A New Synthesis of Pantethine and Some Analogues thereof.

By R. E. Bowman and J. F. Cavalla.

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A synthesis of pantethine (II) has been evolved by the condensation of D(−)-pantolactone with di-(2-*N*-β-alanylamidoethyl) disulphide. By this general method, various analogues of pantethine have also been prepared.

Pantetheine, a growth factor of *Lactobacillus bulgaricus*, was first synthesised by Snell, Brown, Peters, Craig, Wittle, Moore, McGlohon, and Bird (*J. Amer. Chem. Soc.*, 1950, **72**, 5349) and later by Baddiley and Thain (*J.*, 1952, 800). It possesses the structure (I), interconvertible with the disulphide, pantethine (II).

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

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

The earlier synthesis (Snell *et al.*, *loc. cit.*) involved reaction between methyl pantothenate and 2-mercaptoethylamine, to give poor yields of (I) which was isolated only with difficulty. The synthesis due to Baddiley and Thain (*loc. cit.*) required simple condensation of D(−)-pantolactone (αγ-dihydroxy-ββ-dimethylbutyric lactone) with *N*-β-alanyl-2-mercaptoethylamine at 100°, substantially pure (I) being obtained.

Since this work was completed various other syntheses of pantethine have appeared.* Thus, novel methods have been described by Wieland and Bokelmann (*Naturwiss.*, 1951, **38**, 384), Schwyzer (*Helv. Chim. Acta*, 1952, **35**, 1903), King, Stewart, and Cheldelin (*J. Amer. Chem. Soc.*, 1953, **75**, 1290); and, in a preliminary communication, Viscontini, Adank, Merckling, Ehrhardt, and Karrer (*Helv. Chim. Acta*, 1953, **36**, 835) have announced the development of a synthesis similar to that described in this work. At the outset, however, only the earlier American route was known and since this gave poor yields it was decided to evolve a synthesis which besides giving a pure product could, by modification, be used to prepare analogues which might have enhanced growth-promoting or, possibly, reversed (bacteriostatic) properties. This was achieved by the preparation in good yield of the dihydrochloride of di-(2-*N*-β-alanylamidoethyl) disulphide (III) by the Sheehan and Frank's modification (*J. Amer. Chem. Soc.*, 1949, **71**, 1856) of the Gabriel phthalimido-method. Cystamine, di-2-aminoethyl disulphide (Mills and Bogert, *J. Amer. Chem. Soc.*, 1940, **62**, 1173), was treated with β-phthalimidopropionyl chloride in the presence of

* Mention of the present work has been made in a footnote to the paper of Wittle, Moore, Stipek, Peterson, McGlohon, Bird, Brown, and Snell (*J. Amer. Chem. Soc.*, 1953, **75**, 1694).

magnesium oxide to give the derivative (IV), which on fission with hydrazine hydrate in the usual manner followed by treatment with dilute hydrochloric acid gave (III).

$$(HCl,NH_2\cdot CH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot S)_2 \quad \text{(III)}$$

$$[o\text{-}C_6H_4(CO)_2N\cdot CH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot S]_2 \quad \text{(IV)}$$

Condensation of the free base of (III) with D(−)-pantolactone in the absence of solvent gave good yields of pantethine. Paper chromatography of this crude condensation product and detection with a sodium cyanide–sodium nitroprusside spray showed three spots having R_F values, 0·18, 0·47, and 0·78 respectively. The first two of these were of relatively low intensity, yet detection with ninhydrin showed the first two spots clearly with only a very faint indication of the third visible after prolonged heating. The spot of R_F 0·18 was shown to be due to unchanged di-(2-*N*-β-alanylamidoethyl) disulphide and that of R_F 0·78 to pantetheine. The spot of R_F 0·47, mentioned but unidentified both by Baddiley and Thain (*loc. cit.*) and by Schwyzer (*loc. cit.*), is considered to be due to the monosubstituted disulphide (V) arising by incomplete reaction of the D(−)-pantolactone and the amide.

