N-Carboxy-anhydrides Derived from *threo*- and *erythro*-β-Hydroxyaspartic Acids and Poly-β-methyl Hydrogen *threo*-β-Methoxy-DL-aspartate

By **Y. Liwschitz** and **A. Singerman**, Department of Organic Chemistry, The Hebrew University, Jerusalem, Israel

The *N*-carboxy-anhydrides derived from either *threo*- or *erythro*-β-hydroxy-DL-aspartic acid in which the hydroxy-group is protected by an acetyl group and the β-carboxy-group by a benzyl or methyl ester do not undergo polymerisation in solution by basic catalysts or in bulk. This is due to an *O* → *N*-acetyl shift. Polymerisation does take place after methylation of the hydroxy-group.

α-AMINO-ACIDS possessing one additional functional group have been polymerised by means of suitably protected *N*-carboxy-anhydrides.[1] However, the preparation of a polymer from a tetrafunctional α-amino-acid has not yet been reported.† It therefore seemed of interest to synthesise *N*-carboxy-anhydrides from *threo*- and *erythro*-β-hydroxyaspartic acid with the β-carboxy- and hydroxy-groups reversibly masked, and investigate their polymerisation. In order to pave the way for the preparation of the much less readily obtainable optically active substances, we restricted ourselves to the use of racemic *threo*- and *erythro*-β-hydroxyaspartic acids prepared stereospecifically.[3] In the *threo*-series, we first aimed at protecting the β-carboxy-group by the benzyl ester and the hydroxy-group by an acetyl group Esterification of the β-carboxy-group was performed directly in accordance with the method of Katchalski for aspartic acid.[4] Verification of the structure of the ester (Ia) was carried out in the following manner. Ammonolysis gave an amide (IV) whose i.r. spectrum was compared with that of α- and β-asparagines, implying β-structure. Preparation of the *N*-benzyloxycarbonyl derivative of the ester resulted in an oily substance from which a crystalline dibenzylammonium salt (VII) was obtained. On the other hand, *threo*-β-hydroxyaspartic acid was converted into its *N*-benzyloxycarbonyl derivative (V) and this into the dibenzyl ester (VI). Partial hydrolysis removed the α-ester, in analogy with the findings of Frankel and Berger,[5] and the dibenzyl-

† We do not include dihydroxy-phenylalanines [2] among tetrafunctional amino-acids since the second hydroxy-function does not create additional synthetic problems.

[1] (*a*) E. Katchalski and M. Sela, *Adv. Protein Chem.*, 1958, **13**, 243; (*b*) E. Katchalski, M. Sela, H. I. Silman, and A. Berger, in "The Proteins," ed. H. Neurath, Academic Press, New York, 2nd edn., 1964, vol. II, p. 406.

[2] H. J. Harwood and H. G. Cassidy, *J. Amer. Chem. Soc.*, 1957, **79**, 4360.

[3] Y. Liwschitz, Y. Rabinsohn, and A. Haber, *J. Chem. Soc.*, 1962, 3589.

