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] $[\alpha]^{27}D$ −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.

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(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.

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(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-

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ficulty of obtaining pure samples of the synthetic intermediate N-propargyl-2-pyrrolidone. We now report an improved synthesis of this intermediate which enables oxotremorine to be obtained in an over-all yield of 46% based on the starting material 2-pyrrolidone.

Experimental

N-Propargyl-2-pyrrolidone.—A solution of 28 g. of A.R. KOH in 100 ml. of dry methanol was added to 45 g. of 2-pyrrolidone. The solvent was slowly removed under reduced pressure from the stirred solution at an internal temperature not exceeding 25°. When solid began to separate, 200 ml. of toluene was added and toluene was then continuously added during the course of all subsequent distillations. When 500 ml. of toluene had distilled, the pressure was allowed to rise and distillation was continued at an internal temperature of 90°. Finally 700 ml. of toluene was collected at atmospheric pressure. The suspension was cooled and treated at 40° with 60 g. of propargyl bromide over a period of 1 hr. The reaction mixture was then heated to 67° for 30 min., cooled, and filtered. The toluene was removed under reduced pressure, and the product was distilled. It had b.p. 77° (0.05 mm.), $n^{25}D$ 1.4970, yield 40 g. (61%).

1-(2-Oxopyrrolidino)-4-pyrrolidino-2-butyne, Oxotremorine.—A solution of 30 g. of N-propargyl-2-pyrrolidone, 9.2 g. of paraformaldehyde, and 23 ml. of pyrrolidine in 60 ml. of dioxane was heated at 100° for 12 hr. in an atmosphere of nitrogen. The solvent was removed under reduced pressure, and the residue was dissolved in 60 ml. of 5 *N* HCl. After three extractions with ether, the aqueous solution was made alkaline with 33% aqueous NaOH and extracted with chloroform. Distillation of the chloroform extracts yielded a fraction having b.p. 124° (0.1 mm.), $n^{25}D$ 1.5156, yield 38 g. (76%).

Anal. Calcd. for $C_{12}H_{18}N_2O$: C, 69.87; H, 8.80. Found: C, 69.89; H, 8.59.

Perchloric Acid in the Preparation of 2′,3′-Isopropylidene 6-Thioinosine

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The preparation of the title compound reported in the literature[2-4] can be greatly improved by using perchloric acid[5] as a catalyst for the condensation of 6-thioinosine and acetone. Adequate amounts of this isopropylidene intermediate, required for chemotherapeutic work,[6] can be prepared readily following the procedure described in the Experimental section.

Experimental

The extent of formation of the isopropylidene derivatives was estimated by paper chromatography of an aliquot of the reaction mixture on Schleicher and Schuell No. 589 paper (orange ribbon) by the descending method. The solvent system used throughout consisted of the standard isopropyl alcohol–water–ammonia mixture (70:20:10). For each isolated product, the R_f value was compared with an authentic sample of the same material prepared by previously reported procedures.

2′,3′-O-Isopropylidene 6-Thioinosine. A. Small Scale.—To 100 ml. of acetone (dried with $MgSO_4$), 0.35 ml. of 2,2-dimethoxypropane was added, followed by 0.45 ml. of 70% aqueous perchloric acid. After 5 min. 244 mg. (1 mmole) of 6-thioinosine was added rapidly, and the reaction flask was stoppered and swirled for approximately 5 min. until the solids had dissolved. After 10 min. the solution was neutralized with 0.35 ml. of pyridine and concentrated under diminished pressure to approximately 20 ml. A 10% Na_2CO_3 solution (5 ml.) was added and the remaining acetone was removed. The aqueous solution was extracted twice with 5-ml. portions of dichloromethane and acidified with glacial acetic acid (about 0.7 ml.) to pH 6. After 2 hr. at 4°, the crystalline product was filtered off, washed with water, and dried *in vacuo* at room temperature. The material obtained showed a single spot on paper chromatography (R_f 0.72) and weighed 230 mg. (yield, 80%). Calculated on the basis of the ultraviolet absorption, the aqueous filtrate contained another 30 mg. of product, but no starting material (single spot).

B. "Large" Scale.—To avoid foaming, it was necessary to replace the Na_2CO_3 with an NH_4OH solution. To 2400 ml. of dry acetone was added 65 ml. of 2,2-dimethoxypropane followed by 87.2 ml. of 70% perchloric acid solution. After 5 min., 47.3 g. (0.166 mole) of 6-thioinosine was added quickly with continuous stirring. After 20 min., the clear, yellow reaction mixture was quenched by the addition of 194 ml. of pyridine which had been dried with calcium hydride. A white precipitate formed immediately. Water (920 ml.) was added, followed by 65 ml. of 15 *N* NH_4OH solution, and the suspension was concentrated under reduced pressure until all of the acetone had been removed. Another 65-ml. portion of 15 *N* NH_4OH was added, and the clear solution obtained was extracted with two 325-ml. portions of dichloromethane. The aqueous layer was filtered, chilled, and acidified to pH 6 with glacial acetic acid. After standing for 18 hr. at 4°, the white cyrstalline solid was collected by filtration, washed with water, and dried *in vacuo* at 78°. The product weighed 46.5 g. 86%; m.p. 238–240° dec.; λ_{max} ($\epsilon \times 10^3$): pH 1, 322 (24.0); pH 7, 318 (22.7); pH 13, 310 (23.4). The material showed a single spot on paper chromatography (R_f 0.72).

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Synthesis of Some Amino Acid Thiol Esters[1a]

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In connection with a study of certain aspects of the mechanism of action of sulfhydryl enzymes, several amino acid thiol esters have been prepared in this laboratory. These compounds are of particular interest since amino acid thiol esters serve as important model compounds for the study of amino acid–sulfhydryl enzyme interaction. Certain of the N-acyl compounds have been shown to be active substrates for proteolytic enzymes.[2,3]

The syntheses of amino acid thiol esters which have been reported have been for the most part restricted to N-acylamino acid thiol esters. Thiol esters of hippuric acid have been reported by Jeger, *et al.*,[4] by Johnston,[2] and by Schwyzer and Hürlimann.[5] A number of amino acid thiolesters of thiophenol and cysteamine have been prepared by Wieland.[6] Thiol ester

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