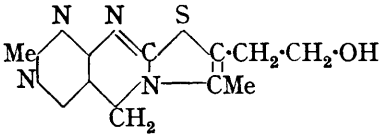
7. *Aneurin. Part IX. A New Synthesis of Thiochrome. Note on the Synthesis of Aneurin.*

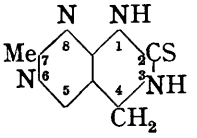
By F. Bergel and A. R. Todd.

A synthesis of thiochrome from 2-thio-7-methyl-1 : 2 : 3 : 4-tetrahydro-1 : 3 : 6 : 8-benztetrazine and methyl α-chloro-γ-acetoxypropyl ketone is described. *O*-Acetylaneurin is shown to be an intermediate product in the synthesis of aneurin described in Part VII (J., 1937, 364). The existence of a low-melting modification of aneurin chloride hydrochloride is confirmed.

The structure of thiochrome (I), the blue-fluorescent oxidation product of aneurin (vitamin B_1), has been established by its synthesis from 4-chloro-5-chloromethyl-2-methylpyrimidine and 2-amino-4-methyl-5-β-hydroxyethylthiazole (Todd, Bergel, Fraenkel-Conrat, and Jacob, J., 1936, 1601). This synthesis was, however, unsatisfactory as a practical method for the preparation of the pigment. As larger quantities of thiochrome were required for various purposes, we endeavoured to synthesise it by adapting the procedure used in the synthesis of 3-methylthiazolobenzimidazole (Todd, Bergel, and Karimullah, *Ber.*, 1936, **69**, 217). 2-Thio-7-methyl-1 : 2 : 3 : 4-tetrahydro-1 : 3 : 6 : 8-benztetrazine (II) was heated with methyl α-chloro-γ-acetoxypropyl ketone either alone or better in presence of glacial acetic acid; a product was obtained identical with thiochrome prepared from aneurin,

the initially formed acetyl derivative being hydrolysed during the process of working up. The yield in this synthesis was again disappointing; further attempts to improve the yield are not contemplated, as the ready accessibility of synthetic aneurin makes the preparation of thiochrome from it an economical process, the yield being 70—80%.

(I.) 

 (II.)

The preparation of 8-thiopurines by heating 4 : 5-diaminopyrimidines with thiourea (Gabriel, *Ber.*, 1901, **34**, 1254) is a satisfactory process, but fusion of 4-amino-5-aminomethyl-2-methylpyrimidine with thiourea gave a very small yield of the benztetrazine derivative (II). Condensation of the hydrochloride of 4-amino-5-aminomethyl-2-methylpyrimidine with potassium thiocyanate, and heating of the substituted thiourea so formed, gave (II) in a yield of 45%. It seems from these results that the benztetrazine ring system is much less readily formed than the purine nucleus.

Note on the Synthesis of Aneurin.—In Part VII (J., 1937, 364) we described a synthesis of aneurin in which 4-amino-5-thioformamidomethyl-2-methylpyrimidine was condensed with methyl α-chloro-γ-acetoxypropyl ketone. The crude product yielded the vitamin chloride after several recrystallisations from alcohol containing hydrogen chloride. At some stage in this process the acetyl group originally present in the chloro-ketone must have been removed. A probable explanation seemed that it was eliminated by hydrolysis during the crystallisation process. In support of this view we have isolated from the crude reaction mixture *O-acetylaneurin chloride hydrochloride*. This substance is readily hydrolysed by acids, and yields a crystalline, sparingly soluble picrate, m. p. 176°. The residual material after removal of the *O*-acetylaneurin yielded, on being worked up as described in Part VII (*loc. cit.*), aneurin chloride hydrochloride. When absolute alcohol was used in the crystallisation processes the vitamin chloride had m. p. 236—237°, unchanged by further recrystallisation from the same solvent. On recrystallisation from 90% alcohol or by seeding with the natural material it was readily converted into a product, m. p. 246—247°, identical with the usual form of the vitamin. The m. p. of aneurin chloride hydrochloride is not a very good criterion of purity, as m. p.'s ranging from 245° to 250° can be obtained according to the rate of heating. The possibility that our low-melting material is contaminated with *O*-acetylaneurin is unlikely, as treatment of it with aqueous picric acid yields at once a crystalline picrate, m. p. 203—204° without further purification. In our experience direct production of a crystalline picrate of this m. p. is only possible with very pure aneurin. Moreover, the same low-melting form of the vitamin has been obtained and examined by Williams and Cline (*J. Amer. Chem. Soc.*, 1937, **59**, 216) in their synthesis of aneurin; in this case there is no possibility of acetylaneurin being present as an impurity.

Experimental.

