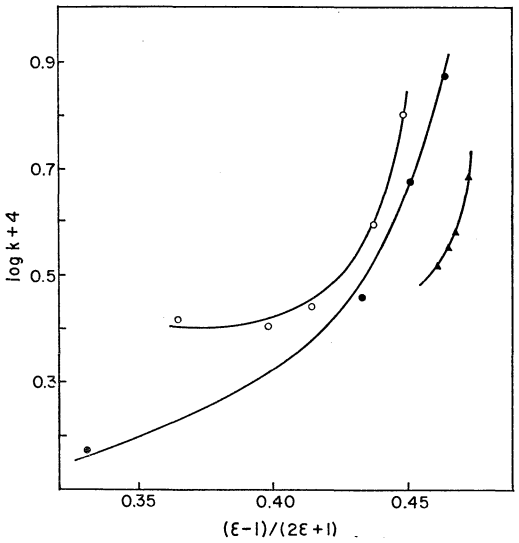

FIG. 1. ○, 1-propanol; ●, ethanol; and ▲, methanol.

activation on ascending the homologous series of alcohols may indicate the increasing amount of order necessary to effect hydrogen bonding with structurally more complex alcohols.

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DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF VICTORIA,
VICTORIA, BRITISH COLUMBIA.

Structural biochemistry. VI. Synthesis of 2′-(L-prolyl-L-prolyl)emetine[1,2]

GEORGE R. PETTIT AND S. K. GUPTA

Peptides of the ergot type[3] represent an interesting aspect of alkaloid bio-organic chemistry.[4] The occurrence of such substances in the plant kingdom is suggestive of an intermediate in alkaloid biosynthesis and (or) an evolutionary stage in alkaloid formation. Part of a sequence in alkaloid transport or involvement in enzymatic reactions are also reasonable possibilities. To study the effect of small peptide units bound to an alkaloid (originally isolated in the non-conjugated state), we undertook the preparation of the title substance. The *Cephaelis ipecacuanha* alkaloid emetine (I*a*) was selected on the basis of its free secondary amino group and its use in the treatment of, for example, amebic hepatitis (cf. ref. 1). The diprolyl unit was chosen because of its frequent occurrence in natural

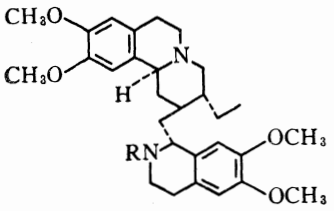
[1]For part V in this series, see G. R. Pettit and A. K. Das Gupta, Can. J. Chem., 45, 567 (1967).

[2]We are grateful to Merck and Co., Rahway, New Jersey, and to the National Science Foundation (research grant GB-4939) for support of this program.

[3]A study of acid-catalyzed isomerization involving ergot alkaloidal peptides has recently been reported (6). One member of this group (ergotamine) containing a partially transformed proline residue has been obtained by total synthesis (7). A review of the ergot peptide alkaloids has been prepared by Stoll and Hofmann (8).

[4]For recent structural studies in the field of alkaloidal peptides, consult ref. 9. The syntheses of several peptide derivatives of salsolidine have also been described recently (10).

peptide hormones[5] and the involvement of proline in the biosynthesis of certain alkaloids.[6]



I*a* R = H
I*b* R = Z-Pro-
I*c* R = *t*-butoxycarbonyl-Pro-
I*d* R = Pro-
I*e* R = *t*-butoxycarbonyl-Pro-Pro-
I*f* R = Pro-Pro-

