145. *Coenzyme A. Part V.* A New and Convenient Synthesis of Pantetheine* (Lactobacillus bulgaricus *Factor*).

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Pantetheine (*Lactobacillus bulgaricus* factor) (I) has been prepared in good yield by an adaptation of the Bergmann peptide synthesis. Reaction of carbobenzyloxy-β-alanine azide with 2-benzylthioethylamine (III) gave the amide (IV). Benzyl and carbobenzyloxy-groups were removed by reduction with sodium in liquid ammonia, and the resulting amino-thiol, *N*-β-alanyl-2-mercaptoethylamine (V) on being heated with D(−)-pantolactone gave pure pantetheine directly.

A growth factor for *Lactobacillus bulgaricus* was isolated from natural materials by McRorie, Masley, and Williams (*Arch. Biochem.*, 1950, **27**, 471) and by Brown, Craig, and Snell (*ibid.*, p. 473). It was recognised as a pantothenic acid derivative and shown later by synthesis to possess the structure (I). Originally called the "*L. bulgaricus* factor" (LBF), the names pantetheine and pantethine have been suggested by Snell and his collaborators for the two interconvertible forms (I) and (II) in which the growth factor is known to exist (Snell, Brown, Peters, Craig, Wittle, Moore, McGlohon, and Bird, *J. Amer. Chem. Soc.*, 1950, **72**, 5349). The synthesis involved reaction between methyl pantothenate and 2-mercaptoethylamine and the yield of biologically active material was less than 10% before purification. The final isolation of the synthetic material in a state of purity involved partition chromatography and counter-current distribution which are not well adapted to laboratory-scale preparation (Snell *et al.*, *loc. cit.*).

$$HO\cdot CH_2\cdot CMe_2\cdot CH(OH)\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot SH \quad (I)$$

$$[HO\cdot CH_2\cdot CMe_2\cdot CH(OH)\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot S]_2 \quad (II)$$

Our interest in this substance was aroused by its established relation to coenzyme A (Brown, Craig, and Snell, *loc. cit.*; Baddiley and Thain, *Chem. and Ind.*, 1951, 337; *J.*, 1951, 2253) in which it is probably attached through a pyrophosphate linkage involving the

* Part IV, *J.*, 1951, 3425.

primary hydroxyl group to adenosine-2′(or 3′) : 5′ diphosphate. As we required quantities of this substance, not only for biological studies, but also as an intermediate in various synthetic experiments an improved synthesis seemed desirable. In view of the difficulties encountered in the first synthesis an alternative route giving high yields would also dispel any doubts as to the correctness of the structures (I) and (II).

It seemed likely that one drawback of the synthesis was the rather unreactive nature of methyl pantothenate towards amines and the subsequent difficulty in removing unchanged ester. Our synthesis therefore envisaged reaction between the more reactive pantolactone and *N*-β-alanyl-2-mercaptoethylamine (V).

For the preparation of the amine (V) we adapted the Bergmann peptide synthesis in a manner similar to that suggested by Sifferd and du Vigneaud (*J. Biol. Chem.*, 1935, **108**, 753) for sulphur-containing peptides. 2-Benzylthioethylamine (III) was prepared in almost quantitative yield by reaction of 2-bromoethylamine hydrobromide and toluene-ω-thiol in the presence of sodium ethoxide in alcohol, a very considerable improvement on the earlier method (Michels, *Ber.*, 1892, **25**, 3048). Sodium in liquid ammonia removed the benzyl group from (III), giving 2-mercaptoethylamine (cysteamine), also in high yield. This is the most convenient method for the preparation of 2-mercaptoethylamine. Reaction of (III) with carbobenzyloxy-β-alanine azide in chloroform gave an excellent yield of 2-benzylthio-*N*-(carbobenzyloxy-β-alanyl)ethylamine (IV), from which the benzyl and the carbobenzyloxy-group were removed by reduction with sodium in liquid ammonia. The resulting *N*-β-alanyl-2-mercaptoethylamine (V) was almost pure as isolated from the reaction mixture, but final purification was effected by vacuum-sublimation. The structure of the amino-thiol was established by acetylation to the *SN*-diacetyl compound which was shown to be identical with that prepared by condensing *N*-acetyl-β-alanine methyl ester with 2-mercaptoethylamine and acetylating the product (Baddiley and Thain, *J.*, 1951, 3425). The free thiol (V) was unstable in air, being readily oxidised to the disulphide (VI).

(III) $H_2N{\cdot}CH_2{\cdot}CH_2{\cdot}S{\cdot}CH_2Ph$ $Ph{\cdot}CH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}S{\cdot}CH_2Ph$ (IV)

(V) $H_2N{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}SH$ $(H_2N{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}S)_2$ (VI)

D(−)Pantolactone reacted readily with *N*-β-alanyl-2-mercaptoethylamine (V) at 100° in the absence of solvent. The product was indistinguishable from pantetheine when examined by paper chromatography, only traces of impurities being detected. When tested as a growth stimulant for *L. bulgaricus* it showed an activity of 26 000 units/mg. Snell *et al.* (*J. Amer. Chem. Soc., loc. cit.*) report an activity of about 29 000 units/mg. which suggests that their preparation is somewhat purer. However, the accuracy of such methods is rather limited.