$$\begin{matrix} HO\cdot CH_2\cdot CMe_2\cdot CH(OH)\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot S \\ NH_2\cdot CH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot S \end{matrix} \quad \text{(V)}$$

By this means pantethine, having an activity of 22,600 units/mg. when tested as a growth stimulant for *Lactobacillus helveticus* 80, was obtained. This value was lower than obtain by Baddiley and Thain (*loc. cit.*) but, as these authors state, the accuracy of the microbiological method of testing is somewhat limited.

Simple analogues were prepared by this method by variation of the lactone moiety. In this manner, the corresponding γ-butyrolactone (VI; R = H) and γ-valerolactone (VI; R = Me) derivatives were obtained as colourless solids.

$$[R\cdot CH(OH)\cdot CH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot S]_2 \quad \text{(VI)}$$

Barnett and Robinson (*Biochem. J.*, 1942, **36**, 357) have reported the preparation of 5-hydroxy-4 : 4-dimethylpent-2-enolactone (VII; R = H) by the condensation of β-hydroxy-αα-dimethylpropaldehyde with malonic acid under acid conditions followed by decarboxylation and lactonisation. Difficulty was experienced in repeating this work and an alternative method was sought by condensing the aldehyde with ethyl cyanoacetate (Cope's method, *J. Amer. Chem. Soc.*, 1941, **63**, 3452), to obtain 2-cyano-5-hydroxy-4 : 4-dimethylpent-2-enolactone (VII; R = CN). This on acid hydrolysis and decarboxylation gave the unsaturated lactone (VII; R = H) as an oil, which could be catalytically hydrogenated to the corresponding saturated lactone.

Silberstein (*Monatsh.*, 1904, **25**, 13), condensing the hydroxyaldehyde with malonic acid under ammoniacal conditions, reported a lactone (VII; R = H) as a crystalline solid, m. p. 177°; Barnett and Robinson (*loc. cit.*) also obtained a crystalline solid, m. p. 115°. By analogy with similar homologues the likelihood that a simple pentenolactone of this type should be a high-melting solid appears remote, and in neither case was hydrogenation to the corresponding saturated lactone attempted, as is reported in this work.

$$\begin{matrix} CH_2\cdot CMe_2\cdot CH{:}CR \\ O\text{———}CO \end{matrix} \quad \text{(VII)}$$

$$(HO\cdot CH_2\cdot CMe_2\cdot CH{:}CH\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot S)_2 \quad \text{(VIII)}$$

Condensation of (VII; R = H) with di-(2-*N*-β-alanylamidoethyl) disulphide gave the required analogue (VIII) as a resin; with the saturated lactone the corresponding analogue was obtained as a crystalline solid.

The first analogue of pantothenic acid found to be a growth inhibitor was *N*-pantoyltaurine, which was shown by Snell (*J. Biol. Chem.*, 1941, **139**, 975; 1941, **141**, 121) and almost simultaneously by Kuhn, Wieland, and Möller (*Ber.*, 1941, **74**, 1705) to inhibit the growth of lactic acid bacteria. Barnett and Robinson (*Biochem. J.*, 1942, **36**, 364) confirmed these findings and showed that the compound was active in inhibiting the growth *in vitro* of micro-organisms for which pantothenic acid was essential. With this in

mind, di-(2-*N*-taurylamidoethyl) disulphide (IX) was prepared by condensing 2-phthalimidoethane-1-sulphonyl chloride with cystamine, to give the derivative (X). This resisted

$(NH_2 \cdot CH_2 \cdot CH_2 \cdot SO_2 \cdot NH \cdot CH_2 \cdot CH_2 \cdot S)_2$ (IX)

$[o\text{-}C_6H_4(CO)_2N \cdot CH_2 \cdot CH_2 \cdot SO_2 \cdot NH \cdot CH_2 \cdot CH_2 \cdot S]_2$ (X)

