The Nitro Enol Ether 4-Nitro-1-cyclohexyl-3-ethoxy-2-oxo-3-pyrroline. Synthesis and Use as a Reagent for Amino Group Protection[1]

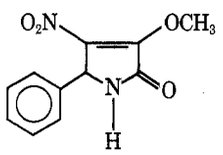
Philip L. Southwick,* Richard F. Dufresne, and John J. Lindsey

Department of Chemistry, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213

Received June 3, 1974

The nitro enol ether 4-nitro-1-cyclohexyl-3-ethoxy-2-oxo-3-pyrroline (IV) was prepared from enolic 4-carbethoxy-1-cyclohexyl-2,3-dioxopyrrolidine by a three-step sequence in which the final reaction was direct nitration of 1-cyclohexyl-3-ethoxy-2-oxo-3-pyrroline-4-carboxylic acid. The enol ether IV served as an active reagent for the attachment of the 4-nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl group (NOPY group) to amino functions, from which it could be removed by the action of ammonia at room temperature. The possibility of using the NOPY group for the protection of amino groups during synthesis was demonstrated in the preparation of two simple dipeptide esters.

It was recently shown that the nitro enol ether 3-methoxy-4-nitro-5-phenyl-2-oxo-3-pyrroline (I) reacts under



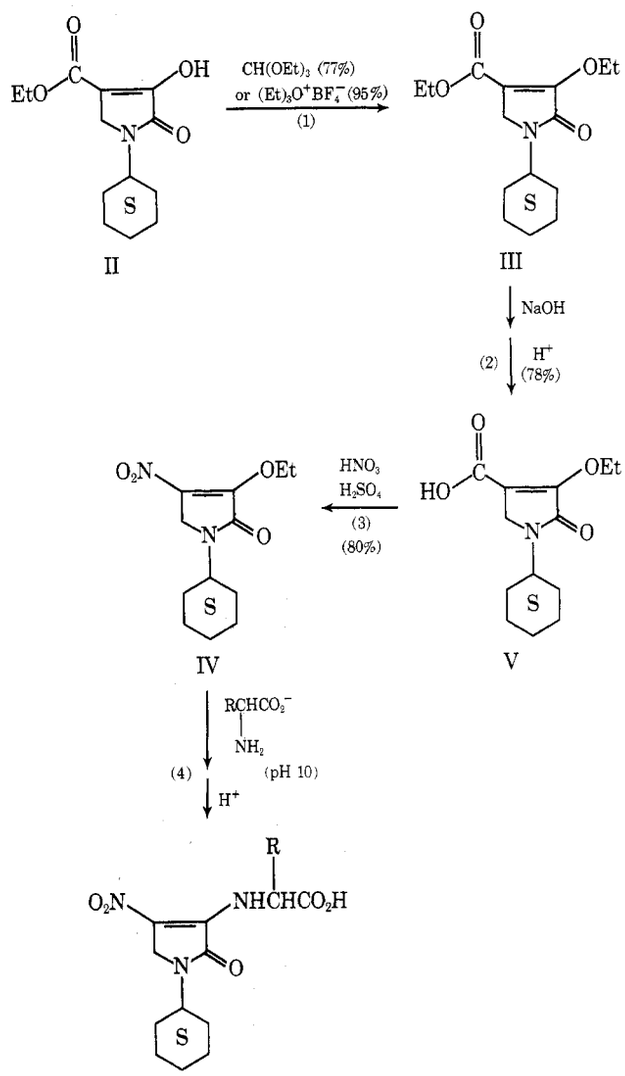
I

mild conditions with ammonia and primary or secondary amines to undergo replacement of the methoxy group by an amino group or substituted amino group.[2] In order to investigate the possible utility of nitro enol ethers in synthetic applications it was desirable to obtain a representative compound of that type which was more easily prepared and which, unlike compound I, did not contain a chiral center to contribute toward unwanted stereochemical complications. The work described here was concerned with the synthesis and reactions of such a compound, 4-nitro-1-cyclohexyl-3-ethoxy-2-oxo-3-pyrroline (IV).

