 c.c.). The yellow solution was cooled, acidified with acetic acid, and concentrated in a vacuum to about half the original volume. On standing, 4-*hydroxy*-5-*cyano*-2-*methylpyrimidine* separated; it crystallised from water in fine colourless needles or rods (9 g.), m. p. 233—235° (Found: C, 53·3; H, 4·0; N, 30·8. $C_6H_5ON_3$ requires C, 53·3; H, 3·7; N, 31·1%).

Efforts were made to cause direct production of the pyrimidine so as to avoid, if possible, the losses involved in the ring closure of the intermediate ester with sodium hydroxide; for this purpose condensations were made at various temperatures with various amounts of sodium ethoxide but without satisfactory results.

4-*Chloro*-5-*cyano*-2-*methylpyrimidine* (VI).—When 4-hydroxy-5-cyano-2-methylpyrimidine (5 g.) was heated under reflux with phosphoryl chloride (15 c.c.) during 30 minutes, most of it dissolved to give a dark brown solution. After removal of phosphoryl chloride in a vacuum, the mixture was poured into ice-water, neutralised with potassium carbonate, and extracted with ether. After drying of the extract over sodium sulphate and removal of solvent, the *chloro*-compound remained as a reddish-yellow resin, pure enough for amination purposes. Recrystallised from light petroleum, it formed long colourless rods, m. p. 63—64° (Found: Cl, 22·6. $C_6H_4N_3Cl$ requires Cl, 23·1%). Yield, 60—70%.

4-*Amino*-5-*cyano*-2-*methylpyrimidine* (VII).—The above chloro-compound (2 g.) was heated with absolute-alcoholic ammonia (6 c.c. saturated at 0°) in a sealed tube at 100° during 4 hours. After removal of the alcohol and ammonia in a vacuum, the residue was boiled with *ca.* 100 c.c. of chloroform, and the solution filtered from ammonium chloride and evaporated. On recrystallising from methyl alcohol, the product formed colourless needles, m. p. 249° with partial sublimation (Grewe, *loc. cit.*, gives m. p. 249°) (Found: C, 54·0; H, 4·8. Calc. for $C_6H_6N_4$: C, 53·7; H, 4·5%). Yield, 40%.

Ethyl 4-*Hydroxy*-2-*methylpyrimidine*-5-*carboxylate* (X).—To a solution of sodium (12·8 g.) in absolute alcohol (500 c.c.) at 0° were added acetamidine hydrochloride (26·3 g.) and ethyl ethoxymethylenemalonate (60 g.) (Claisen, *Ber.*, 1893, **26**, 2731). After standing for 1 hour at room temperature, the mixture was heated on a water-bath under reflux for a further hour. After removal of most of the alcohol in a vacuum, the residue was diluted with water, and unchanged ester removed by extraction with ether. The aqueous solution was acidified with acetic acid, and the *pyrimidine* extracted with ethyl acetate. The residue obtained on evaporating the dried ethyl acetate solution crystallised from acetone in long woolly needles, m. p. 191° (Found: C, 52·8; H, 5·9. $C_8H_{10}O_3N_2$ requires C, 52·7; H, 5·5%). Yield, 60%.

Ethyl 4-*Amino*-2-*methylpyrimidine*-5-*carboxylate* (XI).—A mixture of the above ester (96 g.) and phosphoryl chloride (250 c.c.) was heated under reflux for 30 minutes. The red solution so formed was evaporated in a vacuum to remove phosphoryl chloride, and the resinous residue treated with a little ice-water, made alkaline with potassium carbonate, and extracted with chloroform. The dried chloroform solution on evaporation left the chloro-ester as a reddish oil, which without further purification was heated in an autoclave at 100° during 3 hours with 10 times its volume of absolute-alcoholic ammonia (4*N*). After cooling, the alcohol and excess of ammonia were removed under reduced pressure, and the residue recrystallised several times from water. The *product* formed long colourless needles, m. p. 120° (Found: N, 23·2. $C_8H_{11}O_2N_3$ requires N, 23·2%). Yield, 65%.

4-*Amino*-2-*methylpyrimidine*-5-*carboxyamide* (XII).—The above finely powdered amino-ester (50 g.) was shaken at room temperature with aqueous ammonia (320 c.c., *d* 0·880) during 36 hours. The needle-shaped crystals of the initial material disappeared gradually, although no apparent dissolution was observed. The *amide* was collected and recrystallised from absolute alcohol, forming small prisms, m. p. 264—265° (Found: N, 36·7. $C_6H_8ON_4$ requires N, 36·8%). Yield, 65%. A further small quantity was obtained by concentrating the ammoniacal mother-liquor.

