acetic acid. A gummy precipitate appeared which was completely soluble in sodium hydroxide, indicating absence of the starting compound and of its dihydro derivative. The sodium hydroxide solution was brought back to *p*H 7 by acetic acid and the pale pink precipitate was washed with a little water and dried at 0.1 mm. at room temperature. The dried solid decomposed gradually at about 160–170°, giving off a gas and leaving a dark red liquid. A methanol solution of the compound produced a nearly colorless spot on filter paper. After exposure to air for several weeks the spot exhibited a salmon color similar to that produced by a solution of a known sample of II.

Anal. Calcd. for $C_{23}H_{27}N_3SO_2 \cdot 1^1/_2H_2O$: C, 63.56; H, 6.96; S, 7.38. Found: C, 63.44; H, 6.70; S, 7.25, 7.32.[10]

Reaction rates were compared by use of a Bausch and Lomb Spectronic 20 colorimeter. A measured volume of glacial acetic acid containing a known concentration of the styrylquinoline was mixed with a measured volume of boiled or distilled water or of glacial acetic acid containing a known weight of cysteine or cysteineamine hydrochloride and diluted to a predetermined final volume in a volumetric flask. After mixing, the clear sample was kept at approximately 20° in a cabinet between readings. The air was swept out of the flasks and tubes with butane gas to prevent oxidation of cysteine to cystine by oxygen, but sometimes a white deposit believed to be cystine did form in the test tubes. Also, the original cysteine contained a trace of cystine and it was usually necessary to filter or centrifuge the water or acetic acid solution before mixing it with the styrylquinoline solution. Optical densities were read at 480, 510, and 540 mμ. The wave length corresponding to the greatest absorption was not the same for all the compounds, but the rate of change of absorption with time differed little from one wave length to another. The logarithms of the optical densities were plotted against time. The quantity of cysteine used was so much larger than the quantity of the styrylquinoline that a psuedo first order reaction curve was obtained and the straight line drawn through the points near the beginning of the curve plotted on semilogarithmic paper was used to estimate by extrapolation the 50% reaction time. In fact, however, the curves eventually turned to parallel the time axis, indicating that an equilibrium was approached.

DEPARTMENT OF CHEMISTRY
CARSON-NEWMAN COLLEGE
JEFFERSON CITY, TENN.

Simplified Synthesis of the C-Terminal Tripeptide Sequence of Oxytocin

WILLIAM D. CASH

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A variety of preparative methods have been utilized in the synthesis of ethyl carbobenzoxy-L-prolyl-L-leucylglycinate and carbobenzoxy-L-prolyl-L-leucylglycinamide, intermediates in the synthesis of oxytocin.[1] However, in all of these methods the use of protected L-leucine is required and at some stage in the synthesis the protecting group has to be removed. The present report describes a more direct synthesis in which the blocking of the amino group of L-leucine is not necessary.

Carbobenzoxy-L-prolyl-L-leucine was prepared by the mixed anhydride method[2] from carbobenzoxy-L-proline and L-leucine. The recrystallized product agreed well in melting point and optical rotation with the product obtained on hydrolysis of methyl carbobenzoxy - L - prolyl - L - leucinate prepared by the nitrophenyl ester method.[1f] The protected dipeptide was coupled by the mixed anhydride method with ethyl glycinate hydrochloride to yield ethyl carbobenzoxy - L - prolyl - L - leucyl glycinate or with glycinamide hydrochloride to yield carbobenzoxy - L - prolyl - L - leucylglycinamide. Melting points and optical rotations of the protected tripeptides were in close agreement with literature values.

EXPERIMENTAL

All melting points were determined in capillary tubes and are corrected.

Methyl carbobenzoxy-L-prolyl-L-leucinate. Methyl L-leucinate hydrochloride[3] (10.9 g.) was dissolved in water (100 ml.). Potassium bicarbonate (8.5 g.) was added and the resulting mixture was extracted four times with 30-ml. portions of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous magnesium sulfate and filtered. *p*-Nitrophenyl carbobenzoxy-L-prolinate[1f] (18.5 g.) was added to the filtrate. The resulting solution was concentrated *in vacuo* to about 30 ml. and was left standing at room temperature for 3 days. Ethyl acetate (100 ml.) was added and the resulting solution was extracted with 30 ml. portions of *N* ammonium hydroxide until colorless extracts were obtained. The ethyl acetate solution was washed twice with water, was dried over anhydrous magnesium sulfate, and was filtered. The filtrate was concentrated *in vacuo* and hexane was added to the residue. The crystalline product was filtered off; wt. 14.9 g., m.p. 75.5–78°. This material was recrystallized from ethyl acetate (50 ml.) and hexane (150 ml.); wt. 10.7 g., m.p. 76.5–78°, $[\alpha]_D^{21}$ −69.0° (*c* 1, ethanol).

