New Compounds

Synthesis of Tripeptides of 1-Aminocyclopentane-1-carboxylic Acid

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In the course of studies designed to investigate peptide transport in biological systems,[1] tripeptides of 1-aminocyclopentane-1-carboxylic acid (cycloleucine)[2] were needed. The present report is concerned with the synthesis of glycylcycloleucyl-L-valine and glycylcycloleucyl-L-alanine. A number of other cycloleucine peptides have previously been synthesized by authors interested in the tumor growth inhibition shown by cycloleucine.[3-6] Transport studies with *Lactobacillus casei* 7469 have shown that this organism has pathways for the uptake of tripeptides distinct from those for the uptake of free amino acids.[7]

Experimental[8]

Carbobenzoxyglycylcycloleucine Ethyl Ester (I).—To a solution containing 5.8 g. (0.03 mole) of cycloleucine ethyl ester hydrochloride,[3] 4.2 ml. (0.03 mole) of triethylamine, and 6.3 g. (0.03 mole) of carbobenzoxyglycine in 150 ml. of methylene chloride, 6.2 g. (0.03 mole) of dicyclohexylcarbodiimide was added. The mixture was stirred for 16 hr. in a cold room (4°), filtered to remove dicyclohexylurea, washed successively with 0.5 *N* HCl, 0.5 *N* $NaHCO_3$, and water, and dried overnight (Na_2SO_4). The solvent was removed *in vacuo*. Recrystallization of the residue from ethanol–ethyl ether yield 6.9 g. (66%) of product, m.p. 100°.

Anal. Calcd. for $C_{18}H_{24}N_2O_5$: C, 62.07; H, 6.89; N, 8.04. Found: C, 62.10; H, 6.97; N, 8.30.

Carbobenzoxyglycylcycloleucine (II).—I (4.05 g., 0.012 mole) was added to a mixture of 15 ml. of 1 *N* NaOH and 20 ml. of ethanol and stirred until dissolved. The chilled solution was acidified with 1 *N* HCl. A crystalline precipitate appeared which was recrystallized from ethanol–ethyl ether; yield 1.97 g. (53%), m.p. 174°, lit.[4] m.p. 176°.

Carbobenzoxyglycylcycloleucyl-L-alanine Benzyl Ester (III).—To a solution of 1.0 g. (0.003 mole) of II and 0.003 mole of L-alanine benzyl ester [freshly prepared from 1.03 g. (0.003 mole) of L-alanine benzyl ester benzenesulfonate[9] and 0.44 ml. (0.003 mole) of triethylamine] in 150 ml. of acetonitrile was added 0.66 g. (0.0032 mole) of dicyclohexylcarbodiimide. The mixture was stirred at 4° for 60 hr.; the precipitate of dicyclohexylurea was then filtered off, and the filtrate was reduced to dryness *in vacuo*. The residue was dissolved in ethyl acetate, washed with 0.5 *N* HCl, 0.5 *N* $NaHCO_3$, and water, and dried overnight (Na_2SO_4). The product crystallized upon cooling and addition of petroleum ether, and was recrystallized from ethyl acetate–petroleum ether; yield 0.93 g. (62%), m.p. 175°.

Anal. Calcd. for $C_{26}H_{31}N_3O_6 \cdot H_2O$: C, 62.51; H, 6.66; N, 8.41. Found: C, 62.79; H, 6.40; N, 8.27.

Glycylcycloleucyl-L-alanine (IV).—III (0.25 g., 0.00053 mole) was dissolved in 65 ml. of 10% glacial acetic acid in methanol and 0.17 g. of freshly prepared palladium black was added. Hydrogen was bubbled into the mixture, which was vigorously agitated with the aid of a Vibromixer.[10] After 1 hr. of hydrogenation, the catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. A fluffy precipitate appeared, which was recrystallized from water–acetone; yield 0.117 g. (87%), m.p. 236–242° dec.,[11] $[\alpha]^{25}D$ −18.0° (*c* 1, water), R_f 0.55.

Anal. Calcd. for $C_{11}H_{19}N_3O_4$: C, 51.15; H, 7.80; N, 16.27. Found: C, 51.16; H, 7.59; N, 16.29.