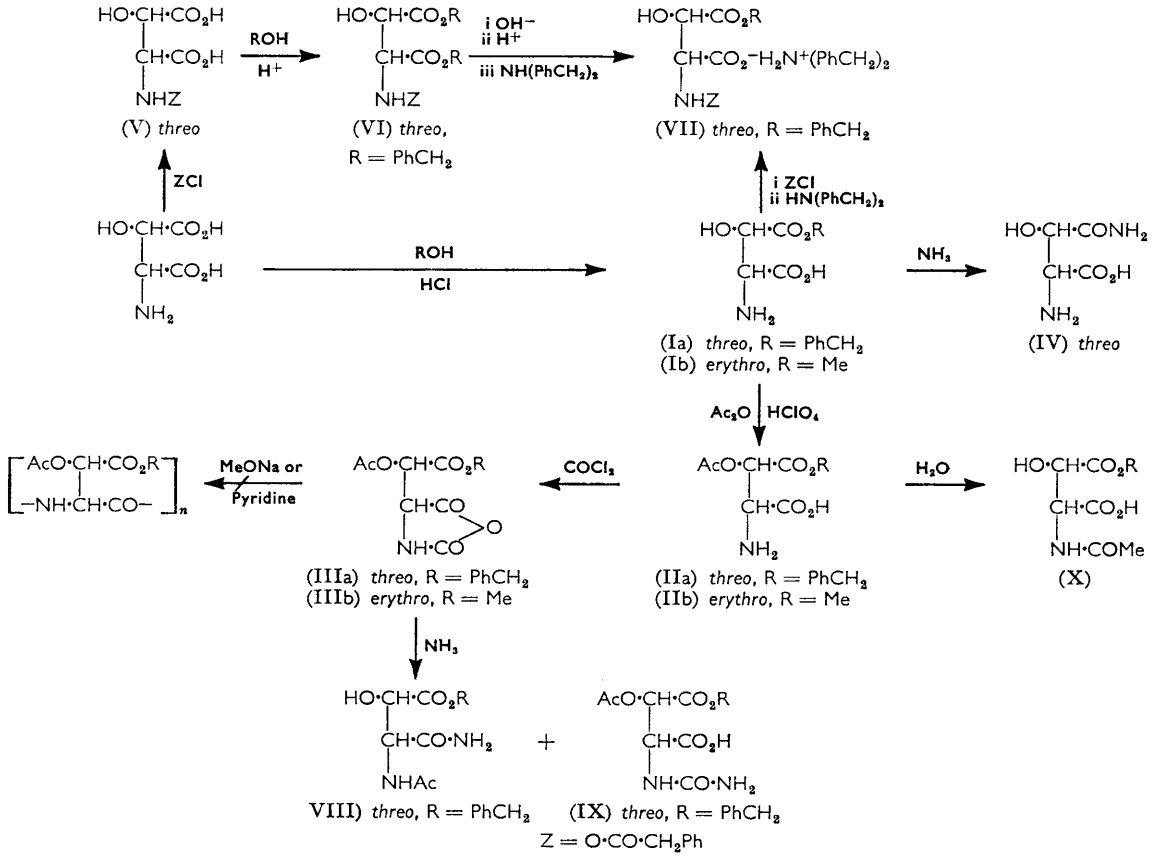
[4] E. Katchalski, private communication.

[5] M. Frankel and A. Berger, *J. Org. Chem.*, 1951, **16**, 1513.

ammonium salt of the resulting *N*-benzyloxycarbonyl ester (VII) was shown to be identical with the compound described above (m. p. and i.r. spectrum). Further proof for the β-structure of the ester was obtained by the method of Larsen and Kjaer,[6] by which α-monoamino-acids can be distinguished from other ninhydrin-positive substances by treatment with methanolic cupric nitrate before development of the ninhydrin colour which then either is absent or appears at most as a very faint violet spot, in contrast to other amino-acids in which the carboxy-group is farther from the amino-function so that complex formation with copper does not then occur.

aqueous solution. Similar behaviour has not been reported for *O*-acetylserine or *O*-acetylthreonine derivatives. Treatment of (IIa) in dioxan with phosgene produced the *N*-carboxy-anhydride (IIIa). This was readily purified by dissolving in ethyl acetate and precipitation with light petroleum until the presence of chloride ions could no longer be detected. The substance decomposed at 134° and its i.r. spectrum confirmed the existence of the substituted oxazolidine-2,5-dione ring and thus constituted additional proof for the β-structure of the original benzyl ester.