The synthesis of 2′-(L-prolyl-L-prolyl)-emetine (I*f*) was achieved as follows. First, two routes to 2′-(L-prolyl)emetine (I*d*) were evaluated. Emetine hydrochloride was converted into the free base in tetrahydrofuran by using triethylamine, and condensed with carbobenzoxy-L-proline in the presence of dicyclohexylcarbodiimide. Proline amide I*b* was obtained in a good yield (85%), and catalytic hydrogenolysis of the carbobenzoxy protecting group gave amide I*d*, which was characterized as the hydrochloride salt. The sequence was repeated starting with emetine and *t*-butoxycarbonyl-L-proline (in methylene chloride), Sheehan's (2) 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride being used as condensing agent. Conversion into *t*-butoxycarbonyl-protected proline I*c* was also realized in a satisfactory yield (82%). Allowing amide I*c* to react with trifluoroacetic acid at room temperature for 2 h provided, after conversion of the trifluoroacetate salt into the hydrochloride derivative, the same substance (I*d* hydrochloride) obtained by using the carbobenzoxy protecting group. Experimental ease during the cleavage step allowed the selection of the *t*-butoxycarbonyl group for the next step. For this purpose, the anhydride prepared from *i*-butyl chloroformate and *t*-butoxycarbonyl-L-proline in tetrahydrofuran was allowed to react with amine I*d* in the presence of triethylamine. The resulting diprolyl peptide I*e* was also obtained by an alternate route.

By using the *N*-methylmorpholine (3) modification of the mixed-anhydride technique, *t*-butoxycarbonyl-L-proline was condensed with L-proline methyl ester in tetrahydrofuran. The oily *t*-butoxycarbonyl-L-prolyl-L-proline[7] methyl ester obtained (70% yield) by this means was saponified to give crystalline (m.p. 187–188°) *t*-butoxycarbonyl-L-prolyl-L-proline. By the water-soluble carbodiimide method, the latter dipeptide was condensed with emetine to yield (93%) dipeptide amide I*e*. The product (I*e*) was identical with the dipeptide obtained via intermediate I*c*. Removal of the protecting group from dipeptide I*e* gave, after conversion into the free base, the required dipeptide I*f* as a dihydrate melting at 133–135°.

EXPERIMENTAL

All solvents and trifluoroacetic acid were redistilled. In addition, tetrahydrofuran was passed through a column of activated alumina. L-Proline, emetine hydrochloride, and 1-ethyl-3-(3′-dimethyl aminopropyl)carbodiimide hydrochloride were used as received, respectively, from Nutritional Biochemicals Corp. (Cleveland, Ohio), Mann Research Laboratories (New York, New York), and Cyclo Chemical Corp. (Los Angeles, California). Extracts of aqueous solutions were dried over magnesium sulfate. Activated alumina refers to Merck (Rahway, New Jersey) "suitable for chromatography". Thin-layer chromatograms were prepared on microscope slides (the commercial microscope slides were ground to a uniform surface) with silica gel G (E. Merck, AG., Darmstadt, Germany). Before use, the plates were dried in a desiccator over calcium sulfate. Thin-layer chromatograms of *N*-protected proline derivatives were developed with iodine, and those of the unprotected derivatives were developed with ninhydrin.

Each analytical sample was colorless and displayed a single spot on a thin-layer chromatogram. Melting points were recorded on a Kofler melting point apparatus. Before elemental analysis, all analytical samples were redried *in vacuo* at room temperature to constant weight. Elemental microanalyses were

[5]For example, the 188-unit polypeptide of human pituitary growth hormone has a prolyl-prolyl unit (11). Refer also to Bodanszky and Lande (12) and to a review by Schwyzer (13). For other examples see ref. 14.

[6]Refer to Mothes and Schütte (15) for a useful review of alkaloid biosynthesis.

[7]Both benzyl and carbobenzoxy derivatives of L-prolyl-L-proline have been reported (16–18).

performed in the laboratory of Dr. A. Bernhardt, Max-Planck Institut, Mülheim, Germany. Optical rotatory dispersion measurements were performed by Miss K. Reimer on a JASCO ORD-UV-5 instrument at 25° in methanol. Other optical rotation measurements (at 20° in chloroform), reported at the sodium D-line, were provided by Dr. P. Demoen, Analytical Department, Janssen Laboratories, Beerse, Belgium. Infrared spectra were determined by the Nujol mull technique or (for comparison spectra) in chloroform solution.

