83. EXPERIMENTS ON THE BIOGENESIS OF VITAMIN B₁

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WE have recently suggested [Harington & Moggridge, 1939; compare also Bonner & Buchman, 1938] that the 4-methyl-5-(β -hydroxyethyl)-thiazole (I) of aneurin might arise *in vivo* from α -amino- β -(4-methylthiazole-5)-propionic acid (II) by a reaction analogous to the formation of "fusel oil" in alcoholic fermentation. The fact that yeast, which is pre-eminently able to effect this type of degradation, is a rich source of aneurin added plausibility to this hypothesis. The synthesis of the amino-acid (II) was described in our last communication. It has now been found that the compound II is in fact broken down by yeast to give 4-methyl-5-(β -hydroxyethyl)-thiazole (I) which was isolated as the picrate; the yield amounted to nearly 60% on the assumption that only one of the optical isomerides was attacked.

Laevorotatory α -amino- β -(4-methylthiazole-5)-propionic acid of specific rotation -9.0° could also be isolated from the fermented liquor: it is thought that this compound is optically pure, since the rotation was not appreciably altered by a second fermentation with yeast. The latter observation is of interest in that it settles the configuration of the optical isomerides of the amino-acid (II): that corresponding to the naturally occurring amino-acids—presumably the one attacked by yeast—must be dextrorotatory while the isomeride isolated will be the *d*(-)-form.

(I)

(II)

(III)

(IV)

It was now decided to try to extend this work by preparing the amino-acid (III) analogous to aneurin. If this compound could be obtained it would be of interest both to see if it could replace aneurin in animal experiments, and to study its fermentation by yeast, with which it might be expected to give both aneurin and cocarboxylase. The work has had to be abandoned for the time being without complete characterization of the compound III: the opportunity is taken here, however, to record the initial stages of the synthesis.

In spite of repeated attempts no conditions could be found in which 2-methyl-4-amino-5-bromomethylpyrimidine hydrobromide (IV) would react with the amino-acid (II). The contrast with the ease of interaction of the pyrimidine (IV) with thiazoles in general suggested that the condensation must in this case be prevented by either the amino- or carboxyl-group in the side chain of II. It was accordingly decided to prepare an ester and an *N*-acylated derivative of II, and to investigate the condensation of these compounds with the pyrimidine (IV). α -Amino- β -(4-methylthiazole-5)-propionic acid methyl ester hydrochloride was obtained without difficulty by the usual method: attempts to prepare the *N*-carbobenzyloxy- or *N*-acetyl-derivatives of this compound by the action of the respective chlorides, however, met with failure. The behaviour of the thiazole amino-acid towards acid chlorides is reminiscent of that of histidine and other glyoxalines, and it seems probable that the acyl chlorides may also attack the basic thiazole ring. An unsuccessful attempt was next made to prepare the *N*-acetylated methyl ester of II from 4-methylthiazole-5-aldehyde. This was condensed with acetylglycine to give the azlactone (V), from which the acetamido-acrylic ester (VI) was easily obtained. No method of reduction of this compound to the required α -acetamido- β -(4-methylthiazole-5)-propionic acid methyl ester could, however, be found.

The amino-acid could be acetylated in excellent yield by boiling with acetic acid containing one equivalent of acetic anhydride, and it was found that the condensation of the pyrimidine (IV) with the α -acetamido- β -(4-methylthiazole-5)-propionic acid proceeded smoothly to give the *N*-acetyl derivative (VII) of the required product. This compound was hydrolysed by hydrobromic acid, and a picrate was isolated which analysed as the tripicrate of compound III. III itself has proved hard to purify, and at the stage which the work has reached has not been obtained crystalline in large enough quantities for satisfactory analysis. No claim can therefore be made that the compound is fully characterized.