The *N*-carboxy-anhydride in the *erythro*-series (IIIb)



We then tried to prepare the *O*-acetyl derivative by reaction with acetyl chloride in glacial acetic acid,[7] and according to the method of Sheehan *et al.*,[8] but these reactions were unsuccessful because of partial acidolysis of the β-benzyl ester group. However, by use of perchloric acid and acetic anhydride [9] the desired β-benzyl hydrogen *threo*-β-acetoxy-DL-aspartate (IIa) was obtained. This substance was very sensitive to alkaline conditions, undergoing an $O \longrightarrow N$-shift to the β-benzyl hydrogen *N*-acetyl-*threo*-β-hydroxy-DL-aspartate (X) as might be expected.[10] This rearrangement, however, took place even on heating in neutral

was synthesised similarly. However, since its β-benzyl ester could not be prepared by direct esterification, probably owing to interference of the hydroxy-group, we used the β-methyl ester (Ib), obtained in good yield by the direct method. Selective acetylation of the hydroxy-group and reaction with phosgene finally yielded the *N*-carboxy-anhydride (IIIb) which was purified like the *threo*-isomer, giving colourless crystals decomposing at 124°.

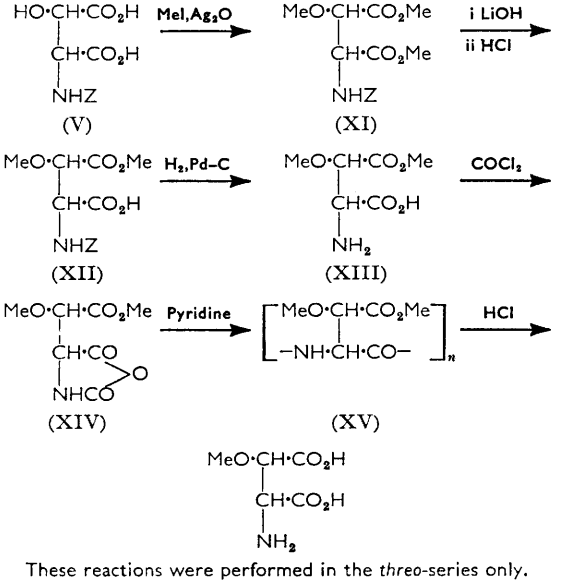
We then tried to polymerise the *N*-carboxy-anhydrides in dioxan solution with sodium methoxide as initiator

[6] P. O. Larsen and A. Kjaer, *Biochim. Biophys. Acta*, 1960, **38**, 148.
[7] Y. Liwschitz, A. Zilkha, and I. Shahak, *J. Org. Chem.*, 1956, **21**, 1530.
[8] J. C. Sheehan, M. Goodman, and G. P. Hess, *J. Amer. Chem. Soc.*, 1956, **78**, 1377.
[9] W. Sakami and G. J. Toennis, *J. Biol. Chem.*, 1942, **144**, 203.
[10] D. F. Elliott, *J. Chem. Soc.*, 1949, 589.

or alternatively in pyridine. When a negative biuret reaction and lack of carbon dioxide evolution showed that no polypeptides had resulted, we suspected an $O \longrightarrow N$-shift of the acetyl group used for the protection of the hydroxy-group. We therefore decided to investigate the reaction between the *N*-carboxy-anhydride of β-benzyl hydrogen *threo*-β-acetoxy-DL-aspartate (IIIa) in dioxan solution and one equivalent of ammonia, *i.e.*, under weakly basic conditions. From the reaction mixture two substances were isolated. The β-benzyl hydrogen *N*-carbamoyl-*threo*-β-acetoxy-DL-aspartate (IX), which resulted from a nucleophilic attack of ammonia on the 2-carbonyl group, and N^2-acetyl-*threo*-β-hydroxy-DL-α-asparagine β-benzyl ester (VIII) which was formed by attack of ammonia on the 5-carbonyl group with simultaneous $O \longrightarrow N$-shift of the acetyl group. This, of course, proved that such a shift could partly be responsible for the complete failure of the *N*-carboxy-anhydrides to polymerise although steric factors might also be contributory. (The *N*-carboxy-anhydride derived from 2-amino-2-methylpropanoic acid is extremely difficult to polymerise.[11])

Polymerisation in bulk in a high vacuum was also unsuccessful. On titrating the *N*-carboxy-anhydride derived from β-benzyl hydrogen *threo*-β-acetoxy-DL-aspartate (IIIa) with sodium methoxide, according to the method of Berger,[12] two equivalents of alkali were used up, which shows that not only the α-carboxy-group involved in the anhydride formation, but also the acetic acid formed by hydrolysis of the *O*-acetyl group, was titrated.