2'-(N$^{\alpha}$-Carbobenzoxy-prolyl)emetine (Ib)

A cool (ice-bath) suspension of emetine (I*a*) hydrochloride (3.4 g) in tetrahydrofuran (40 ml) was treated with triethylamine (1.4 ml). The solution was filtered and the filtrate concentrated *in vacuo*. A cool (−10°, ice–salt bath) solution composed of the foamy residue and carbobenzoxy proline (1.25 g) was stirred (magnetic bar method) while dicyclohexylcarbodiimide (1.1 g) in 10 ml of chloroform was added. Stirring at ice-bath temperature was continued for 5 h. Excess dicyclohexylcarbodiimide was transformed by using three drops of glacial acetic acid, and dicyclohexylurea was collected. After the solution was washed successively with water, 1% aqueous sodium bicarbonate, and water, it was concentrated *in vacuo*. The residual oil crystallized from benzene – diethyl ether (1:4) as fine needles melting at 119–120°, yield 3.0 g (85%). Two recrystallizations from the same solvent gave an analytical sample, m.p. 121–122°; optical rotatory dispersion: $[\alpha]_{650}$ −20.9°, $[\alpha]_{589}$ −41.9°, $[\alpha]_{400}$ −72.8°, and $[\alpha]_{350}$ −125.7° (*c*, 1.67).

Anal. Calcd. for $C_{42}H_{53}N_3O_7$: C, 70.88; H, 7.45; N, 5.90; O, 15.75. Found: C, 70.94; H, 7.65; N, 6.00; O, 16.14.

2'-(N$^{\alpha}$-t-Butoxycarbonyl-prolyl)emetine (Ic)

To a solution of dry emetine (I*a*) hydrochloride (3.4 g) in methylene chloride (25 ml) at ice-bath temperature was added, with stirring, triethylamine (1.4 ml). Fifteen minutes later *t*-butoxycarbonyl-L-proline (1.0 g) (4) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (1.05 g) (2) were added. The resulting solution was stirred for 2 h at room temperature and then washed with water, 2% aqueous sodium bicarbonate, and water. Removal of the solvent *in vacuo* gave a foamy solid weighing 2.8 g. Recrystallization from petroleum ether gave colorless crystals melting at 133–135°. Another recrystallization from the same solvent gave an analytical sample with the same melting point, $[\alpha]_D$ −50.4° (*c*, 3.57).

Anal. Calcd. for $C_{39}H_{55}N_3O_7$: C, 69.14; H, 8.12; N, 6.20; O, 16.54. Found: C, 68.95; H, 8.30; N, 6.16; O, 16.31.

2'-(Prolyl)emetine (Id) Dihydrochloride

Method A, from 2'-(N$^{\alpha}$-Carbobenzoxy-prolyl)emetine

Hydrogen was passed through a sintered disk into a mixture composed of carbobenzoxy derivative I*b* (1.0 g) in methanol (75 ml) and suspended 10% palladium on charcoal (0.2 g) for 6 h. The evolution of carbon dioxide (exit gases were passed into a barium hydroxide solution) was essentially complete during the first 4.5 h. The solution was filtered and the filtrate concentrated to a foamy solid weighing 0.60 g, m.p. 77–79°. A solution of the base in benzene (20 ml) was treated with ethereal hydrogen chloride to yield 0.65 g of a powder melting at 215–217° (sintering at 210°). Two recrystallizations from methanol – diethyl ether gave a pure sample, m.p. 215–216°; thin-layer chromatography solvent, methanol; $[\alpha]_D$ −46.3° (*c*, 4.60).

Anal. Calcd. for $C_{34}H_{49}Cl_2N_3O_5 \cdot H_2O$: C, 61.07; H, 7.65; Cl, 10.62; N, 6.28. Found: C, 61.53; H, 7.80; Cl, 10.22; N, 6.08.

Attempts to recrystallize the crude free base (and again after the base was chromatographed on activated alumina) were unsuccessful. Generally, a viscous oil was obtained which, when left in a vacuum, would eventually yield a foamy solid melting at 77–79°.