EXPERIMENTAL

Pressed top yeast (40 g.) was added to α -amino- β -(4-methylthiazole-5)-propionic acid (1 g.) and loaf sugar (80 g.) in water (700 ml.). The mixture was kept for 5 days at room temperature, when fermentation was complete and the yeast was filtered off. The filtrate was acidified with 5*N* HCl (5 ml.) and concentrated *in vacuo* to about 400 ml.: sugar (80 g.) and NaH₂PO₄ (1 g.) in water (300 ml.) were added, the pH was adjusted to 6.0, and a further batch of yeast (40 g.) added. After a further 5 days' fermentation the yeast was filtered off,

the filtrate acidified and concentrated *in vacuo* to 20 ml. Excess of solid Na₂CO₃ was now added, and the mixture extracted with alcohol. The insoluble portion was worked up as described below. The alcoholic solution was treated with a little aqueous HCl and concentrated to low bulk. The aqueous residue was treated with excess Na₂CO₃ and repeatedly extracted with ether. The extracts were dried, concentrated to low bulk and treated with a solution of picric acid (1 g.) in ether; the picrate of 4-methyl-5-(β -hydroxyethyl)-thiazole was deposited as prisms m.p. 162°, 575 mg. or a yield of 57.5% calculated on one only of the optical isomerides of the original amino-acid. (Picric acid found 61.2%: C₆H₅ONS.C₆H₃O₇N₃ requires picric acid 61.6%.) A mixed m.p. determination showed identity with the picrate of the synthetic compound.

From this picrate the picrolonate (m.p. 184°, decomp.) and *p*-nitrobenzoate (m.p. 125°) were obtained by the usual methods (yields 71 and 64% respectively). In both cases mixed m.p. determinations showed identity with the corresponding derivatives of authentic 4-methyl-5-(β -hydroxyethyl)-thiazole. The *p*-nitrobenzoate gave C, 53.6; H, 4.14; S, 10.75%. (C₁₃H₁₂O₄N₂S requires C, 53.4; H, 4.11; S, 10.97%.)

A blank experiment, identical with the above except that no amino-acid was added, gave none of the picrate of 4-methyl-5-(β -hydroxyethyl)-thiazole.

d(-)- α -Amino- β -(4-methylthiazole-5)-propionic acid. The alcohol-insoluble residue of the fermentation product (see above) was treated with water, and insoluble material filtered off. The filtrate was acidified to pH 1.5 with H₂SO₄ and phosphotungstic acid (20% in water) added until no further immediate precipitation occurred. The precipitate was filtered off and washed once with a little water; the filtrate was treated with excess Ba(OH)₂, and after removal of barium phosphotungstate the Ba was quantitatively precipitated with H₂SO₄. The filtrate was concentrated *in vacuo* to low bulk, acidified to pH 7.0–7.4 with acetic acid, treated with an equal volume of alcohol and cooled. The amino-acid separated as colourless prisms (175 mg.): $[\alpha]_D = -9.0^\circ$ (1% solution in *N* H₂SO₄). (Found: N, 15.01; S, 17.26; amino-N (Van Slyke) 7.35%. C₇H₁₀O₂NS requires N, 15.05; S, 17.2; amino-N, 7.5%.) The compound gave a strongly positive ninhydrin reaction.

In an experiment in which the second fermentation was omitted the amino-acid isolated had $[\alpha]_D = -9.5^\circ$. The fact that the second fermentation does not appreciably alter the optical activity suggests that these specimens are optically pure.

α -Amino- β -(4-methylthiazole-5)-propionic acid methyl ester dihydrochloride. The amino-acid (II) (0.5 g.) was suspended in anhydrous methyl alcohol (20 ml.). HCl was passed in for 30 min. at room temperature and a further 30 min. with heating on the water bath. The solvent was removed *in vacuo* and the residue crystallized from methyl alcohol-ether. Yield 90%; prisms, m.p. (decomp.) 187°. (Found: N, 10.15; Cl, 26.2%. C₈H₁₄O₂N₂SCl₂ requires N, 10.25; Cl, 26.0%.) In two experiments this compound was dissolved in chloroform containing diethylamine (1 equiv.), and benzyl chloroformate or acetyl chloride (1 equiv.) added. In neither case could a crystalline product be isolated.

The azlactone (V). 4-Methylthiazole-5-aldehyde [Harrington & Moggridge, 1939] (1.0 g.), acetylglycine (0.92 g.), sodium acetate (3.2 g.) and acetic anhydride (9.4 ml.) were heated at 100° for 2 hr. On treatment with water the product separated in 25–30% yield. Needles from acetic acid, m.p. 157.5°. (Found: N, 13.5%. C₁₁H₈O₂N₂S requires N, 13.48%.)