$[HO \cdot CH_2 \cdot CMe_2 \cdot CH(OH) \cdot CO \cdot NH \cdot CH_2 \cdot CH_2 \cdot SO_2 \cdot NH \cdot CH_2 \cdot CH_2 \cdot S]_2$ (XI)

the normal removal of the phthaloyl groups by hydrazine hydrate but with boiling concentrated hydrobromic acid gave (IX) as the dihydrobromide. Condensation with D(−)-pantolactone gave the required analogue (XI) as a gum, which was purified by partition between *n*-butanol and water as in the case of pantethine.

Finally, modifications were made in the termination of the pantethine molecule. By using the homologue of cystamine, di-(4-aminobutyl) disulphide (Dirscherl and Weingarten, *Annalen*, 1951, **574**, 131), the homologue (XII) was obtained as an amber resin, after purification by partition between *n*-butanol and water.

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

The anilide and dimethylamide of β-alanine were prepared by hydrogenation of the corresponding cyanoacetic acid derivatives in the presence of Raney nickel, being isolated as the hydrochloride and hydrobromide respectively. β-Alanine anilide was also obtained by the phthalimido-route from β-phthalimidopropionyl chloride and aniline, the phthaloyl group being removed with hydrazine hydrate. The free bases were condensed with D(−)-pantolactone to give, after the usual purification, pantothenic acid anilide as a crystalline solid and the dimethylamide as a resin.

Apart from pantethine itself, none of the analogues described above was significantly active in the promotion or inhibition of the growth of *Lactobacillus helveticus* 80.

Experimental

Except where otherwise stated the purification of the uncrystallisable analogues of pantethine was effected by counter-current distribution (13 tap funnels). The moving phase was the lower layer of the partitioning mixture which passed through the system and was collected on issuing from the last funnel. The process was continued until 12 lower layers had been collected.

Di-(2-β-alanylamidoethyl) Disulphide Dihydrochloride (III).—To a solution of cystamine (Mills and Bogert, *loc. cit.*) (7·6 g., 0·05 mole) in water (100 ml.) was added magnesium oxide (6 g., 0·15 mole), and the stirred suspension treated at 0° dropwise during 90 min. with a solution of β-phthalimidopropionyl chloride (24 g., 0·1 mole) in dry dioxan (180 ml.). The mixture was stirred at this temperature for 30 min.; N-hydrochloric acid (80 ml.) was then added during 15 min. The precipitated solid was filtered off, washed with water, and dried (22 g., 80%); m. p. 193—200°. Four crystallisations from chloroform–ethanol (1 : 4) gave colourless needles of *di-(2-β-phthalimidopropionamidoethyl) disulphide* (IV), m. p. 211—213° (Found: C, 56·2; H, 5·1; N, 9·7; S, 11·4. $C_{26}H_{26}O_6N_4S_2$ requires C, 56·3; H, 4·7; N, 10·1; S, 11·6%). Crude (IV) (19 g.) was refluxed for 3 hr. with hydrazine hydrate (3·4 g.) in ethanol (540 ml.). The suspension was cooled in ice-water and filtered, to give a white solid, m. p. >360° (10 g.). The filtrate on concentration *in vacuo* gave a gummy solid to which the previous product was added, and the mixture was shaken with 2N-hydrochloric acid (50 ml.) at room temperature for 2 hr. The suspension was cooled and filtered from phthalhydrazide, m. p. >360° (9 g.; theor., 11·1 g.). The filtrate was concentrated *in vacuo* to a gummy solid, which was dissolved in water (50 ml.) and warmed therein to 50°. Ethanol was added to incipient crystallisation; needles (4 g.) separated, having m. p. 204—207°. Concentration of the mother-liquor gave a less pure product (5 g.), m. p. 202—206° (total yield 72%). Three crystallisations from aqueous ethanol gave short needles of *di-(2-β-alanylamidoethyl) disulphide dihydrochloride*, m. p. 221—222° (Found: C, 33·0; H, 6·7; N, 14·9; S, 16·8; Cl, 18·9. $C_{10}H_{24}O_2N_4S_2Cl_2$ requires C, 32·7; H, 6·6; N, 15·3; S, 17·4; Cl, 19·3%).