4-*Amino*-5-*cyano*-2-*methylpyrimidine* (VII).—The above amide (2 g.) was heated under reflux with phosphoryl chloride (15 c.c.) during 2—3 hours, and the mixture then poured on ice, made

alkaline with potassium carbonate, and extracted with chloroform. After drying over sodium sulphate, the chloroform was removed; the residue crystallised from methyl alcohol in needles, m. p. 249° with partial sublimation, not depressed by 4-amino-5-cyano-2-methylpyrimidine prepared as described above. Yield, 50%.

When large quantities of material were used in this preparation, the yield of product diminished considerably; this may be due to the insolubility of the amide in phosphoryl chloride, and phosphorus pentachloride may be preferable on the large scale.

4-Amino-5-aminomethyl-2-methylpyrimidine Hydrochloride.—The above amino-nitrile in acetic acid solution was subjected to catalytic hydrogenation in presence of palladised charcoal (cf. Grewe, *loc. cit.*). The product had m. p. 264—265°. The same result was achieved with a platinum oxide catalyst, though the reduction was slower.

4-Amino-5-thioformamidomethyl-2-methylpyrimidine (I).—An aqueous solution of the above hydrochloride was neutralised with potassium bicarbonate, and a slight excess (*ca.* 1·2 mols.) of potassium dithioformate added. After a short time the *thioformyl* derivative separated. It crystallised from alcohol in colourless platelets, m. p. 187° (decomp.) (Found: C, 46·1; H, 5·5. $C_7H_{10}N_4S$ requires C, 46·1; H, 5·5%).

Aneurin Chloride (III).—A mixture of 4-amino-5-thioformamidomethyl-2-methylpyrimidine (500 mg.) and methyl α-chloro-γ-acetoxypropyl ketone (600 mg.) (Part III, *loc. cit.*) was heated in a paraffin-bath at 115—120° during 15 minutes. The mixture became liquid and then brownish and viscous, a thiazole-like odour becoming noticeable. The mass was cooled and triturated repeatedly with dry ether; it then fell to a yellowish-brown powder. This was collected and heated with *ca.* 3 c.c. of absolute alcohol containing a little hydrogen chloride; after a few moments the product began to crystallise without having completely dissolved. After cooling, it was collected and separated from a small amount of sparingly soluble 4-amino-5-aminomethyl-2-methylpyrimidine hydrochloride by fractional crystallisation from absolute alcohol. The product had m. p. 233—234°, unchanged by recrystallisation (Found: C, 40·5; H, 6·0; N, 15·5; S, 8·5; Cl, 20·1. Calc. for $C_{12}H_{18}ON_4Cl_2S,H_2O$: C, 40·6; H, 5·6; N, 15·8; S, 9·0; Cl, 20·0%).

Comparison of Natural and Synthetic Aneurin Chloride.—In addition to the tests mentioned on p. 365, the following experiments were made. Treatment with cold aqueous picrolonic acid caused with each sample separation of yellow needles of a picrolonate, m. p. and mixed m. p. 164—165°; recrystallisation of this product from water in the ordinary way gave in each case a mixture of needles and prisms, m. p. 170—180°, not depressed on mixing. When this material was heated for 5 minutes with a small amount of water—insufficient to dissolve it completely—and the solution filtered hot, the residue consisted of prisms, m. p. 228—229° (decomp.). A mixed m. p. showed no depression. These results correspond exactly to the data for the dimorphous aneurin picrolonate described by Windaus, Tschesche, Laqueur, and Schultz (*Z. physiol. Chem.*, **1933**, **204**, 123).

The only apparent difference in the two chlorides lay in the m. p., our specimen from natural sources having m. p. 249—250°, the synthetic material 233—234°, and a mixture of the two 243—246°. On seeding a solution of the synthetic product with a crystal of the natural, we obtained a product, m. p. 245—247°; a solution of the natural vitamin seeded with the synthetic gave crystals, m. p. 241—244°.* In all cases the crystals appeared to be colourless platelets. In view of these facts and the formation of the dimorphous picrolonate we are of the opinion that aneurin chloride is itself dimorphous.

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* (Note added, March 2nd). Since the above was written the interconversion of the two forms of the chloride has also been reported by Williams and Cline (*J. Amer. Chem. Soc.*, 1937, **59**, 216).

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