threo-β-Hydroxyaspartyl Dipeptides

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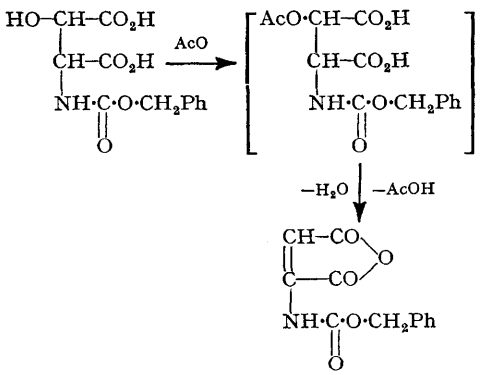
Some dipeptides of *threo*-β-hydroxyaspartic acid were synthesised, by use of β-benzyl *N*-benzyloxycarbonyl-*threo*-β-hydroxyaspartate, with carbodi-imides as condensing agents.

THE synthesis of peptides containing amino-acids with an alcoholic hydroxy-group, such as serine or threonine, presents certain difficulties due to the reactivity of the hydroxy-group, the lability of the peptide bonds (in the case of serine [1]), and the tendency for β-elimination reactions and N ⟶ O (or O ⟶ N) acyl shifts.[2] Further complications are encountered in the synthesis of peptides containing the β-hydroxyaspartic acids; these possess an additional carboxy-group which makes them tetrafunctional. Only the *erythro*-form of β-hydroxyaspartic acid has been isolated from natural sources,[3] but both the *threo*- and *erythro*-isomers, first synthesized by Dakin,[4] can now be prepared stereospecifically.[5] We have attempted to synthesise dipeptides containing the *threo*-isomer, with a view to preparing the β-hydroxy-L-aspartyl analogue of the physiologically active oligopeptide angiotensin II, an octapeptide with L-aspartic acid at its *N*-terminal end. We first used the more readily available racemic form to test the methods and then the L-isomer, obtained by resolution of *N*-benzyl-*threo*-β-hydroxy-DL-aspartic acid with L-ephedrine.[6]

The synthesis of peptides containing hydroxy-amino-acids does not necessarily require protection of the hydroxy-group. On the contrary, the use of acyl groups for this purpose increases the possibility of β-elimination. Etherification does not suffer from this disadvantage, but the benzyl ethers cannot be prepared readily from the hydroxy-amino-acids themselves. The hydroxy-group was therefore not protected in this work.

One of the classical methods for the production of α- and β-aspartyl peptides is based on the cyclic anhydride obtained when *N*-benzyloxycarbonylaspartic acid reacts with acetic anhydride.[7] This procedure, when applied to *N*-benzyloxycarbonyl-β-hydroxyaspartic acid, produced benzyloxycarbonylaminomaleic anhydride by *O*-acetylation followed by β-elimination.

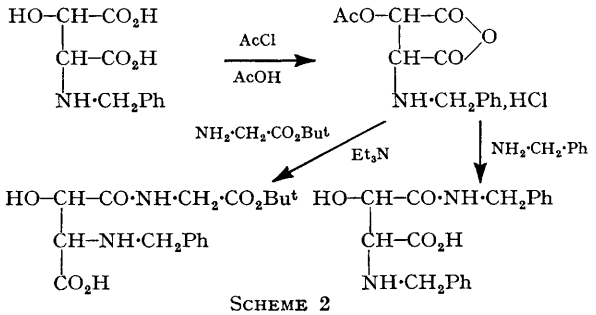


SCHEME 1

We also tried to prepare the cyclic anhydride of *N*-benzyl-*threo*-β-hydroxy-DL-aspartic acid by use of acetyl chloride–glacial acetic acid (3 : 1).[8] This procedure caused *N*-acetylation as well as β-elimination of the acetylated hydroxy-group, and gave *N*-benzylacetamidomaleic anhydride. However, if the reaction was carried out with a 1 : 1 reagent mixture, *O*-acetyl-*N*-benzyl-*threo*-β-hydroxy-DL-aspartic anhydride hydrochloride was obtained. When this was heated with benzylamine or t-butyl glycinate the amide or the dipeptide derivative was produced in low yield with concomitant removal of the *O*-acetyl group by the basic reagents. Since *N*-benzyl amino-acids give a positive ninhydrin reaction on paper chromatograms, we were able to use the method of Larsen and Kjaer [9] on these *N*-benzyl derivatives to