In order to overcome the difficulties inherent in the use of the acetyl group for the protection of the hydroxy-group we decided to mask the latter by methyl ether * and thus to prepare the *N*-carboxy-anhydride of β-methyl hydrogen *threo*-β-methoxy-DL-aspartate (XIV) by the following series of reactions.



These reactions were performed in the *threo*-series only.

Z = O·CO·CH₂Ph

N-Benzyloxycarbonyl-*threo*-β-hydroxy-DL-aspartic acid (V) was treated with methyl iodide and silver oxide in acetone. The resulting dimethyl *N*-benzyloxycarbonyl-*threo*-β-methoxy-DL-aspartate (XI) was hydrolysed with lithium hydroxide in aqueous acetone to the β-monoester (XII) which was purified by chromatography on alumina. After removal of the benzyloxycarbonyl group by catalytic hydrogenolysis β-methyl hydrogen *threo*-β-methoxy-DL-aspartate (XIII) was obtained. This substance, suspended in dioxan, was treated with phosgene, yielding the *N*-carboxy-anhydride (XIV) which was purified by dissolving in ethyl acetate and precipitation with light petroleum.

Since at this stage we were mainly interested to find out whether polymerisation would take place at all, and not to obtain high polymers, we dissolved the *N*-carboxy-anhydride in pyridine, a method which, as a rule, only leads to low polymers. This time the anhydride appeared to polymerise, indicated by evolution of carbon dioxide. After a few hours the polymer (XV) was isolated by precipitation with dry ether. It was yellowish and very hygroscopic, giving a strong biuret colour and a negative Van Slyke α-amino-nitrogen reaction which indicated it to be of at least moderate length. Hydrolysis of the polypeptide (XV) with 5-hydrochloric acid yielded only *threo*-β-methoxy-DL-aspartic acid, identified by chromatography with an authentic sample obtained by alkaline hydrolysis of β-methyl hydrogen β-methoxy-DL-aspartate.

EXPERIMENTAL

Melting points were determined in a Fisher-Johns apparatus. Micro-combustion analyses were carried out by Mrs. M. Goldstein of the Microanalytical Laboratory of the Hebrew University, to whom our thanks are due.

β-Benzyl Hydrogen threo-β-*Hydroxy*-DL-*aspartate* (Ia).—To *threo*-β-hydroxy-DL-aspartic acid (23 g.) suspended in benzyl alcohol (200 ml.) was added with mechanical stirring concentrated hydrochloric acid (27 ml.) and stirring was continued for 2 hr. at 70°. After removal of water *in vacuo* concentrated hydrochloric acid (13 ml.) was added and the mixture evaporated to dryness as before. To the residue a 20% solution of potassium carbonate was added to adjust the pH to 6. After addition of ethanol (1 l.) and cooling the *ester* crystallised. It was recrystallised from water (26·5 g., 72%), m. p. 207° [Found: C, 55·1; H, 5·4; N, 5·2; N (Van Slyke), 5·2. $C_{11}H_{13}NO_5$ requires C, 55·1; H, 5·4; N, 5·8; N (Van Slyke), 5·8%].

threo-β-*Hydroxy*-DL-β-*asparagine* (IV).—The ester (Ia) (0·5 g.) was dissolved in 25% ammonium hydroxide (5 ml.) and left at room temperature for 5 days. After evaporation to dryness *in vacuo*, the residue was dissolved in water and the pH brought to 5 by addition of concentrated hydrochloric acid. The *amide* which precipitated was recrystal-