α -Acetamido- β -(4-methylthiazole-5)-acrylic acid methyl ester (VI). The azlactone (V) was dissolved in methyl alcohol containing sodium methoxide (1 equiv.).

After 1 hr. at room temperature the mixture was acidified, the solvent removed *in vacuo*, and the residue recrystallized from water: needles m.p. 160°, yield 75–80 %. (Found: N, 11.85 %. $C_{10}H_{13}O_3N_2S$ requires N, 11.7 %.)

α-Acetamido-*β*-(4-ethylthiazole-5)-propionic acid. The amino-acid (II) (1 g.), glacial acetic acid (10 ml.) and acetic anhydride (0.59 ml.) were boiled together for 1 min. The solvent was removed *in vacuo* and the residue crystallized from an ethyl acetate-petroleum mixture containing a little acetic acid. Needles m.p. 191°; 85 % yield. (Found: N, 12.1 %; equiv. by titration 226.5. $C_9H_{12}O_3N_2S$ requires N, 12.3 %; equiv. 228.)

2-Methyl-4-amino-5-bromomethylpyrimidine hydrobromide (IV). This compound was prepared by the following modification of the method of Andersag & Westphal [1937]. 2-Methyl-4-amino-5-aminomethylpyrimidine dihydrochloride (2.11 g.) in water (8 ml.) was held at 60° with stirring while barium nitrite (1.237 g.) in water (10 ml.) was added gradually. The solution was kept at 60° for another hour, 2*N* H_2SO_4 (5.0 ml.) was added, the $BaSO_4$ filtered off and the filtrate concentrated *in vacuo*. The residual 2-methyl-4-amino-5-hydroxymethylpyrimidine hydrochloride was recrystallized from aqueous alcohol; yield 60 %. This compound was heated with a 10 % solution of HBr in acetic acid (40 vol.) at 100° for 2 hr. The precipitate was collected and recrystallized from methyl alcohol-ether. Yield 85–90 %.

Attempts to condense the pyrimidine (IV) with the amino-acid (II). In various experiments the pyrimidine (IV) was mixed with equimolecular proportions of both the amino-acid (II) and the amino-acid monohydrobromide, and the mixtures heated (a) in water at 130°, and (b) without solvent at 150°. In no case was any evidence of reaction obtained; the amino-acid could be recovered as its picrate in yields in one case as high as 80 %.

The N-acetamido-acid (VII). *α*-Acetamido-*β*-(4-methylthiazole-5)-propionic acid (0.794 g.) and 2-methyl-4-amino-5-bromomethylpyrimidine hydrobromide (1.0 g.) were ground together and heated without solvent to 160° for 30 min. with occasional stirring. The product was dissolved in methyl alcohol and carefully precipitated by addition of ether: yield 50 %. It was recrystallized by the addition of acetone to the solution in methyl alcohol containing one drop of conc. aqueous HBr: small rod-like crystals, decomposing sharply at 260°. (Found: N, 13.4; Br, 31.1 %. $C_{15}H_{21}O_3N_5SBr_2$ requires N, 13.7; Br, 31.3 %.)

This compound (0.1 g.) was refluxed with *N* HBr (2.5 ml.) for 1 hr. the solvent removed *in vacuo* and the residue rubbed up with acetone: the precipitate was dissolved in water and treated with picric acid (0.148 g.) and 0.2*N* NaOH (3.2 ml.). The tripicrate of the amino-acid (III) was precipitated as an oil that hardened readily: m.p. (decomp.) 164°: yield 77.5 %. (Found: picric acid 68.8 %; $C_{13}H_{17}N_5O_2S \cdot 3C_6H_3O_7N_3$ requires picric acid 69.1 %.) The amino-acid tribromide (III), as prepared either directly from the hydrolysis of the *N*-acetyl derivative (VII) or from the tripicrate, was usually obtained as small homogeneous but amorphous particles: in one experiment plates, m.p. (decomp.) 233°, were isolated, but in quantities too small for satisfactory analysis.

SUMMARY

Yeast fermentation of *dl*-*α*-amino-*β*-(4-methylthiazole-5)-propionic acid leads to 4-methyl-5-(*β*-hydroxyethyl)-thiazole and the *d*(–)-form of the amino-acid.

The initial stages of a synthesis of the *α*-aminopropionic acid analogous to aneurin are described.

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