2-Thio-7-methyl-1 : 2 : 3 : 4-tetrahydro-1 : 3 : 6 : 8-benztetrazine (II).—(A) A mixture of 4-amino-5-aminomethyl-2-methylpyrimidine hydrochloride (1 g.) and powdered thiourea (2 g.) was heated at 180—200° until evolution of ammonia ceased (*ca.* 2½ hours). The melt solidified on cooling and was extracted twice with small amounts of boiling water (10 c.c.). The combined extracts deposited, on standing, a brownish crystalline product, which was extracted with acetone, the acetone-insoluble portion being discarded. The extract was evaporated; the residue, recrystallised from acetone–glacial acetic acid, formed faintly yellowish needles (35 mg.), m. p. 272—274°. When heated with chloroacetone, it yielded a product showing strong blue fluorescence in neutral or alkaline solution.

(B) A solution of 4-amino-5-aminomethyl-2-methylpyrimidine hydrochloride (3 g.) and potassium thiocyanate (3·6 g.) in water (15 c.c.) was evaporated to dryness on the water-bath, and the residue extracted with absolute alcohol. After filtering from potassium chloride, the extract was evaporated to dryness, and the residue heated at 170° for *ca.* 30 minutes. The mass at first liquefied and evolved gas, then resolidified towards the end of the heating. The product was cooled and twice extracted with boiling water (180 c.c. in all). The extract slowly

deposited yellowish crystals on cooling; these were collected and separated from insoluble material by extraction (Soxhlet) with acetone. The acetone extract gave on evaporation the *benztetrazine* (II), which separated from glacial acetic acid–acetone (1 : 1) in colourless soft needles, m. p. 275—277° (decomp.) (yield 1·2 g., *i.e.*, 45%) (Found in material dried at 80° : C, 46·3; A, 4·5; S, 17·4. $C_7H_8N_4S$ requires C, 46·6; H, 4·5; S, 17·8).

Thiochrome (I).—A mixture of the above thiobenztetrazine (1·2 g.), methyl α-chloro-γ-acetoxypropyl ketone (1·8 g.), and glacial acetic acid (16 c.c.) was heated under reflux during 10 hours. The initially clear solution began to deposit solid material after 1 hour, and after 8 hours a small amount (0·5 g.) of the chloro-ketone was added to ensure complete reaction. The mixture was cooled, excess of dry ether added, and the precipitate collected, washed with ether, dissolved in water (20 c.c.), and filtered from insoluble material (0·3 g.). Hydrochloric acid was added to the brown filtrate to give an acid concentration of 8% and after a few hours the solution was evaporated to dryness in a vacuum, and the residue washed with a little butyl alcohol, treated with alcoholic potassium hydroxide solution (15 c.c. of 20%), and extracted rapidly with chloroform (*ca.* 2 l.). The blue-fluorescent chloroform extract was dried and evaporated in a vacuum to *ca.* 50 c.c. Thiochrome slowly separated in faintly yellow, flaky crystals. Recrystallised from chloroform, it had m. p. 224—225°, undepressed by thiochrome prepared by oxidation of natural aneurin (yield, 0·22 g.) (Found : C, 55·5; H, 5·6. Calc. for $C_{12}H_{14}ON_4S$: C, 54·9; H, 5·3%).

When treatment of the crude product with hydrochloric acid was omitted, a product, m. p. 203°, probably acetylthiochrome, was sometimes obtained.

Synthesis of Aneurin : Isolation of O-Acetylaneurin.—A mixture of 4-amino-5-thioformamidomethyl-2-methylpyrimidine (1 g.), methyl α-chloro-γ-acetoxypropyl ketone (1·2 g.), and glacial acetic acid (5 drops) was heated at 118—119° for 25 minutes; the mixture, at first liquid, became viscous. After cooling, the product was washed with ether and warmed with absolute alcohol (8 c.c.) containing alcoholic hydrogen chloride (1 c.c. of 4%). After standing for 12 hours, the crystalline hygroscopic material (0·5 g.) was collected and redissolved in absolute alcohol (5 c.c.) containing a trace of hydrogen chloride. In the course of several hours O-*acetylaneurin chloride hydrochloride* (0·1 g.) separated; recrystallised from absolute alcohol, it formed colourless leaflets, m. p. 205—207° (Found in material dried at 40° : N, 13·7. $C_{14}H_{19}O_2N_4SCl,HCl,H_2O$ requires N, 14·1%). The picrate had m. p. 176°. When a larger concentration of hydrogen chloride than the above-mentioned was used in preliminary treatment of the reaction mixture, only traces of acetylaneurin were obtained.

From the acetylaneurin mother-liquors and from the original mother-liquor more solid material separated on standing. Repeated crystallisation from absolute alcohol containing hydrogen chloride gave aneurin chloride hydrochloride, m. p. 236—237°, indistinguishable save in m. p. from the natural vitamin.

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