[1] J. I. Harris, R. D. Cole, and N. G. Pon, *Biochem. J.*, 1956, **62**, 154.
[2] D. F. Elliott, *Biochem. J.*, 1952, **50**, 542; L. A. Cohen and B. Witkop, *Angew. Chem.*, 1961, **73**, 253.
[3] H. J. Sallach and M. I. Kornguth, *Biochim. Biophys. Acta*, 1959, **34**, 582.
[4] H. D. Dakin, *J. Biol. Chem.*, 1921, **48**, 273.
[5] Y. Liwschitz, Y. Rabinsohn, and A. Haber, *J. Chem. Soc.*, 1962, 3589.
[6] Y. Liwschitz, Y. Edlitz-Pfeffermann, and A. Singerman, *J. Chem. Soc.*, 1967, 2104.
[7] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
[8] A. Zilkha and Y. Liwschitz, *J. Chem. Soc.*, 1957, 4397.
[9] P. O. Larsen and A. Kjaer, *Biochem. Biophys. Acta*, 1960, **38**, 148.

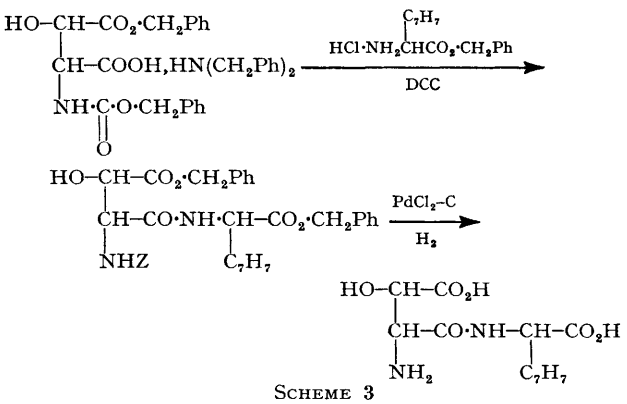
determine whether the α- or the β-carboxy-group remained free. This method distinguishes α-monoamino-acids from other ninhydrin-positive substances on paper chromatograms. Treatment with methanolic cupric nitrate before development of the ninhydrin colour results in complex formation, and the colour is largely or completely suppressed in the case of the α-acids. The test showed in our case that a β-amide (peptide) was formed.



SCHEME 2

Since yields by this method were low, and in order to avoid the possibility of producing mixtures of α- and β-peptides, we decided to use the dibenzylammonium salt of β-benzyl *N*-benzyloxycarbonyl-*threo*-β-hydroxy-aspartate.[10] Coupling with esters of other amino-acids in the form of their hydrochlorides did not then necessitate the use of a tertiary amine to bind the hydrochloric acid. As a coupling agent dicyclohexylcarbodi-imide was used, but in some cases the soluble *N*-cyclohexyl-*N'*-(4-ethyl-2-morpholinyl)carbodi-imide methotoluene-*p*-sulphonate was found to give better yields. When it turned out that alkaline hydrolysis of the protected β-hydroxyaspartyl dipeptides involved great losses, the benzyl ester of L-phenylalanine was used in the coupling reaction instead of the methyl ester, thus allowing the removal of all protecting groups at once by catalytic hydrogenolysis.

The following fully protected α-dipeptides of *threo*-β-hydroxyaspartic acid (Hyasp) were synthesized: DL-Hyasp-Gly, DL-Hyasp-β-Ala, DL-Hyasp-L-Leu, DL-Hyasp-L-Phe, DL-Hyasp-L-Tyr, L-Hyasp-L-Phe, and L-Hyasp-L-Tyr. The free dipeptides were obtained only in the above mentioned cases where the benzyl esters were employed.



SCHEME 3

EXPERIMENTAL

M.p.s were determined with a Fisher-Johns apparatus. Microcombustion analyses were carried out by Mrs. M. Goldstein of the Microanalytical Laboratory of the Hebrew University, to whom our thanks are due.

Benzyloxycarbonylaminomaleic Anhydride.—*N*-Benzyloxycarbonyl-*threo*-β-hydroxy-DL-aspartic acid (2 g.) was heated in acetic anhydride (6 ml.) until it dissolved. Plates of the *anhydride* (1·3 g., 75%) separated from the cooled solution, and were filtered off and washed with dry ether; m.p. 163° (from chloroform) (Found: C, 58·3; H, 3·9; N, 5·7. $C_{12}H_9NO_5$ requires C, 58·2; H, 3·7; N, 5·7%). The substance decolourised a dilute solution of potassium permanganate.