Pantethine (I).—Di-(2-β-alanylamidoethyl) disulphide dihydrochloride (0·46 g.), suspended in absolute methanol (15 ml.), was treated with a 1·37N-solution of sodium methoxide in absolute methanol (1·85 ml.). To the clear solution was added D(−)-pantolactone (0·325 g.);

the solvent was evaporated and the residue kept in anhydrous conditions at 100° for 2·5 hr. The resulting clear glass was substantially pantethine and sodium chloride. Paper chromatography of the material with *n*-butanol–acetic acid–water (4 : 1 : 5) gave spots having R_F 0·78, 0·47, and 0·18, only the first of which was strong when detected with sodium cyanide–sodium nitroprusside spray. The glass was dissolved in aqueous *n*-butanol (15 ml.) and partitioned between *n*-butanol and water, the method outlined above being used. Paper chromatography of the various eluants and funnel contents showed the pantethine to lie in funnels 4—9 with no detectable impurity. The contents of these funnels were concentrated *in vacuo* to give pure pantethine as a colourless glass, $[\alpha]_D$ +15° (*c*, 3·2 in H_2O) (Found: C, 46·8; H, 7·4; N, 9·8; S, 10·9. Calc. for $C_{22}H_{42}O_8N_4S_2$: C, 47·6; H, 7·6; N, 10·1; S, 11·6%).

Di-(2-N′-4′-hydroxybutyryl-β-alanylamidoethyl) Disulphide (VI; R = H).—A suspension of (III) (3·67 g.) in absolute methanol (25 ml.) was treated with a 1·37N-solution of sodium methoxide in absolute methanol (14·6 ml.); to the clear solution was added γ-butyrolactone (1·72 g.) in absolute methanol (25 ml.), and the mixture refluxed for 3 hr., then concentrated *in vacuo* to a white solid, which was leached from sodium chloride with boiling absolute ethanol. Concentration of the extracts and cooling gave a white powder, m. p. 152—162° (1·5 g.), which on threefold crystallisation from absolute ethanol furnished the colourless *disulphide*, m. p. 192—193° (Found: C, 46·0; H, 7·4; N, 11·6; S, 13·3. $C_{18}H_{34}O_6N_4S_2$ requires C, 46·3; H, 7·3; N, 12·0; S, 13·7%).

Di-(2-N′-4′-hydroxyvaleryl-β-alanylamidoethyl) Disulphide (VI; R = Me).—By the method described in the preceding paragraph γ-valerolactone (2·0 g.) and (III) (3·67 g.) gave the required *product*, m. p. 177—179° (from absolute ethanol) (Found: C, 48·6; H, 7·5; N, 11·1; S, 12·7. $C_{20}H_{38}O_6N_4S_2$ requires C, 48·6; H, 7·7; N, 11·3; S, 13·0%.

