100. α-*Amino*-β-(4-*methylthiazole*-5)-*propionic Acid, a Possible Precursor of Aneurin.*

By C. R. Harington and R. C. G. Moggridge.

The presence of a hydroxyethyl side chain in the thiazole component of the aneurin molecule suggests the possible biological formation of this compound from the corresponding α-aminopropionic acid; a further extension of this idea brings the aneurin thiazole into hypothetical relationship with the known natural product methionine. *α-Amino-β-(4-methylthiazole-5)-propionic acid* has been synthesised with the objects of studying (a) its power to replace the aneurin thiazole as a growth factor for organisms which can utilise the two components of aneurin separately, and (b) its possible conversion by yeast into the aneurin thiazole.

In none of the work which had been published on aneurin until a few months ago had serious consideration been given to the biogenesis of the vitamin; the present communication represents an effort in this direction.*

* At a late stage of this work we learnt from Dr. B. C. J. G. Knight that he had been supplied by Dr. E. R. Buchman with samples of the thiazole amino-acid and ethylamine derivatives which had been prepared by the latter in unpublished experiments. Correspondence between one of us (C. R. H.) and Dr. Buchman has revealed that the latter had arrived at a similar biogenetic hypothesis (compare Bonner and Buchman, *Proc. Nat. Acad. Sci.*, 1939, **24**, 431). Dr. Buchman's experiments are being published elsewhere and we wish here to express our great appreciation of the courtesy which he has shown us in this matter.

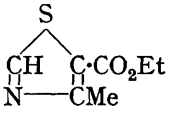
From a biological point of view the most striking feature of the aneurin molecule is the occurrence in it of a thiazole group which appears here for the first time among natural products; moreover the thiazole group is itself remarkable in that it carries a β-hydroxyethyl side chain—which is unusual among compounds of physiological importance but seems in this case to be essential to biological activity.

There is, however, one well-known biochemical reaction which might give rise to the 4-methyl-5-(β-hydroxyethyl)thiazole of aneurin, namely, the fermentative degradation of the corresponding α-aminopropionic acid; such a degradation occurs generally when α-amino-acids are subjected to the action of actively fermenting yeast (Ehrlich, *Ber.*, 1911, **44**, 139; 1912, **45**, 884) and is in fact the reaction which is responsible for the formation of "fusel oil" in alcoholic fermentation; it can also be brought about by the action of certain bacteria on amino-acids.

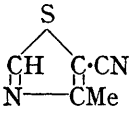
The fact that yeast, which is the organism pre-eminently capable of effecting this particular degradation of amino-acids, is itself a rich source of aneurin, lends further plausibility to the view that the thiazole component of the vitamin may actually arise from *α-amino-β-(4-methylthiazole-5)-propionic acid*; moreover the introduction of the latter compound into a scheme for the biogenesis of aneurin brings this process into hypothetical relation with known natural products, since it is not altogether unreasonable to picture the thiazole amino-acid as arising itself from methionine, acetaldehyde and ammonia.

In order to test the hypothesis outlined above, the thiazole amino-acid has been synthesised with the objects of studying (1) its power of replacing 4-methyl-5-(β-hydroxyethyl)thiazole in the *Staphylococcus aureus* growth test (Knight, *Biochem. J.*, 1937, **31**, 966), (2) its metabolism by fermenting yeast, and (3) its possible occurrence in nature. The first of these tests has been kindly carried out for us by Dr. B. C. J. G. Knight with negative results; this failure is, however, not necessarily significant in relation to the general hypothesis, since it may mean no more than the inability of a particular micro-organism to effect the degradation of the amino-acid side chain; our intention is therefore now to pursue the further biochemical programme indicated above.

The synthesis of the amino-acid has followed conventional lines and calls for no detailed comment. The starting material was ethyl 4-methylthiazole-5-carboxylate (I), which was converted through the *amide* into the *nitrile* (II) and thence into the *aldehyde* (III); the amino-acid (IV) was prepared from the last by the modified Erlenmeyer synthesis (Harington and McCartney, *Biochem. J.*, 1927, **21**, 852). The process involves the successful application in a new series of the method of Stephen (J., 1925, **127**, 1874) for the preparation of aldehydes.



(I.)



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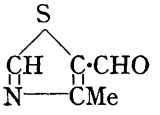

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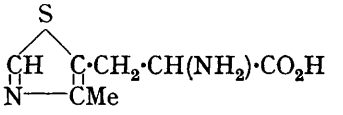

(IV.)