Method B, from 2'-(N$^{\alpha}$-t-Butoxycarbonyl-prolyl)-emetine

A solution of *t*-butoxycarbonyl derivative I*c* (2.0 g) in trifluoroacetic acid (10 ml) was allowed to stand at room temperature for 2 h. The remaining trifluoroacetic acid was removed *in vacuo*, and a solution of the oily residue in water (5 ml) was neutralized with cold aqueous sodium hydroxide. A benzene (2 × 50 ml) extract of the aqueous mixture was washed with water and concentrated to approximately 10 ml. After the addition of ethereal hydrogen chloride, the solid (1.0 g) was collected and recrystallized from chloroform – diethyl ether to yield the hydrochloride derivative of alkaloid I*d* melting at 215–220°. Two recrystallizations from methanol – diethyl ether gave an analytical sample of fine crystals, m.p. 220–222°; thin-layer chromatography solvent, 5:1:4 *n*-butanol – acetic acid – water; optical rotatory dispersion: $[\alpha]_{650}$ +8.5°, $[\alpha]_{589}$ +19.2°, and $[\alpha]_{400}$ +29.1° (*c*, 11.68).

Anal. Found: C, 61.23; H, 7.70.

The substance (I*d* dihydrochloride monohydrate) obtained by method B was identical with that prepared by method A, as evidenced by comparison infrared spectra, thin-layer chromatography, and mixture melting point determination (mixture melting at 220–222°).

2'-(N$^{\alpha}$-t-Butoxycarbonyl-prolyl-prolyl)emetine (Ie)

Method A

To a cool (−5°, ice–salt bath) solution composed of tetrahydrofuran (10 ml), *t*-butoxycarbonyl-L-proline (0.27 g), and triethylamine (0.2 ml) was added *i*-butyl chloroformate (0.2 g). After 10 min emetine derivative I*d* (0.72 g) in tetrahydrofuran (10 ml) was added, and stirring was continued for 2 h at −5° and for 3 h at room temperature. The solvent was then removed, and the residue was dissolved in benzene (50 ml) and successively washed with water, 1% aqueous sodium bicarbonate, and water. The benzene solution was chromatographed on activated alumina. Elution with benzene gave a colorless foam

which recrystallized from benzene – petroleum ether as colorless crystals, m.p. 133–136°, weighing 0.20 g. Two recrystallizations from benzene–hexane gave an analytical sample melting at 133–135°; thin-layer chromatography solvent, 4:1 chloroform–methanol.

Anal. Calcd. for $C_{44}H_{62}N_4O_8$: C, 68.19; H, 8.06; N, 7.23; O, 16.51. Found: C, 68.04; H, 8.21; N, 7.14; O, 16.34.

Method B

Triethylamine (1.4 ml) was added to a solution of dry emetine hydrochloride (3.4 g) in methylene chloride (35 ml). The solution was cooled in an ice bath, and *t*-butoxycarbonyl-L-prolyl-L-proline (1.7 g) was added. The dipeptide was prepared as described in the experiment outlined below. Stirring was continued for 15 min, and 1-ethyl-3-(3′-dimethylaminoprolyl)carbodiimide hydrochloride (1.6 g) was added. Stirring was continued at ice-bath temperature for 1 h and at room temperature for 1.5 h. Methylene chloride (50 ml) was added, and the solution was washed with water, 1% aqueous sodium bicarbonate, and water. Removal of the solvent *in vacuo* gave an oil which solidified upon trituration with petroleum ether. After being washed with petroleum ether and dried, the solid weighed 3.6 g (93%) and melted at 122–125°. Recrystallization from diethyl ether (Norit A) – petroleum ether gave 3.0 g of colorless crystals, m.p. 125–126°. Two recrystallizations from the same solvent led to a pure specimen melting at 131–133°; thin-layer chromatography solvent, 4:1 chloroform–methanol; optical rotatory dispersion: $[\alpha]_{650}$ −52.3°, $[\alpha]_{589}$ −104.7°, and $[\alpha]_{450}$ −128.6° (*c*, 2.1).