2-Cyano-5-hydroxy-4 : 4-dimethylpent-2-enolactone (VII; R = CN).—To a solution of β-hydroxy-αα-dimethylpropaldehyde (61·2 g., 0·6 mole) in benzene (75 ml.) was added ethyl cyanoacetate (56·5 g., 0·5 mole), ammonium acetate (3·85 g., 0·05 mole), and glacial acetic acid (6·0 g., 0·1 mole), and the whole refluxed in an oil-bath (135°) under a Dean and Stark apparatus. Water was evolved immediately on boiling and within 1 hr. 12 ml. were collected. Refluxing was continued for a further hour and the mixture cooled, whereupon beautiful long needles of a substance separated (10 g.), m. p. 185°, which will be described in another communication. This material was filtered off, and the filtrate diluted with benzene (150 ml.), successively extracted with dilute aqueous sodium carbonate (2 × 50 ml.) and water (3 × 100 ml.), and dried (Na_2SO_4); the solvent was removed and the residue distilled *in vacuo*. A small initial fraction of the compound, m. p. 185°, sublimed followed by the major fraction, b. p. 166°/2 mm., which set to a solid (55 g.). Crystallisation from ethanol gave glistening plates of the *cyano-lactone*, m. p. 92° (Found: C, 63·8; H, 6·1; N, 9·5. $C_8H_9O_2N$ requires C, 63·6; H, 6·0; N, 9·3%). Ultra-violet absorption: max. at 255 mμ (ε 7078 in EtOH).

2-Cyano-5-hydroxy-4 : 4-dimethylpentanolactone.—Reduction of (VII; R = CN) (2 g.) in alcohol (50 ml.) over 10% palladised charcoal (0·2 g.) occurred smoothly at atmospheric pressure. Removal of the catalyst and concentration of the solution gave the saturated *lactone* as cubes (1·2 g.), m. p. 77—78° (Found: C, 62·9; H, 7·1; N, 8·9. $C_8H_{11}O_2N$ requires C, 62·7; H, 7·2; N, 9·1%).

5-Hydroxy-4 : 4-dimethylpent-2-enolactone (VII; R = H).—2-Cyano-5-hydroxy-4 : 4-dimethylpent-2-enolactone (10 g.) was refluxed for 18 hr. with constant-boiling hydrochloric acid (100 ml.), the solution was cooled and extracted with ether (3 × 50 ml.), the ethereal extracts were washed with water (3 × 50 ml.) and dried (Na_2SO_4), the ether was removed, and the residual oil was distilled *in vacuo*, to give the required *lactone* (4 g.), b. p. 52°/0·4 mm., n^{20} 1·4662 (Found: C, 66·8; H, 8·0. $C_7H_{10}O_2$ requires C, 66·6; H, 8·0%). Ultra-violet absorption: max. at 216 mμ (ε 7032 in H_2O containing 1% of EtOH).

5-Hydroxy-4 : 4-dimethylpentanolactone.—Hydrogenation of 5-hydroxy-4 : 4-dimethylpent-2-enolactone (5 g.), as in the previous example, gave the saturated *lactone* as an oil, b. p. 64°/0·7 mm., n^{20} 1·4520 (Found: C, 65·9; H, 9·8. $C_7H_{12}O_2$ requires C, 65·6; H, 9·4%), having no absorption in the range 215—300 mμ.

Di-[2-N′-(5′-hydroxy-4′ : 4′-dimethylpent-2′-enoyl)-β-alanylamidoethyl] Disulphide (VIII).—A suspension of (III) (0·736 g.) in methanol (20 ml.) was treated with a 1·37N-solution of sodium methoxide in methanol (2·92 ml.), followed by 5-hydroxy-4 : 4-dimethylpent-2-enolactone (0·504 g.) in methanol (20 ml.) under the conditions used in the preparation of pantethine. The resulting gum was dissolved in *n*-butanol (50 ml.) and washed with water saturated with *n*-butanol (15 × 20 ml.). Concentration of the organic layer gave a pale yellow glass which was substantially the required *analogue* (Found: C, 53·3; H, 8·4; N, 10·8; S, 12·8.

$C_{24}H_{42}O_6N_4S_2$ requires C, 52·7; H, 7·8; N, 10·2; S, 11·7%). Attempts to obtain a crystalline specimen by chromatographic or other means were unsuccessful.