N-Benzylacetamidomaleic Anhydride.—To *N*-benzyl-*threo*-β-hydroxy-DL-aspartic acid (2 g.) was added a mixture of freshly distilled acetyl chloride (12 ml.) and glacial acetic acid (4 ml.). After 15 min. the substance had dissolved completely and the mixture was evaporated *in vacuo* to leave an oil which was dissolved in dry ether. Addition of light petroleum precipitated *needles*, m.p. 64°, which reduced a potassium permanganate solution (Found: C, 63·2; H, 4·8; N, 5·8. $C_{13}H_{11}NO_4$ requires C, 63·5; H, 4·5; N, 5·7%).

O-*Acetyl*-N-*benzyl*-threo-β-*hydroxy*-DL-*aspartic Anhydride Hydrochloride.*—To *N*-benzyl-*threo*-β-hydroxy-DL-aspartic acid (4 g.) was added a mixture of freshly distilled acetyl chloride (15 ml.) and glacial acetic acid (15 ml.). When the substance had dissolved (*ca.* 15 min.) the mixture was evaporated *in vacuo* to leave an oil which was poured into dry ether (100 ml.). The viscous oil which resulted was triturated with dry ether and yielded hygroscopic *crystals* (3·2 g., 52%) which were dried *in vacuo* (P_2O_5) (Found: N, 4·6. $C_{13}H_{14}ClNO_5$ requires N, 4·7%).

NN'-*Dibenzyl*-threo-β-*hydroxy*-DL-*asparagine.*—The above anhydride (1·5 g.) was dissolved in dry acetone (15 ml.) and benzylamine (3·2 ml.) was added. The mixture was heated under reflux for 2 hr. The *product* was deposited by the cooled solution (0·6 g., 35%); m.p. 183° (from aqueous ethanol) (Found: C, 62·3; H, 6·4; N, 8·2; O, 23·2. $C_{18}H_{20}N_2O_5$ requires C, 61·9; H, 5·8; N, 8·2; O, 23·2%).

t-Butyl N-*Benzyl*-threo-β-*hydroxy*-β-DL-*aspartylglycinate.*—The above anhydride (1·5 g.) was dissolved in dry chloroform (10 ml.) and triethylamine (1·1 ml.) and t-butyl glycinate (1·1 ml.) were added. The mixture was heated under reflux for 2 hr. It was then cooled, extracted with water to remove triethylamine hydrochloride, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residual oil was triturated with light petroleum to yield a *solid* (0·19 g., 10%), m.p. 85° (from aqueous acetone) (Found: C, 55·2; H, 7·1; N, 7·5. $C_{17}H_{24}N_2O_6,H_2O$ requires C, 55·1; H, 7·3; N, 7·6%).

N-*Benzyloxycarbonyl*-threo-β-*hydroxy*-(β-*benzyl*)-α-DL-*aspartyl*-L-*phenylalanine Benzyl Ester.*—To dibenzylammonium β-benzyl-*N*-benzyloxycarbonyl-*threo*-β-hydroxy-DL-aspartate [10] (1·14 g., 2 mmoles) in dichloromethane (30 ml.) was added benzyl L-phenylalaninate toluene-*p*-sulphonate (0·86 g. 2 mmoles), and the mixture was stirred at room temperature for 25 min. The dibenzylamine toluene-*p*-sulphonate which precipitated was filtered off, and dicyclohexylcarbodi-imide (0·41 g., 2 mmoles) in dichloromethane (10 ml.) was added; the mixture was left overnight. A few drops of acetic acid were then added,

[10] Y. Liwschitz and A. Singerman, *J. Chem. Soc.* (*C*), 1967, 1696.

and the precipitated dicyclohexylurea was filtered off. The filtrate was washed with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water, and dried ($MgSO_4$). The solvent was removed *in vacuo* and the oily residue was dissolved in a small volume of ethyl acetate. When light petroleum was added, the *product* was precipitated (0·46 g., 40%); m.p. 90—92° (Found: C, 69·0; H, 5·9; N, 4·7. $C_{35}H_{34}N_2O_8$ requires C, 68·8; H, 5·6; N, 4·6%).

Data for other β-benzyl *N*-benzyloxycarbonyl-*threo*-β-hydroxy-α-aspartyl dipeptides, prepared similarly, are summarised in the Table.