* It has recently been reported that methyl ethers can be cleaved under mild conditions by boron trichloride (tribromide) [13] or by diborane and halogen.[14]

[11] H. Weingarten, *J. Amer. Chem. Soc.*, 1958, **80**, 352.
[12] A. Berger, *Analyt. Chem.*, 1953, **25**, 1554.
[13] (*a*) T. G. Bonner, E. J. Bourne, and S. McNally, *J. Chem. Soc.*, 1960, 2929; (*b*) A. B. Foster, D. Horton, N. Salim, M. Stacey, and J. M. Webber, *J. Chem. Soc.*, 1960, 2587.
[14] L. H. Long and G. F. Freeguard, *Nature*, 1965, **207**, 403.

lised from water, m. p. 249° (decomp.) [Found: C, 32·6; H, 5·4; N, 18·7; N (Van Slyke), 9·2. $C_4H_8N_2O_4$ requires C, 32·4; H, 5·4; N, 18·9; N (Van Slyke), 9·5%].

N-*Benzyloxycarbonyl*-threo-β-*hydroxy*-DL-*aspartic Acid* (V).—To a suspension of *threo*-β-hydroxy-DL-aspartic acid (9 g.) in water (100 ml.) was added magnesium oxide (8 g.) and ether (70 ml.). After cooling in an ice-bath benzyl chloroformate (25 ml.) was added with stirring during 30 min. Stirring was continued for 1 hr. at room temperature. After acidification to pH 2 with concentrated hydrochloric acid the reaction mixture was extracted three times with ethyl acetate (100 ml.). The combined extracts were washed with hydrochloric acid (1 : 1) and water and after drying ($MgSO_4$) they were concentrated to 50 ml. On addition of light petroleum (100 ml.) and cooling the *acid* crystallised (14 g., 85%), m. p. 186° (Found: Cl, 51·5; H, 4·5; N, 4·6. $C_{12}H_{13}NO_7$ requires C, 51·0; H, 4·6; N, 4·9%).

Dibenzyl N-*Benzyloxycarbonyl*-threo-β-*hydroxy*-DL-*aspartate* (VI).—Into a flask equipped with an azeotropic trap and a reflux condenser were placed the acid (V) (9 g.), dry toluene (50 ml.), toluene-*p*-sulphonic acid (0·5 g.), and benzyl alcohol (40 ml.). The mixture was heated under reflux until the theoretical amount of water had been collected in the trap (3 hr.). After cooling magnesium oxide (1 g.) was added. The filtered solution was evaporated to dryness in a high vacuum and the oily residue crystallised on trituration with light petroleum to give the *ester* (15 g., 90%) as needles, m. p. 88° (ethanol) (Found: C, 66·8; H, 5·1; N, 3·2. $C_{26}H_{25}NO_7$ requires C, 67·4; H, 5·4; N, 3·0%).

Dibenzylammonium β-*Benzyl* N-*Benzyloxycarbonyl*-threo-β-*hydroxy*-DL-*aspartate* (VII).—(a) *From* (Ia). To a suspension of (Ia) (9·6 g.) in 3% sodium hydrogen carbonate (250 ml.) was added benzyl chloroformate (9 g.), with stirring. After 2 hr. the reaction mixture was acidified to pH 2 and extracted with ether. The extract was washed with 5*N*-hydrochlororic acid and water, and after drying ($MgSO_4$) was evaporated to dryness. The oil (7·2 g., 75%) was converted into its dibenzylammonium salt by dissolving in ether and adding an equivalent amount of dibenzylamine whereupon the *salt* was precipitated, m. p. 128° (ethyl acetate) (Found: C, 70·0; H, 5·7; N, 4·9. $C_{33}H_{34}N_2O_7$ requires C, 69·5; H, 5·9; N, 4·9%).