Experimental

2-Benzylthioethylamine Hydrochloride.—Powdered 2-bromoethylamine hydrobromide (10·25 g.) was added to a solution of toluene-ω-thiol (6·2 g.) and sodium (2·3 g.) in alcohol (50 c.c.), and the resulting mixture was refluxed in an atmosphere of nitrogen for 1·5 hours. Sodium bromide was filtered off and washed well with alcohol. Filtrate and washings were combined, acidified with concentrated hydrochloric acid, and evaporated to dryness under reduced pressure. The crystalline residue was dissolved in warm *iso*propyl alcohol, filtered, and evaporated under reduced pressure. The residue consisted of almost pure 2-benzylthioethylamine hydrochloride (10·2 g.). Recrystallised from *n*-butyl alcohol it formed prisms, m. p. 119—120° (Found: C, 53·7; H, 7·4; N, 7·1; S, 15·8. Calc. for $C_9H_{14}NSCl$: C, 54·1; H, 6·9; N, 6·9; S, 15·8%). It was converted in the usual way into a benzoyl derivative, m. p. 78°, for which Michels (*loc. cit.*) reports m. p. 78—80°.

2-Mercaptoethylamine.—Sodium in small pieces was added to a solution of 2-benzylthioethylamine hydrochloride (10 g.) in liquid ammonia (*ca.* 150 c.c.) with gentle swirling until a deep blue colour persisted in the solution for at least 45 minutes. Excess of sodium was destroyed by addition of a little ammonium chloride, and ammonia was then removed by evaporation under reduced pressure. The solid residue was dissolved rapidly in iced water and acidified by cautious addition of concentrated hydrochloric acid. Water was removed by evaporation and the dry residue extracted with hot *iso*propyl alcohol (2 × 75 c.c.). The filtered solution

was evaporated to dryness, the residue redissolved in a little *iso*propyl alcohol, and the solution filtered and evaporated. The remaining syrup which crystallised on being rubbed consisted of fairly pure 2-mercaptoethylamine hydrochloride, m. p. *ca.* 40° (5·4 g., 97%). A sample was converted into the free base by treatment of a solution in methanol with the calculated amount of sodium in methanol, filtration, and evaporation. The base was purified by sublimation at 0·1 mm. It melted at 98—99°, undepressed on being mixed with an authentic sample, m. p. 99°.

*2-Benzylthio-*N-(*carbobenzyloxy-β-alanyl*)*ethylamine.*—A solution of 2-benzylthioethylamine hydrochloride (5 g.) in water (25 c.c.) was treated with a slight excess of 10% sodium hydroxide solution, the liberated 2-benzylthioethylamine extracted with chloroform, and the solution dried (Na_2SO_4). To this was added a freshly prepared dry chloroform solution of carbobenzyloxy-β-alanine azide, prepared from carbobenzyloxy-β-alanylhydrazide (6 g.) by the method of Sifferd and du Vigneaud (*loc. cit.*). The resulting yellow solution was set aside at room temperature for 20 hours with the exclusion of moisture. Chloroform and hydrazoic acid were removed by evaporation under reduced pressure and the crystalline residue was recrystallised from aqueous alcohol. *2-Benzylthio-*N-(*carbobenzyloxy-β-alanyl*)*ethylamine* formed plates, m. p. 122—123° (8·2 g., 91%) (Found: C, 64·3; H, 6·0; N, 7·4; S, 8·9. $C_{20}H_{24}O_3N_2S$ requires C, 64·6; H, 6·5; N, 7·5; S, 8·6%).