Carbobenzoxyglycylcycloleucyl-L-valine Benzyl Ester (V).—II (1.3 g., 0.004 mole) and 0.82 g. (0.004 mole) of dicyclohexylcarbodiimide were dissolved in 100 ml. of acetonitrile. A solution of 0.004 mole of L-valine benzyl ester [freshly prepared as described for alanine benzyl ester above] in 50 ml. acetonitrile was added. After 44 hr. of stirring at 4°, the product was worked up as III above; yield 1.35 g. (66%), m.p. 142.5°.

Anal. Calcd. for $C_{26}H_{35}N_3O_6$: C, 65.99; H, 6.92; N, 8.25. Found: C, 66.29; H, 7.03; N, 8.27.

Glycylcycloleucyl-L-valine (VI).—A solution of 1.1 g. (0.0022 mole) of V in 70 ml. of 10% glacial acetic acid in methanol was hydrogenated as described in the preparation of IV above. Recrystallization from water–acetone yielded 0.67 g. (85%) of the acetate of VI, m.p. 235–237° dec., R_f 0.75, $[\alpha]^{25}D$ −24.0° (*c* 1, water).

Anal. Calcd. for $C_{13}H_{23}N_4O_4 \cdot CH_3COOH \cdot H_2O$: C, 49.57; H, 8.04; N, 11.56. Found: C, 48.39; H, 8.08; N, 11.52.

Peptides Labeled with C^{14}.—By methods almost identical with those outlined above, both tripeptides were prepared with a specific activity of 10 μc./mmole, starting from carboxyl-C^{14}-labeled cycloleucine [New England Nuclear Corp.]. Paper chromatography of the labeled peptides and determination of radioactivity with a paper strip scanner showed single peaks of radioactivity which coincided with the single spots seen after ninhydrin spraying.

(1) E. A. Young, D. O. Bowen, and J. F. Diehl, *Biochem. Biophys. Res. Commun.*, **14**, 250 (1964).

(2) Also termed NSC-1026. The name cycloleucine will be used in this report.

(3) T. A. Connors and W. C. J. Ross, *J. Chem. Soc.*, 2119 (1960).

(4) P. Tailleur and L. Berlinguet, *Can. J. Chem.*, **39**, 1309 (1961).

(5) P. Tailleur and L. Berlinguet, *ibid.*, **40**, 2214 (1962).

(6) S. Shankman, S. Higa, F. Lew, and M. E. Roberts, *J. Med. Pharm. Chem.*, **5**, 42 (1962).

(7) E. A. Young and J. F. Diehl, *Federation Proc.*, **23**, 136 (1964); a detailed report will appear elsewhere.

(8) (a) Melting points were determined on a Fisher-Johns block and are corrected. (b) Specific rotation was determined with a 0.01° Zeiss polarimeter. (c) Elementary analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. (d) R_f values refer to ascending paper chromatography using Whatman No. 1 paper and 2-butanol–formic acid–water (75:15:10) as the solvent system.

(9) J. E. Shields, W. H. Gregor, and F. H. Carpenter, *J. Org. Chem.*, **26**, 1101 (1961).

(10) Vibromixer, Fisher Scientific Co.

(11) Hydrogenation in the presence of acetic acid was expected to yield the tripeptide acetate. Elementary analysis indicates that the free tripeptide was obtained. A second batch of III was hydrogenated in methanol in the absence of acetic acid and the product obtained was identical with the analytical sample, as judged by melting point and mixture melting point.

An Improved Synthesis of Oxotremorine

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Oxotremorine[1] [1-(2-oxopyrrolidino)-4-pyrrolidino-2-butyne], a metabolite of tremorine,[2] is a highly active muscarinic tertiary amine which may be used for studying cholinergic effects in both the peripheral and central nervous systems.[3] Investigations of this nature, however, have been hampered by the lack of general availability of oxotremorine due mainly to the dif-

(1) A. K. Cho, W. L. Haslett, and D. J. Jenden, *Biochem. Biophys. Res. Commun.*, **5**, 276 (1961).

(2) G. M. Everett, *Nature*, **177**, 1238 (1956).

(3) (a) A. K. Cho, W. L. Haslett, and D. J. Jenden, *J. Pharmacol. Exptl. Therap.*, **138**, 240 (1962); (b) K. Krnjević and J. W. Phillis, *J. Physiol.*, **166**, 328 (1963); (c) R. W. Brimblecombe, 4th Meeting of Collegium Internationale Neuro-psychopharmacologicum, Birmingham, England, 1964.