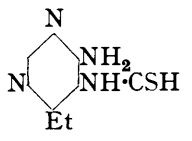
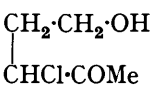
344. *Aneurin. Part V. The Synthesis of 3-Pyrimidylthiazolium Salts, including an Isomer of Aneurin.**

By A. R. Todd and F. Bergel.

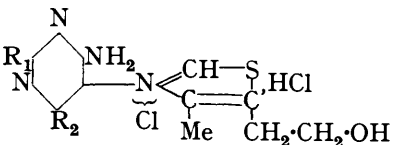
Although 3-pyrimidylthiazolium salts can be synthesised by heating 5-thioformamidopyrimidines with chloroacetone (preceding paper), this simple method cannot be applied when chloroacetone is replaced by methyl α-chloro-γ-hydroxypropyl ketone, owing to the low reactivity of the latter substance. The difficulty 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.)



(II.)



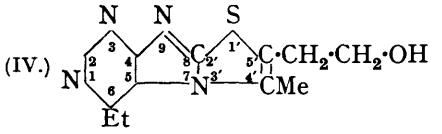
(III.)

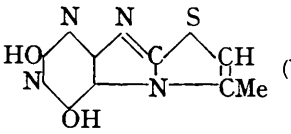
According to the original suggestion of Williams (*J. Amer. Chem. Soc.*, 1935, **57**, 229), (III; R = H, R_2 = Et) should have been identical with the hydrochloride of aneurin (vitamin B_1). This was not the case; in appearance and general solubilities the synthetic substance resembled the natural vitamin 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).

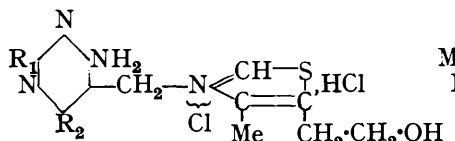
Chem. Soc., 1935, **68**, 1751), and thiochrome (Barger, Bergel, and Todd, *Ber.*, 1935, **68**, 2257) all give negative results.

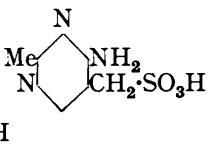
Any possibility that the vitamin might be represented by a closely related structure (III; $R_1 = R_2 = Me$) may be excluded on the following grounds. When synthetic 3-pyrimidylthiazolium salts containing an amino-group in position 6′ are oxidised with alkaline potassium ferricyanide under the conditions 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.*).

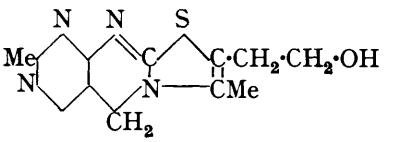
(IV.) 


(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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