threo-β-*Hydroxy*-α-DL-*aspartyl*-L-*phenylalanine*.— *N*-Benzyloxycarbonyl-*threo*-β-hydroxy-(β-benzyl)-α-DL-aspartyl-L-phenylalanine benzyl ester (1·93 g., 3 mmoles) was dissolved in a mixture of methanol (45 ml.), glacial acetic acid (11 ml.), and water (56 ml.), and the catalyst (30% palladium chloride on Darco; 0·5 g.) was added. Hydrogenolysis was carried out in a Parr low-pressure apparatus for 15 hr. at room temperature. Part of the substance precipitated and was filtered off with the catalyst. The filtrate was evaporated and the resulting viscous oil was triturated with ethyl acetate to yield hygroscopic *crystals* which were washed with ethyl acetate and dried *in vacuo* (0·45 g., 56%) [Found: C, 44·9; H, 6·3; N, 7·7; N(Van Slyke), 4·4. $C_{13}H_{16}N_2O_6,3H_2O$ requires C, 44·6; H, 6·3; N, 8·0; N(Van Slyke), 4·0%]. The catalyst, with the adhering substance was heated with a small amount of water and then filtered off. The cooled solution deposited *crystals* (0·09 g., 10%), m.p. 192° [Found: C, 50·9; H, 5·5; N, 9·4; N(Van Slyke), 4·7. $C_{13}H_{16}N_2O_6,\frac{1}{2}H_2O$ requires C, 51·1; H, 5·6; N, 9·2; N(Van Slyke), 4·6%]. T.l.c. of the two differently hydrated dipeptides in two different solvent systems showed them to be identical.

N-Benzyloxycarbonyl-*threo*-β-hydroxy-(β-benzyl)-α-DL-aspartyl-	Yield (%)	M.p.	Found (%) C	H	N	Formula	Required (%) C	H	N
L-Phe-OMe	72	80°	64·8	5·5	5·1	$C_{29}H_{30}N_2O_8$	65·1	5·6	5·2
L-Tyr-OEt	40	52	64·2	6·1	5·3	$C_{30}H_{32}N_2O_9$	63·8	5·7	5·0
β-Ala-OMe [a]	85	69	60·7	6·0	5·9	$C_{23}H_{26}N_2O_8$	60·2	5·7	6·1
L-Leu-OMe [a]	45	53	62·8	6·2	5·6	$C_{26}H_{32}N_2O_8$	62·4	6·4	5·6
Gly-O·CH_2Ph	50	Semi-solid	64·9	5·8	5·9	$C_{28}H_{28}N_2O_8$	64·6	5·4	5·4
N-Benzyloxycarbonyl-*threo*-β-hydroxy-(β-benzyl)-α-L-aspartyl-									
L-Tyr-OEt [b]	60	39	63·5	6·0	5·2	$C_{30}H_{32}N_2O_9$	63·8	5·7	5·0
L-Phe-O·CH_2Ph [c]	70	98	68·0	6·1	5·3	$C_{35}H_{34}N_2O_8$	68·8	5·6	4·6

[a] *N*-Cyclohexyl-*N'*-(4-ethyl-2-morpholinyl)carbodi-imide methotoluene-*p*-sulphonate used as condensing agent. [b] $[\alpha]_D^{16}$ +8·4° (*c* 12·5 in ethyl acetate). [c] $[\alpha]_D^{22}$ −9·0° (*c* 4·7 in ethyl acetate).

β-*Benzyl* threo-β-*Hydroxy*-L-*aspartate*.—This *substance* was prepared (60%) in accordance with the procedure for the racemic compound;[10] $[\alpha]_D^{22}$ +9·4° (*c* 4·5 in N-HCl) (Found: N, 5·9. $C_{11}H_{13}NO_5$ requires N, 5·8%), m.p. 202° (decomp.).

β-*Benzyl* N-*Benzyloxycarbonyl*-threo-β-*hydroxy*-L-*aspartate*.—This *compound* was prepared (75%) from β-benzyl *threo*-β-hydroxy-L-aspartate in accordance with the procedure for the racemic compound;[10] m.p. 83° (from ethyl acetate–light petroleum), $[\alpha]_D^{16}$ −5·5° (*c* 6 in ethyl acetate) (Found: N, 4·0. $C_{19}H_{19}NO_7$ requires N, 3·8%). The dibenzylammonium salt was prepared by dissolving the compound in dry ether and adding dibenzylamine (1 equiv.).

Threo-β-*Hydroxy*-α-L-*aspartyl*-L-*phenylalanine*.— *N*-Benzyloxycarbonyl-*threo*-(β-benzyl)-α-L-aspartyl-L-phenylalanine benzyl ester (0·4 g.) was hydrogenolysed catalytically according to the procedure for the DL-L-isomer (see above) to give the *dipeptide*, m.p. 232° (decomp.) (0·14 g., 70%), $[\alpha]_D^{22}$ +24·8° (*c* 1·7 in N-HCl) [Found: C, 52·8; H, 5·5; N, (Van Slyke), 4·8. $C_{13}H_{16}N_2O_6$ requires C, 52·8; H, 5·4; N (Van Slyke), 4·7%].

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