(b) *By partial hydrolysis of* (VI). The ester (VI) (4·6 g.) was dissolved in dioxan (50 ml.) and water (20 ml.). To this was added a mixture of 2N-sodium hydroxide (5 ml.), water (24 ml.), and dioxan (60 ml.). The solution was left overnight. After the pH had been adjusted to 4 the solution was evaporated to dryness *in vacuo* and the oily residue was dissolved in 1N-potassium hydrogen carbonate (20 ml.) and this was extracted with ether (30 ml.). The aqueous layer was acidified with 5N-hydrochloric acid to pH 2. The viscous oil which separated (1·5 g.) was converted into its dibenzyl amine salt as in (a) to give an identical product (mixed m. p., i.r. spectrum, and elemental analysis).

β-*Benzyl Hydrogen* threo-β-*Acetoxy*-DL-*aspartate* (IIa).—A solution was prepared [9] by mixing 60% perchloric acid (65 ml.) with acetic anhydride (8·1 ml.) and diluting with glacial acetic acid to 100 ml.

The ester (Ia) (5 g.) was dissolved in this solution (45 ml.). After cooling in an ice-bath, acetic anhydride (11 ml.) was added in small portions and the reaction mixture left for 2 hr. at room temperature. Water (1 ml.), tri-n-butylamine (8·6 ml.), and ether (400 ml.) were added and after cooling and scratching the *product* crystallised, and was filtered off and washed with ethanol (5 g., 85%). Analytical sample from aqueous ethanol, m. p. 130° (decomp.) [Found: C, 55·5; H, 5·4; N, 4·8; N (Van Slyke), 4·8. $C_{13}H_{15}NO_6$ requires C, 55·5; H, 5·4; N, 5·0; N (Van Slyke), 5·0%].

O ⟶ N-*Acetyl Shift in* (IIa).—The acetoxy-ester (IIa) was heated in water at the boiling point for several minutes. After cooling β-*benzyl* N-*acetyl*-threo-β-*hydroxy*-DL-*aspartate* (X) crystallised as the semihydrate in plates, m. p. 140°. This substance gave a negative ninhydrin test (Found: C, 54·1; H, 5·5; N, 4·6. $C_{13}H_{15}NO_6,\frac{1}{2}H_2O$ requires C, 53·9; H, 5·5; N, 4·8%).

β-*Benzyl Dihydrogen* N-*Carboxy*-threo-β-*acetoxy*-DL-*aspartate* N,α-*Anhydride* (IIIa).—Into a suspension of (IIa) (3·7 g.) in dry dioxan (100 ml.) stirred at 50° was bubbled dry phosgene for 1 hr. A clear solution was obtained after 5 min. Dry nitrogen was passed through the reaction mixture for 2 hr. at room temperature. After evaporation to dryness *in vacuo* the residue solidified. The *product* was purified until free from chloride ions by repeatedly dissolving it in dry ethyl acetate and precipitating it with light petroleum (3·1 g., 75%), m. p. 134° (Found: C, 54·7; H, 4·3; N, 4·6. $C_{14}H_{13}NO_7$ requires C, 54·7; H, 4·2; N, 4·6%). The i.r. spectrum showed absorption peaks at 1780 and 1860 $cm.^{-1}$, characteristic for the two carbonyl stretches of the five-membered oxazolidine-2,5-dione ring and distinction from the six-membered anhydrides derived from β-amino acids which absorb [15] at 1710 and 1780 $cm.^{-1}$.

Reaction Between (IIIa) *and Ammonia.*—(IIIa) (1·4 g.) was dissolved in dry dioxan (10 ml.) and 25% ammonium hydroxide (0·8 ml.) was added. Colourless needles started to be deposited in the reaction mixture, and were filtered off after 5 hr. (0·8 g., 60%). This compound, which was the ammonium salt of the ureido-acid (IX), was dissolved in a small volume of water and on addition of concentrated hydrochloric acid to pH 2 β-*benzyl* N-*carbamoyl*-threo-β-*acetoxy*-DL-*aspartate* (IX) was obtained in quantitative yield, m. p. 203°. The substance gave a negative ninhydrin reaction (Found: C, 51·2; H, 4·7; N, 8·5. $C_{14}H_{16}N_2O_7,\frac{1}{2}H_2O$ requires C, 50·7; H, 4·5; N, 8·4%).