Di-[2-N′-(5′-*hydroxy*-4′ : 4′-*dimethylpentanoyl*)-β-*alanylamidoethyl*] *Disulphide*.—A suspension of (III) (0·736 g.) in methanol was treated with sodium methoxide and 5-hydroxy-4 : 4-dimethylpentanolactone (0·512 g.) as for (VIII) above. The resulting resin was dissolved in *n*-butanol (50 ml.) and washed with water saturated with *n*-butanol (10 × 25 ml.). A further 50 ml. of *n*-butanol were then shaken with each aqueous washing, the resin thus being obtained in *n*-butanol solution, concentration of which gave the colourless *analogue*, crystallising from ether–ethanol as needles, m. p. 134—138° (Found: C, 52·8; H, 8·5; N, 9·9; S, 11·5. $C_{24}H_{46}O_6N_4S_2$ requires C, 52·3; H, 8·4; N, 10·2; S, 11·6%).

Di-(2-*taurylamidoethyl*) *Disulphide Dihydrobromide*.—To a solution of cystamine (0·76 g.) in dry dioxan (50 ml.) was added triethylamine (2·02 g.), followed at −5° by a solution of 2-phthalimidoethanesulphonyl chloride (2·73 g.) in dry dioxan (50 ml.) added dropwise during 1 hr. with stirring. The mixture was stirred at this temperature for a further hour, filtered from some amorphous material, and concentrated *in vacuo* to a gummy solid. Trituration under water gave a colourless solid (2·0 g.), m. p. 140—147°, which on two crystallisations from aqueous acetone gave feathery needles of *di*-(2-2′-*phthalimidoethylsulphonamidoethyl*) *disulphide*, m. p. 163—164° (Found: C, 46·0; H, 4·5; N, 9·1; S, 20·8. $C_{24}H_{26}O_8N_4S_4$ requires C, 46·0; H, 4·2; N, 8·9; S, 20·4%). For further synthetical work this was not purified but hydrolysed in the crude state with concentrated hydrobromic acid. It (2 g.) was refluxed for 90 min. with concentrated hydrobromic acid (25 ml.), cooled, diluted with water (15 ml.), cooled in ice-water, and filtered from phthalic acid. The filtrate was concentrated *in vacuo* to a brown oil, which on repeated crystallisation from aqueous ethanol gave light brown plates of the *disulphide dihydrobromide*, m. p. 170—173° (0·9 g.) (Found: C, 18·5; H, 4·7; N, 10·8; S, 24·2; Br, 30·0. $C_8H_{24}O_4N_4S_4Br_2$ requires C, 18·2; H, 4·6; N, 10·6; S, 24·3; Br, 30·3%).

Di-[2-N′-(D-2′ : 4′-*dihydroxy*-3′ : 3′-*dimethylbutyryltaurylamidoethyl*] *Disulphide* (XI).—A suspension of the foregoing dihydrobromide (1·06 g.) in absolute methanol (20 ml.) was treated with a 1·37N-solution of sodium methoxide in absolute methanol (2·96 ml.) and to the resulting clear solution was added D(−)-pantolactone (0·52 g.). The solvent was evaporated and the residual gum held at 100° for 3 hr. Examination of this material by paper chromatography with *n*-butanol–acetic acid–water (4 : 1 : 5) and detection with sodium cyanide–sodium nitroprusside revealed a major component having R_F 0·75 and two minor ones having R_F 0·46 and 0·23 respectively. By comparison with the pure substance, the last of these was shown to be due to di-(2-taurylamidoethyl) disulphide. When the spots on a duplicate paper were detected with ninhydrin only the two minor components were visible. Partition of the gum between *n*-butanol and water by the general method resulted in the analogue collecting in funnels 3—7. Concentration of these solutions gave the required *disulphide* as an uncrystallisable pale yellow glass, $[\alpha]_D$ +18° (*c*, 2·7 in H_2O) (Found: C, 39·0; H, 6·9; N, 8·7; S, 19·8. $C_{20}H_{42}O_{10}N_4S_4$ requires C, 38·3; H, 6·7; N, 8·9; S, 20·5%).