The opportunity was also taken to prepare β-(4-methylthiazole-5)-ethylamine, which is formed smoothly on heating the amino-acid (IV) in admixture with diphenylamine. This compound, which requires only deamination to convert it into the thiazole component of aneurin, has also been tested for us by Dr. Knight, who finds that it is inactive in promoting the growth of *Staph. aureus*.

Experimental.

Ethyl 4-Methylthiazole-5-carboxylate Hydrochloride.—The free ester has been prepared in 48% yield by Clarke and Gurin (*J. Amer. Chem. Soc.*, 1935, **57**, 1876). We have obtained better results by the following more rapid method. Phosphorus pentasulphide (36 g.), formamide (60 g.), and anhydrous ether (600 c.c.) were shaken together for 2 days; the decanted ethereal solution was concentrated to low bulk, and the residue twice extracted with saturated aqueous ammonium sulphate; the aqueous solution was then extracted thrice with ether, and the combined extracts evaporated. The residual crude thioformamide (23 g.), alcohol (15 c.c.), and ethyl α-chloroacetoacetate (47·5 g.) (Allihn, *Ber.*, 1878, **11**, 567) were mixed, with sufficient

cooling to keep the temperature below 45°. After some hours at ordinary temperature the mixture was chilled to 0°, and the *ester hydrochloride* collected and washed with ether. Yield, 56% of the theoretical of practically pure product. Recrystallised from alcohol, it formed colourless needles, m. p. 155° (Found : Cl, 17·25. $C_7H_9O_2NS$,HCl requires Cl, 17·1%).

4-*Methylthiazole*-5-*carboxyamide*.—The free ester was obtained by shaking an aqueous suspension of the above hydrochloride with excess of sodium carbonate, followed by extraction with ether. The residue left on evaporation of the ether was shaken for 2—3 days with aqueous ammonia (3 vols. of *d* 0·880); the *amide* then crystallised in part. Evaporation of the whole to dryness in a vacuum gave an 80% yield of almost pure amide, which, after recrystallisation from alcohol, was obtained as long needles, m. p. 149° (Found : N, 19·9; S, 22·8. $C_5H_6ON_2S$ requires N, 19·75; S, 22·6%).

5-*Cyano*-4-*methylthiazole*.—The crude amide (10·3 g.) was refluxed with freshly distilled phosphorus oxychloride (50 c.c.) until the solid had all disappeared; the solution on cooling deposited a crystalline precipitate (14·6 g.). This product was hygroscopic; it crystallised from ethyl acetate in colourless plates, m. p. 100°, containing phosphorus and reacting vigorously with water; it appears to be a complex of the nitrile with phosphorus residues. The *nitrile* was obtained by treating the above product with ice-water, basifying with sodium carbonate, and extracting with ether; evaporation of the dried extract left an oil, which soon crystallised. Yield, 60% of the theoretical. The product had b. p. 86—88°/14 mm. and, recrystallised from light petroleum (b. p. 60—80°), formed colourless prisms, m. p. 33·5° (Found : N, 22·4; S, 25·7. $C_5H_4N_2S$ requires N, 22·6; S, 25·85%).

The *hydrochloride* was obtained by passing hydrogen chloride into an ethereal solution of the base; it had m. p. 145° (decomp.) (Found : N, 17·35; Cl, 22·3. $C_5H_4N_2S$,HCl requires N, 17·45; Cl, 22·1%).

4-*Methylthiazole*-5-*aldehyde*.—Dry hydrogen chloride was passed into a mixture of anhydrous ether (50 c.c.) and anhydrous stannous chloride (10 g.) in a pressure bottle until the latter had dissolved; an ethereal solution of the crude nitrile (2·5 g.) was then added; the bottle was immediately corked and shaken until the solid product first formed had given place to a viscous yellow oil (2·5—3 hrs.). The ether was decanted, and the oil taken up in a little water; the solution was cooled to — 10°, treated with excess of chilled aqueous sodium hydroxide (250 c.c. of 40%), and rapidly extracted with ether. Evaporation of the dried (calcium chloride) ethereal extracts after treatment with charcoal gave the crude *aldehyde* in 65% yield. After recrystallisation from light petroleum (b. p. 60—80°) it formed colourless plates, m. p. 72·5° (Found : N, 10·9; S, 25·7. C_5H_5ONS requires N, 11·0; S, 25·2%). The yield of recrystallised product was a little over 40% of the theoretical; the aldehyde gave the Schiff reaction and reduced Fehling's solution; it formed a *phenylhydrazone*, orange plates, m. p. 161°, from aqueous alcohol (Found : N, 19·3. $C_{11}H_{11}N_3S$ requires N, 19·4%), and a *semicarbazone*, colourless plates, m. p. 241°, from aqueous alcohol (Found : N, 30·05. $C_6H_8ON_4S$ requires N, 30·4%). In some preparations of the aldehyde a petroleum-insoluble by-product, m. p. 148°, was encountered; in general, however, the amounts of this compound obtained were negligible and it was not further investigated.