The dioxan mother-liquor was left for 5 days at room temperature and during this time a colourless crystalline substance precipitated which was neutral and gave a negative ninhydrin reaction. This was N^2-*acetyl*-threo-β-*hydroxy*-DL-α-*asparagine benzyl ester* (VIII) (0·25 g., 20%), m. p. 155° (from ethanol) (Found: C, 55·8; H, 5·4; N, 10·3. $C_{13}H_{16}N_2O_5$ requires C, 55·6; H, 5·7; N, 10·0%).

β-*Methyl Hydrogen* erythro-β-*Hydroxy*-DL-*aspartate* (Ib).—To a stirred suspension of *erythro*-β-hydroxy-DL-aspartic (16·5 g.) in dry methanol (165 ml.) was added concentrated hydrochloric acid (19 ml.) and the reaction mixture was heated at reflux for 3 hr. After evaporation to dryness *in vacuo* the solid residue was dissolved in ethanol (150 ml.) and the pH was brought to 8 by addition of pyridine. After standing overnight in a refrigerator the *product* precipitated and was recrystallised from ethanol (14 g., 78%), m. p. 205° (decomp.) (Found: C, 36·2; H, 5·5; N, 8·4. $C_5H_9NO_5$ requires C, 36·8; H, 5·5; N, 8·6%).

β-*Methyl Hydrogen* erythro-β-*Acetoxy*-DL-*aspartate* (IIb).—This *compound* was prepared in the same manner as the *threo*-derivative (IIa) (quantitative yield), m. p. 137° (decomp.) (ethanol) [Found: N (Van Slyke), 6·5. $C_7H_{11}NO_6$ requires N (Van Slyke), 6·8%].

[15] A. Zilkha and Y. Burstein, *Biopolymers*, 1964, **2**, 147.

β-*Methyl Dihydrogen* N-*Carboxy*-erythro-β-*acetoxy*-DL-*aspartate* N,α-*Anhydride* (IIIb).—Preparation of this *compound* was as for the *threo*-derivative (IIIa), yield 70%, m. p. 124° (Found: C, 41·6; H, 3·8; N, 6·5. $C_8H_9NO_7$ requires C, 41·6; H, 3·9; N, 6·1%).

Dimethyl N-*Benzyloxycarbonyl*-threo-β-*methoxy*-DL-*aspartate* (XI).—A mixture of (V) (23 g.) in dry acetone (120 ml.), methyl iodide (120 ml.), and silver oxide (120 g.) was heated at 37° for 5 hr. with occasional shaking. After cooling it was filtered and the precipitate was washed with dry acetone. The combined filtrates were evaporated to dryness *in vacuo*. The oily residue was dissolved in acetone (120 ml.) and treated with methyl iodide (100 ml.) and silver oxide (100 g.), as before, but only for 3 hr. This time removal of the solvent was effected in a high vacuum. The remaining oil was dissolved in benzene and purified by column chromatography on neutral alumina, using benzene as the eluent, to give the *product* (24 g., 90%) (Found: C, 56·0; H, 5·8; N, 4·3. $C_{15}H_{19}NO_7$ requires C, 55·5; H, 5·8; N, 4·3%).

N-*Benzyloxycarbonyl*-threo-β-*methoxy*-DL-*aspartamide*.—This was prepared in 80% yield by ammonolysis of (XI), in methanol saturated with ammonia, for 5 days at room temperature. After removal of the solvent *in vacuo* and trituration of the residue with water, the *product* precipitated, m. p. 206° (ethanol) (Found: C, 53·9; H, 5·9; N, 13·6. $C_{13}H_{17}N_3O_5$ requires C, 53·0; H, 5·8; N, 14·2%).