Di-(4-β-*alanylamidobutyl*) *Disulphide Dihydrochloride*.—Di-(4-aminobutyl) disulphide dihydrobromide (7·3 g.) (Dirscherl and Weingarten, *loc. cit.*), dissolved in water (100 ml.), was treated with magnesium oxide (2·4 g.) and, dropwise during 1 hr. at 0—5°, with β-phthalimidopropionyl chloride (9·6 g.) in dry dioxan (80 ml.). The mixture was stirred at this temperature for 30 min., then N-hydrochloric acid (100 ml.) was added, and the whole was stirred for 15 min., diluted with water (100 ml.), and filtered. The colourless solid crystallised from ethanol to give small needles of *di*-(4-*phthalimidopropionamidobutyl*) *disulphide*, m. p. 175° (Found: C, 59·1; H, 5·6; N, 9·4; S, 10·4. $C_{30}H_{34}O_6N_4S_2$ requires C, 59·0; H, 5·6; N, 9·2; S, 10·5%).

The phthaloyl groups were removed from this compound as for (III), and the resulting *di*-(4-β-*alanylamidobutyl*) *disulphide dihydrochloride* crystallised from ethanol–ether as needles, m. p. 159—160° (Found: C, 39·7; H, 7·6; N, 13·2; S, 15·1; Cl, 17·0. $C_{14}H_{32}O_2N_4S_2Cl_2$ requires C, 39·7; H, 7·5; N, 13·2; S, 15·1; Cl, 16·8%).

Di-[4-N′-(D-2′ : 4′-*dihydroxy*-3′ : 3′-*dimethylbutyryl*)-β-*alanylamidobutyl*] *Disulphide* (XII).—By the method given above for pantethine (II), the foregoing dihydrochloride (0·53 g.) was treated with sodium ethoxide and then coupled with D(−)-pantolactone (0·325 g.). The resultant gum on paper chromatography [pentan-1-ol–acetic acid–water (4 : 1 : 5)] showed spots having R_F 0·82, 0·40, and 0·09 respectively, detected with sodium cyanide–sodium nitroprusside; with ninhydrin only the last two spots were apparent. Partitioning as above between *n*-butanol and water gave the required *analogue* which was found chromatographically pure in the first three funnels and recovered as a pale yellow resin, $[\alpha]_D$ +12° (*c*, 1·3 in H_2O) (Found: C, 51·7; H, 8·4; N, 8·9; S, 10·3. $C_{26}H_{50}O_8N_4S_2$ requires C, 51·1; H, 8·3; N, 9·2; S, 10·5%).

β-*Alanine Anilide Hydrochloride.*—(*a*) A solution of cyanoacetanilide (5 g.) in alcohol (200 ml.) was shaken at 40° with Raney nickel (W7) in the presence of hydrogen at atmospheric pressure, the theoretical volume of hydrogen being rapidly absorbed. The filtered solution was concentrated *in vacuo* to an oil, which was dissolved in a ethanolic N-hydrochloric acid (30 ml.). On addition of ether to turbidity, β-*alanine anilide hydrochloride* crystallised as plates (4 g.), m. p. 201—202° (Found: C, 53·9; H, 6·8; N, 13·8; Cl, 17·8. $C_9H_{13}ON_2Cl$ requires C, 53·9; H, 6·5; N, 14·0; Cl, 17·7%).

(*b*) To a stirred suspension of aniline (1·86 g.) in water (25 ml.) at 10° was added a solution of β-phthalimidopropionyl chloride (4·75 g.) in dry dioxan (30 ml.) dropwise during 30 min., simultaneously with N-sodium hydroxide (20 ml.). The mixture was stirred for 15 min., poured into water (250 ml.), and filtered and the resulting solid crystallised from chloroform–ethanol (1 : 4), to give glistening needles (3·6 g.) of β-*phthalimidopropionanilide*, m. p. 191—192° (Found: C, 69·5; H, 4·7; N, 9·7. $C_{17}H_{14}O_3N_2$ requires C, 69·4; H, 4·8; N, 9·5%). Reaction of this compound in the usual manner with hydrazine hydrate followed by treatment with hydrochloric acid gave β-alanine anilide hydrochloride, m. p. 201—202°, identical with that prepared as above.