Anal. Calcd. for $C_{44}H_{62}N_4O_8$: C, 68.19; H, 8.06; N, 7.23; O, 16.51. Found: C, 68.04; H, 7.97; N, 7.24; O, 16.56.

The samples of dipeptide I*e* prepared by methods A and B were found (by comparison infrared spectra and thin-layer chromatograms) to be identical.

t-Butoxycarbonyl-prolyl-proline

To a cool (ice-bath) solution of *t*-butoxycarbonyl-L-proline (4.3 g) in tetrahydrofuran (50 ml) was added *N*-methylmorpholine (2.4 ml). The solution was cooled to −10° and *i*-butyl chloroformate (3.0 g) was added, with stirring. Stirring was continued for 10 min, and a cold (ice-bath) solution prepared from dimethylformamide (25 ml), L-proline methyl ester hydrochloride (3.6 g) (5), and *N*-methylmorpholine (2.2 ml) was added. The mixture was stirred at −5° for 4 h and at room temperature for 1 h. The solid which separated was collected and the filtrate evaporated *in vacuo*. A solution of the oily residue in ethyl acetate (300 ml) was successively washed with water, 1% aqueous citric acid, water, 1% aqueous sodium bicarbonate, and water. Removal of the solvent *in vacuo* gave the dipeptide methyl ester as an oil, yield 4.4 g (70%). Although this substance gave one spot on a thin-layer chromatogram (4:1 benzene–methanol as solvent), attempts at crystallization were unsuccessful.

A solution of the methyl ester (4.2 g) in methanol (25 ml) – water (5 ml) containing sodium hydroxide (2.0 g) was allowed to remain at room temperature for 1.5 h. After dilution with water (50 ml) and extraction with ethyl acetate (2 × 25 ml), the organic phase was discarded. The aqueous portion was cooled, acidified to pH 2 with citric acid, saturated with sodium chloride, and extracted with chloroform (3 × 50 ml). The combined chloroform extracts were washed with water and concentrated to an oily residue which crystallized from ethyl acetate – petroleum ether as colorless cubic crystals weighing 2.4 g (60%), m.p. 180–181°. Two recrystallizations from ethyl acetate gave an analytical sample melting at 187–187.5°; thin-layer chromatography solvent, 5:1:4 *n*-butanol – acetic acid – water; optical rotatory dispersion: $[\alpha]_{650}$ −91.4°, $[\alpha]_{589}$ −171°, and $[\alpha]_{400}$ −279.2° (*c*, 1.86).

Anal. Calcd. for $C_{15}H_{24}N_2O_5$: C, 57.72; H, 7.68; N, 8.97. Found: C, 57.90; H, 7.77; N, 8.91.

2′-(Prolyl-prolyl)emetine (If)

A 1.8 g sample of dipeptide I*e* was allowed to react with trifluoroacetic acid (20 ml) for 2 h at room temperature. The solution was diluted with diethyl ether (100 ml) and cooled. The solid which separated was collected and washed several times with diethyl ether. A solution of the trifluoroacetate derivative in water (25 ml) was cooled and neutralized with ammonium hydroxide. The oil which separated was extracted with benzene, and the combined extracts were washed with water. Removal of the solvent gave 0.90 g (60%) of a colorless foam; two recrystallizations from diethyl ether – pentane yielded pure crystals of the dihydrate, m.p. 133–135°; thin-layer chromatography solvent, 5:1:4 *n*-butanol – acetic acid – water; optical rotatory dispersion: $[\alpha]_{650}$ −56.4°, $[\alpha]_{589}$ −120.9°, and $[\alpha]_{450}$ −145.2° (*c*, 0.62).

Anal. Calcd. for $C_{39}H_{54}N_4O_6 \cdot 2H_2O$: C, 65.89; H, 8.22; N, 7.88. Found: C, 65.65, 65.81; H, 7.78, 7.89; N, 7.36.

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DEPARTMENT OF CHEMISTRY,
ARIZONA STATE UNIVERSITY,
TEMPE, ARIZONA.