β-*Methyl Hydrogen* N-*Benzyloxycarbonyl*-threo-β-*methoxy*-DL-*aspartate* (XII).—(XI) (22 g.) was dissolved in acetone (400 ml.) and water (30 ml.). To this was added at room temperature with stirring during 30 min. aqueous 1N-lithium hydroxide solution (20 ml.). After 1 hr. further the acetone was evaporated *in vacuo* at 40° and the residue was extracted with two portions of ether (20 ml.). The aqueous solution was acidified with concentrated hydrochloric acid to pH 2. The oil which separated was extracted with ether and after drying ($MgSO_4$) the solvent was evaporated leaving a viscous oily *product* (15 g., 70%) (Found: C, 52·7; H, 5·2; N, 4·5. $C_{14}H_{17}NO_7$ requires C, 54·0; H, 5·5; N, 4·5%).

N^2-*Benzyloxycarbonyl*-threo-β-*methoxy*-DL-β-*asparagine*.—This was prepared by ammonolysis of (XII) by methanolic ammonia at room temperature for 5 days. After removal of the solvent *in vacuo* the residue was dissolved in a small volume of water and acidified to pH 2. After 1 day the *product* precipitated, m. p. 184° (ethanol) (Found: C, 52·8; H, 5·4; N, 9·5. $C_{13}H_{16}N_2O_6$ requires C, 52·7; H, 5·4; N, 9·5%).

β-*Methyl Hydrogen* threo-β-*Methoxy*-DL-*aspartate* (XIII).—(XII) (13 g.) was dissolved in 80% methanol (100 ml.) and 10% palladium–charcoal (1 g.), was added. Hydrogenation was carried out at room temperature for 10 hr. After separation of the catalyst by filtration, the solvent was evaporated off *in vacuo*. In order to remove water completely the residue was taken up in dry ethanol and this was again evaporated *in vacuo*. The glassy material which remained was dissolved in hot, dry ethanol (20 ml.). After cooling dry ether was added and the hygroscopic *product* precipitated (5·5 g., 75%) [Found: C, 40·1; H, 6·5; N, 7·7; N (Van Slyke), 7·5. $C_6H_{11}NO_5$ requires C, 40·6; H, 6·2; N, 7·9; N (Van Slyke), 7·9%].

β-*Methyl Dihydrogen* N-*Carboxy*-threo-β-*methoxy*-DL-*aspartate* N,α-*Anhydride* (XIV).—Through a stirred suspension of (XIII) (5 g.) in dry dioxan (100 ml.) was passed dry phosgene at 60° for 1 hr. during which time a clear solution was obtained. Excess of phosgene was removed by bubbling dry nitrogen through the reaction mixture for 3 hr. After evaporation of the solvent *in vacuo* the residual oil was repeatedly dissolved in dry ethyl acetate and precipitated by addition of dry light petroleum until it no longer gave a positive chloride test. The *product* was solid but extremely hygroscopic. Its i.r. spectrum had the typical absorption bands at 1780 and 1860 $cm.^{-1}$ (Found: C, 41·5; H, 5·3; N, 7·5. $C_7H_9NO_6$ requires C, 41·4; H, 4·5; N, 6·9%).

Poly-β-*methyl Hydrogen* threo-β-*Methoxy*-DL-*aspartate* (XV).—The anhydride (XIV) (0·1 g.) was dissolved in dry pyridine (3 ml.) and left at room temperature. Carbon dioxide was evolved from the start. After 24 hr. dry ether was added which precipitated the polymer (0·066 g., 85%). After separation by filtration the polymer was several times triturated with dry ether to remove traces of pyridine. It was very hygroscopic and gave a positive biuret and a negative ninhydrin reaction [Found: C, 44·9; H, 5·9; N, 8·4. Calc. for $(C_6H_9NO_4)_n$: C, 45·2; H, 5·7; N, 8·8%].

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