N-D-2 : 4-*Dihydroxy*-3 : 3-*dimethylbutyryl*-β-*alanine Anilide.*—β-Alanine anilide hydrochloride (0·8 g.) in absolute methanol (20 ml.) was treated with methanolic 1·37N-sodium methoxide (2·96 ml.) and then with D(−)-pantolactone (0·52 g.); the solvent was evaporated and the residual gum held at 100° for 3 hr. The gum was cooled, dissolved in the upper layer from *n*-butanol–acetic acid–water (4 : 1 : 5) (30 ml.), and washed successively with small quantities (12 × 20 ml.) of the lower layer of this mixture; these washings were washed twice with more of the upper layer (30 ml.). Evaporation then gave a resin which on trituration under ether gave a colourless solid, m. p. 101—104° (0·35 g.). This on crystallisation from ethyl acetate gave D-*pantothenanilide* as thick needles, m. p. 106—108°, $[\alpha]_D$ +7° (*c*, 6·5 in H_2O) (Found: C, 60·9; H, 7·6; N, 9·5. $C_{15}H_{22}O_4N_2$ requires C, 61·2; H, 7·5; N, 9·5%).

NN-*Dimethylcyanoacetamide.*—Ethyl cyanoacetate (56·5 g.) was treated with a solution of dimethylamine (45 g.) in ethanol (100 ml.) containing sodium ethoxide (0·5 g.) at room temperature for 3 days. The solution was concentrated *in vacuo* to a brown oil which crystallised from ethanol (50 ml.) at −30°. Recrystallisation from benzene–light petroleum (b. p. 40—60°) gave glistening plates of the *dimethylamide*, m. p. 65—66° (Found: C, 54·0; H, 7·1; N, 25·0. $C_5H_8ON_2$ requires C, 53·6; H, 7·2; N, 25·0%).

β-*Alanine Dimethylamide Hydrobromide.*—Reduction of the foregoing amide (2·7 g.) in ethanol with Raney nickel (W7) as described above for β-alanine anilide gave an oil. This was treated with a dilute solution of hydrobromic acid in ethanol, and ether was added to turbidity. Needles (2 g.) of the *hydrobromide*, m. p. 169—170°, were obtained (Found: C, 30·5; H, 6·6; N, 14·3; Br, 40·4. $C_5H_{13}ON_2Br$ requires C, 30·5; H, 6·7; N, 14·2; Br, 40·6%).

N-(D-2 : 4-*Dihydroxy*-3 : 3-*dimethylbutyryl*)-β-*alanine Dimethylamide.*—β-Alanine dimethylamide hydrobromide (0·79 g.) was treated with 1·37N-sodium methoxide (2·96 ml.) and kept at 100° with D(−)-pantolactone (0·52 g.) for 3 hr. The resulting gum when subjected to paper chromatography (*n*-butanol–acetic acid; ninhydrin) gave only one spot, due to β-alanine dimethylamide, having R_F 0·40. When the paper was kept in the dark for some weeks or heated to 130° for 20 min. a second spot, due to the analogue, appeared having R_F 0·75. The gum was partitioned between *n*-butanol and water as described above, NN-*dimethyl*-D-*pantothenamide*, $[\alpha]_D$ +22° (*c*, 2 in H_2O), being obtained as an almost colourless resin on concentration of the contents of funnels 10—12 (Found: C, 52·9; H, 8·9; N, 10·8. $C_{11}H_{22}O_4N_2$ requires C, 53·6; H, 9·0; N, 11·4%).

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RESEARCH DEPARTMENT, PARKE, DAVIS & CO., LTD.,
HOUNSLOW, MIDDLESEX.

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