N-β-*Alanyl-2-mercaptoethylamine.*—The dry, finely powdered carbobenzyloxy-compound (12 g.) was added in portions to liquid ammonia (*ca.* 150 c.c.). To the suspension was added sodium in small pieces with occasional agitation. As the sodium dissolved, suspended carbobenzyloxy-compound went into solution, then later a solid was deposited. Addition of sodium was continued until the resulting blue solution maintained its colour for at least 45 minutes. Excess of sodium was destroyed by ammonium chloride, and ammonia removed by evaporation under reduced pressure. The solid residue was dissolved in iced water, and the solution acidified (Congo-red) with concentrated hydrochloric acid. Insoluble material was extracted with ether, and the aqueous solution evaporated to dryness under reduced pressure. The dry, powdered residue was extracted with 3 lots of boiling *iso*propyl alcohol (total 200 c.c.), which was then filtered while hot and evaporated to dryness under reduced pressure. This was repeated with a second portion (60 c.c.) of *iso*propyl alcohol. The syrupy residue (6 g., quantitative) was almost pure *hydrochloride* of the amino-thiol (V) (Found: Cl, 19·3. $C_5H_{13}ON_2SCl$ requires Cl, 19·3%). A solution of this salt in alcohol when kept under nitrogen slowly deposited crystals. Recrystallised from 95% alcohol the pure salt had m. p. 214—217° (Found: C, 33·0; H, 6·8; N, 14·7; S, 17·0. $C_5H_{13}ON_2SCl$ requires C, 32·6; H, 7·0; N, 15·1; S, 17·3%). A solution of the hydrochloride (5·8 g.) in methanol (50 c.c.) was mixed with a solution of sodium (0·72 g.) in methanol (20 c.c.). After 10 minutes at room temperature sodium chloride was removed and the filtrate evaporated under reduced pressure to a syrup. This was dissolved in hot *iso*propyl alcohol (40 c.c.), and the solution was filtered and evaporated to dryness. The syrupy residue slowly crystallised. N-β-*Alanyl-2-mercaptoethylamine* obtained in this way had m. p. 88—90° and was pure enough for conversion into pantetheine (yield, almost quantitative). A sample sublimed at 140°/10^{-5} mm. had m. p. 93—95° (Found: C, 40·5; H, 8·1; N, 18·3; S, 20·8. $C_5H_{12}ON_2S$ requires C, 40·5; H, 8·1; N, 18·8; S, 21·5%). The base gave a positive nitroprusside reaction and decolorised a solution of iodine in potassium iodide and so must be a thiol. Longer storage of solutions of the free base deposited the amorphous disulphide.

SN-*Diacetyl-β-alanyl-2-mercaptoethylamine.*—Freshly sublimed, powdered *N*-β-alanyl-2-mercaptoethylamine (0·2 g.) was heated with acetic anhydride (2 c.c.) on a steam-bath for 1 hour. Acetic anhydride was then removed by evaporation under reduced pressure, and the residue was treated with water. *SN*-Diacetyl-β-alanyl-2-mercaptoethylamine, recrystallised from ethyl acetate, had m. p. 139—140°, somewhat higher than was found for the earlier sample (Baddiley and Thain, *J.*, 1951, 3425), but the m. p.s of the samples were undepressed on admixture.

Pantetheine.—D(−)-Pantolactone (1·75 g.) and *N*-β-alanyl-2-mercaptoethylamine (2·0 g.) were heated on a steam-bath for 2·5 hours in an atmosphere of nitrogen. The resinous product was triturated with ether, then traces of solvent were removed under reduced pressure. The yield was quantitative. Examination of this product by paper chromatography as described below indicated that it was essentially homogeneous; it possessed full biological activity. Further purification for analysis was effected by a 12-stage counter-current distribution in *n*-butyl alcohol–water–acetic acid (4 : 5 : 1). Pantetheine was concentrated mainly in fractions 4, 5, and 6. Evaporation of these combined fractions left a resin, $[\alpha]_D^{20}$ +12·9° (*c*, 4·5 in water) (Found: C, 46·8; H, 8·1; N, 9·8; S, 11·0. Calc. for $C_{11}H_{22}O_4N_2S$: C, 47·5; H, 7·9; N, 10·1; S, 11·5%). This probably contains traces of moisture because of its resinous and hygroscopic nature.

Paper Chromatography of Pantetheine.—Ascending-front chromatography on Whatman No. 1 paper was carried out by the usual methods in the following solvent mixtures: *n*-butanol–water, amyl alcohol–water, *n*-butanol–water–acetic acid (4 : 5 : 1). No special precautions were taken for temperature control since substances for comparison were always run alongside those under examination. The following were examined, pantetheine (not purified) as prepared above, synthetic pantetheine (solution in *n*-butanol supplied by Parke, Davis & Co.), pantetheine purified by counter-current distribution, *N*-β-alanyl-2-mercaptoethylamine, and 2-mercaptoethylamine. After the solutions had run overnight, solvent was evaporated from the papers in a steam-oven, and substances were detected by both ninhydrin and cyanide–nitroprusside spraying. The R_F are shown in the Table.

	BuOH–H_2O	BuOH–AcOH	C_5H_{11}·OH–H_2O
Pantetheine (unpurified)	0·7	0·75	0·55
,, (purified)	—	0·75	—
,, (Parke Davis)	0·7	0·75	0·55
N-β-Alanyl-2-mercaptoethylamine	0—0·4	0·1	0—0·15
2-Mercaptoethylamine	0—0·4	0·1	0—0·3

A faint, slow-moving spot, R_F 0·37 in butanol–acetic acid-water and 0·21 in butanol–water, was observed with both the unpurified pantetheine and the Parke, Davis sample, but this was absent from the sample purified by counter-current distribution. In butanol–water and in amyl alcohol–water considerable tailing of 2-mercaptoethylamine and *N*-β-alanyl-2-mercaptoethylamine was observed. This did not occur in butanol–acetic acid–water which produced clear spots of all substances examined. In our hands the cyanide–nitroprusside spray was very satisfactory and sensitive, but the ninhydrin spray was rather insensitive with these compounds, particularly with pantetheine.

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