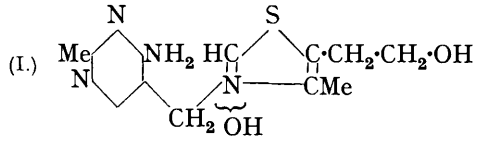
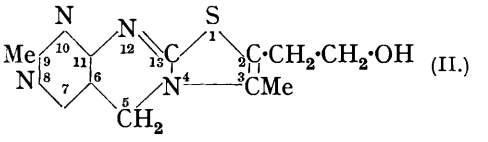
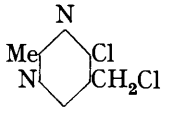
353. *Aneurin. Part VI. A Synthesis of Thiochrome and Related Compounds.**

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

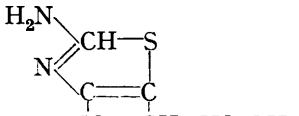
Kuhn, Wagner-Jauregg, van Klaveren, and Vetter (*Z. physiol. Chem.*, 1935, **234**, 196) isolated from yeast a yellow basic substance, $C_{12}H_{14}ON_4S$, whose neutral or alkaline solutions were characterised by intense blue fluorescence; to it they gave the name thiochrome. The same substance was 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.



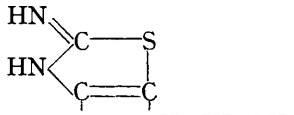




(III.)



(IV*a*.)



(IV*b*.)

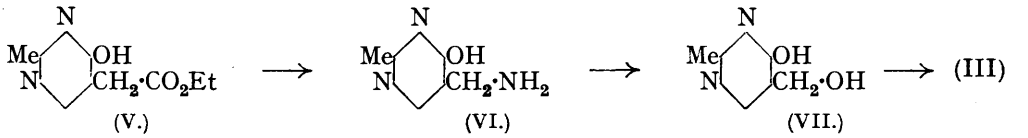
It seemed possible that compounds similar to (II) might be synthesised by condensing 4-chloro-5-chloromethylpyrimidines with 2-aminothiazoles; thiochrome itself would be synthesised in this way from *4-chloro-5-chloromethyl-2-methylpyrimidine* (III) and 2-amino-4-methyl-5-β-hydroxyethylthiazole (IV*a*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

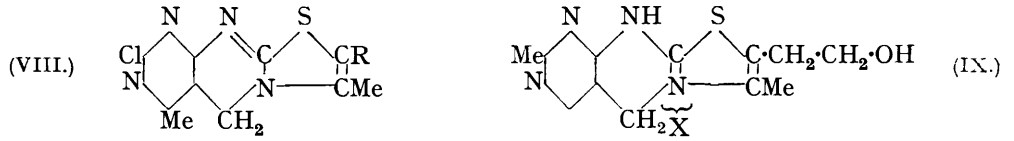
For the synthesis of (III) various methods seemed possible; we adopted the following route, as the earlier intermediates were available in connection with other synthetic investigations. Condensation of acetamidine with ethyl formylsuccinate yielded *ethyl 4-hydroxy-2-methylpyrimidine-5-acetate* (V), 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 = $CH_2 \cdot CH_2 \cdot OH$) 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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MEDICAL CHEMISTRY DEPARTMENT, UNIVERSITY OF EDINBURGH.

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