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

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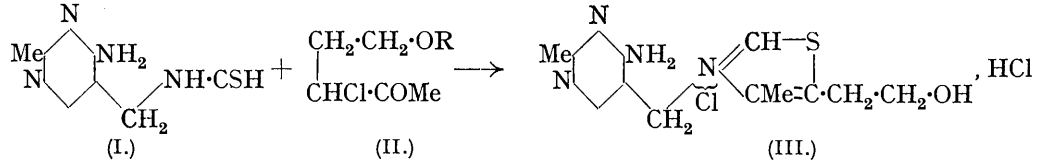
Williams and Cline (*J. Amer. Chem. Soc.*, 1936, **58**, 1504) have reported a synthesis of aneurin (III) and indicated briefly the route they employed. No details have yet been published, nor are details available of the synthesis carried out by the I. G. Farbenindustrie A. G. (cf. Grewe, *Z. physiol. 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.

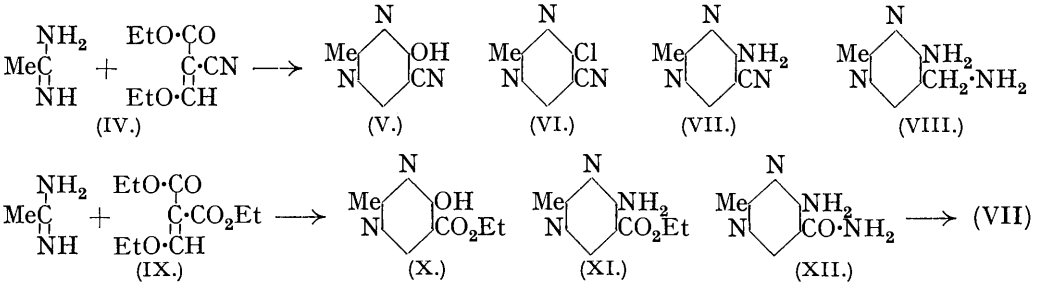
We have synthesised the vitamin by a method which is an extension of that already described for 3-pyrimidylthiazolium salts (Part V; J., 1936, 1559) and depends on the condensation of *4-amino-5-thioformamidomethyl-2-methylpyrimidine* (I) with methyl α-chloro-γ-hydroxypropyl ketone (II; R = H) or one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