Anal. Calcd. for $C_{20}H_{28}O_5N_2$: C, 63.8; H, 7.50; N, 7.44. Found: C, 64.0; H, 7.62; N, 7.44.

Carbobenzoxy-L-prolyl-L-leucine. Method A. Carbobenzoxy-L-proline (12.5 g.) was dissolved in tetrahydrofuran (70 ml.). Triethylamine (7.5 ml.) was added and then the solution was cooled to −10°. Isobutyl chloroformate (6.8 g.) in tetrahydrofuran (30 ml.) was added. The solution was stirred at −10° for 20 min. Then a solution of L-leucine (7.9 g.) and triethylamine (12.6 ml.) in water (65 ml.) was added. Stirring was continued without further cooling for 90 min. The reaction mixture was acidified by the slow addition of concentrated hydrochloric acid. Tetrahydrofuran was removed *in vacuo* and the resulting solid was filtered off. It was dissolved in glacial acetic acid (20 ml.) and was added to water (200 ml.). The crystalline solid was filtered off; wt. 17.0 g. It was

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(2) J. R. Vaughan, Jr. and J. A. Eichler, *J. Am. Chem. Soc.*, **75**, 5556 (1953).

(3) This compound was prepared by the method reported for the preparation of methyl L-valinate hydrochloride (R. A. Boissonnas, St. Guttmann, P.-A. Jaquenoud, and J.-P. Waller, *Helv. Chim. Acta*, **39**, 1421 (1956)). It melted at 149.5–150.5°, reported m.p. 148° (G. Takahashi and T. Yagimuma, *Proc. Imp. Acad. (Tokyo)*, **6**, 75 (1930).

recrystallized from chloroform (25 ml.) and carbon tetrachloride (225 ml.); wt. 13.3 g., m.p. 136.5–138°.

Material with this melting point (37.4 g.) was recrystallized again from chloroform (50 ml.) and carbon tetrachloride (500 ml.); wt. 35.2 g., m.p. 138.5–139.5°, $[\alpha]_D^{22}$ −56.5° (*c* 1, ethanol), $[\alpha]_D^{22}$ −63.0° (*c* 5, methanol). Reported,[4] m.p. 118.5–119.5°, $[\alpha]_D$ −62.7° (methanol).[5]

Anal. Calcd. for $C_{19}H_{26}O_5N_2$: C, 63.0; H, 7.23; N, 7.73. Found: C, 62.8; H, 7.38; N, 7.72.

Method B. Methyl carbobenzoxy-L-prolyl-L-leucinate (3.8 g.) was dissolved in methanol (15 ml.). Normal sodium hydroxide (10.5 ml.) was added and the resulting solution was stirred at room temperature for 2 hr. The solution was then acidified by the slow addition of concentrated hydrochloric acid. Methanol was removed *in vacuo*. The crystalline product was filtered off and was washed with water; wt. 3.6 g., m.p. 136–138°. This material was recrystallized from chloroform (7 ml.) and carbon tetrachloride (50 ml.); wt. 3.2 g., m.p. 138.5–139.5°, $[\alpha]_D^{22}$ −57.5° (*c* 1, ethanol).

Anal. Found: C, 63.0; H, 7.31; N, 7.74.

Ethyl carbobenzoxy-L-prolyl-L-leucylglycinate. Carbobenzoxy-L-prolyl-L-leucine (1.45 g.) was dissolved in tetrahydrofuran (20 ml.). Triethylamine (0.56 ml.) was added and the solution was cooled to −10°. Isobutyl chloroformate (0.55 g.) in tetrahydrofuran (10 ml.) was added. The mixture was stirred for 20 min. at −10°. Then a solution of ethyl glycinate hydrochloride (0.67 g.) and triethylamine (0.70 ml.) in water (5 ml.) was added. Stirring was continued without further cooling for 90 min. Water (25 ml.) was added and the mixture was acidified by slow addition of concentrated hydrochloric acid. Tetrahydrofuran was removed *in vacuo*. The resulting solid was filtered off and washed successively with 30-ml. portions of *N* hydrochloric acid, water, 5% sodium bicarbonate, and water; wt. 1.6 g., m.p. 148–150°. The product was recrystallized from ethanol (15 ml.); wt. 1.3 g., m.p. 150–152°, $[\alpha]_D^{21}$ −83.2 (*c* 2.5, ethanol). Reported, m.p. 148–149°,[1a] 148–149.5°,[1e] 150–151°,[1d] 151–152°,[1f] $[\alpha]_D$ −79.8°,[1a] −81.2°,[1e] −82.6°,[1f] (ethanol).