Azlactone.—The crude aldehyde (2·2 g.), hippuric acid (3·11 g.), acetic anhydride (20·5 c.c.), and fused sodium acetate (7 g.) were heated together on the steam-bath for 15 minutes. Water (300 c.c.) was added to the cooled mixture and after decomposition of the excess of acetic anhydride the crystalline *azlactone* was collected (yield, 70%). The compound formed orange-brown needles, m. p. 199°, from acetic acid (Found : N, 10·4. $C_{14}H_{10}O_2N_2S$ requires N, 10·4%).

α-*Amino*-β-(4-*methylthiazole*-5)-*propionic Acid*.—The azlactone (3·6 g., crude), red phosphorus (3·5 g.), and a mixture (28 c.c.) of equal volumes of hydriodic acid (*d* 1·7) and acetic anhydride were refluxed together for 1 hour. The solution was filtered hot, and the filtrate evaporated in a vacuum, the evaporation being repeated after addition of water. The residue was shaken with water and ether, and the aqueous layer, after re-extraction with ether, was treated with excess of ammonia and again evaporated. The mixture of amino-acid and ammonium salts so obtained was boiled with acetone and left at the ordinary temperature overnight. Next day the precipitate was collected, washed with acetone, and dissolved in boiling water; the *amino-acid* separated from the cooled solution in colourless prisms, m. p. 240° (decomp.); yield, 62% [Found : C, 44·5; H, 5·65; N, 15·1; S, 16·9. $C_7H_{10}O_2N_2S$ requires C, 45·2; H, 5·4; N, 15·05; S, 17·2%. Found : amino-N (van Slyke), 7·15. Calc., 7·5%]. The amino-acid gave consistently low values for carbon on analysis; that this was not due to water of crystallisation was shown by the figures for nitrogen and sulphur, and by the fact that the samples analysed lost no significant weight on drying at 110° in a vacuum.

The amino-acid is sparingly soluble in cold water and readily in hot; it is insoluble in alcohol. It is precipitated from aqueous solution by phosphotungstic acid and mercuric chloride, but not by mercuric sulphate in dilute sulphuric acid. Picric and flavianic acids in cold aqueous solution give no precipitate with the amino-acid, but on admixture of hot concentrated solutions of the amino-acid (1 mol.) and picric acid (2 mols.) a *dipicrate* is formed, which separates on cooling in yellow prisms, m. p. 146° (decomp.) (Found: picric acid, 71·1. $C_7H_{10}O_2N_2S,2C_6H_3O_7N_3$ requires picric acid, 71·1%).

β-(4-*Methylthiazole*-5)-*ethylamine Dihydrochloride*.—α-Amino-β-(4-methylthiazole-5)-propionic acid (0·5 g.) and diphenylamine (10 g.) were heated together in a metal bath; evolution of carbon dioxide began at 200° and proceeded rapidly and smoothly to completion at 220°. The cooled melt was treated with dilute hydrochloric acid and ether, and the aqueous layer, after a second washing with ether, was treated with charcoal and evaporated in a vacuum. The residue was taken up in hot methyl-alcoholic hydrogen chloride (5N); on cooling, the *dihydrochloride* separated in colourless prisms, m. p. 246°; separation was completed by addition of a little dry ether, the total yield being 50% (Found: C, 33·1; H, 5·9; N, 12·9; S, 14·55. $C_6H_{10}N_2S,2HCl$ requires C, 33·5; H, 5·6; N, 13·0; S, 14·85%). The preparation of the above amine by Hofmann or Curtius degradation of the corresponding propionamide is mentioned in Brit. Pat. 456,751 of the I.G.-Farbenindustrie Akt. Ges. (*Chem. Zentr.*, 1937, I, 286).

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