Carbobenzoxy-L-prolyl-L-leucylglycinamide. Carbobenzoxy-L-prolyl-L-leucine (3.6 g.) was dissolved in tetrahydrofuran (25 ml.). Triethylamine (1.5 ml.) was added and the solution was cooled to −10°. Isobutyl chloroformate (1.4 g.) in tetrahydrofuran (20 ml.) was added. The solution was stirred at −10° for 20 min. Then a solution of glycinamide hydrochloride (1.2 g.) and triethylamine (1.6 ml.) in water (10 ml.) was added. Stirring was continued without further cooling for 90 min. Water (25 ml.) was added and the reaction mixture was acidified by the slow addition of concentrated hydrochloric acid. Tetrahydrofuran was removed *in vacuo*. The product was filtered off and was washed successively with 30-ml. portions of *N* hydrochloric acid, water, 10% sodium bicarbonate, and water. After drying, the product was washed by trituration with ethyl acetate (30 ml.); wt. 3.4 g., m.p. 159–161°.

Material with this melting point (6.8 g.) was purified further by stirring in boiling water (125 ml.). After cooling, the product was filtered off; wt. 6.3 g., m.p. 161–163°, $[\alpha]_D^{22}$ −73.3° (*c* 2, 95% ethanol). Reported, m.p. 163–163.5°,[1a] 163°,[1c] 162–163°,[1d] 162–164°,[1f] $[\alpha]_D$ −73.3°[1a] (95% ethanol).

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Department of Biochemistry
Cornell University Medical College
New York 21, N. Y.

(4) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, **82**, 3359 (1960).

(5) The discrepancy in the observed melting point and that reported by Anderson and Callahan is apparently due to dimorphism. When first prepared in this laboratory, the compound melted at 119.5–120.5°. However, since the first recrystallization from acetic acid and water, the higher melting material has always been obtained. F. M. Callahan kindly agreed to recrystallize a sample of his product using seed crystals of our higher melting form. He obtained the higher melting form. When a sample of the lower melting form furnished by F. M. Callahan was recrystallized in this laboratory without the use of seed crystals, the higher melting form was obtained. Attempts in both laboratories to convert the higher melting into the lower melting form have not been successful.

Potential Growth Antagonists. II.[1] A Route to Alkyl Substituted Homoserines[2]

Irwin L. Honigberg, Louis H. Goodson and W. H. Burton

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In connection with the work on substituted glycines as metabolic antagonists,[1] an attempt was made to prepare DL-2-amino-4-hydroxy-2-methylbutyric acid (α-methylhomoserine) from 4-hydroxy-2-butanone (I) *via* the hydantoin (II), followed by hydrolysis to the amino acid (IVa). Unfortunately, the initial reaction of the hydroxy ketone with ammonium carbonate and sodium cyanide did not give the expected hydantoin, but led to an intractable oil. The reaction product also had the characteristic odor of methyl vinyl ketone, which suggests that the basicity of the reaction medium caused dehydration of the hydroxy ketone, followed by polymerization. Hays,[3] in the preparation of 4-hydroxy-2-butanone, has reported the decomposition of the ketone in the presence of base.

In the second attempt to prepare the amino acid, the commercially available 4-acetoxy-2-butanone (I. R = CH_3CO-; R_1 = H) was treated with sodium cyanide and ammonium carbonate in the hope that the rate of ammonolysis of the ester would be slower than the rate of hydantoin formation. However, only a trace of crystalline material was isolated and it had an infrared spectrum suggesting the presence of the unsaturated lactone:

$$\underbrace{CH_2—CH{=}C(CH_3)—CO}_{O}; \lambda_{max}^{Nujol}\ 5.67\ \mu\ (C{=}O)$$

(1) L. H. Goodson, I. L. Honigberg, J. J. Lehman, and W. H. Burton, *J. Org. Chem.*, Paper I, **25**, 1920(1960).

(2) This research was supported by Contract No. SA-43-ph-2394 with the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Bethesda, Md.

(3) J. T. Hays, G. F. Hager, H. M. Engelmann, and H. M. Spurlin, *J. Am. Chem. Soc.*, **73**, 5369 (1951).