The scheme used for the synthesis of IV is outlined in Chart I. The readily available enol of 1-cyclohexyl-4-carbethoxy-2,3-dioxopyrrolidine (II)[3] was alkylated at the enolic hydroxyl either by use of ethyl orthoformate or triethyloxonium fluoroborate. The resulting ethyl enol ether (III) was then saponified with sodium hydroxide to yield 1-cyclohexyl-3-ethoxyl-2-oxo-3-pyrroline-4-carboxylic acid (V). Direct nitration of the latter compound with a mixture of fuming nitric and concentrated sulfuric acids at 0° resulted in introduction of the nitro group at the 4 position with loss of the carboxyl group through decarboxylation. The yields in the several steps are indicated on the chart; they were good enough to make feasible the preparation of IV in the desired amounts.

In order to test in a preliminary way the possibility that compound IV might be a useful reagent in such applications as protein modification or peptide synthesis, reactions with a number of amino acids were investigated. It was found that the amino group of such compounds reacted readily with compound IV at room temperature in aqueous solutions at pH 10 (borate buffer) to which some acetonitrile was added to increase the solubility of IV. The products were acidic substances which yielded analytical and spectroscopic data completely consistent with assignment of their structures as those of *N*-(4-nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl) derivatives (NOPY derivatives) of the amino acids (VI), formed by replacement of the ethoxyl group as in eq 4. The products of type VI were stable crystalline compounds. Specific rotations of these derivatives were higher than those of the incorporated amino acids in the two instances examined.

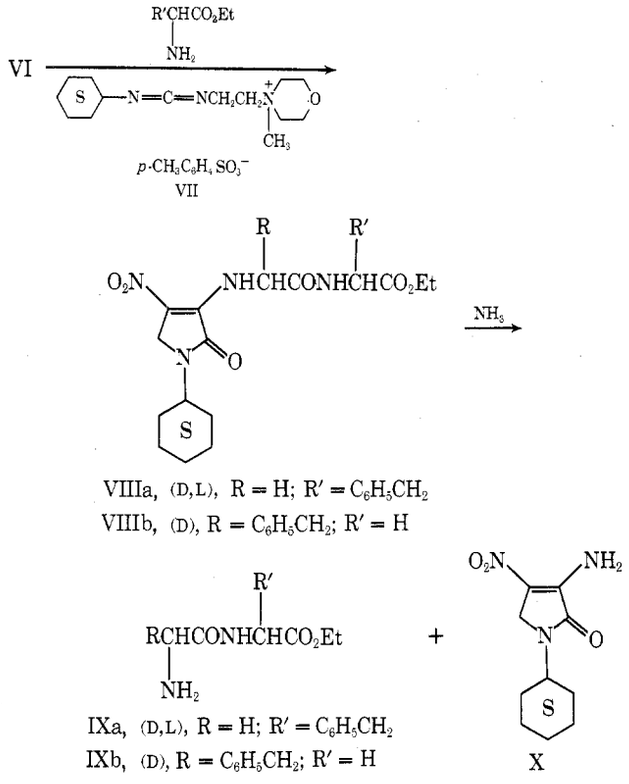
Chart I



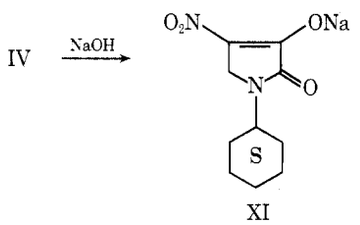
Two simple examples of dipeptide synthesis were chosen to test the possible utility of the 4-nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl (NOPY) group as a removable protecting group for amino functions. Both ethyl glycyl-D,L-phenylalaninate (IXa) and ethylL-phenylalanylglycinate

(IXb) were obtained from the appropriate NOPY-protected amino acids (VIa and VIc) by simple procedures in which coupling with an amino acid ester was conducted with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (VII), and removal of the protecting group was accomplished by treatment with ammonia in acetonitrile at room temperature, as indicated in Chart II. The NOPY dipeptides (VIII) were easily separated from the water-soluble urea derivative formed from VII and were obtained in yields on the order of 75–80%. Cleavage of the NOPY derivatives VIII with ammonia in acetonitrile occurred smoothly and produced the known dipeptide ethyl esters (IXa and IXb) (characterized as hydrobromides). The yield of IXa was 73%; that of IXb was 88%. The other cleavage product was the enamine X. If racemization occurred during coupling of the L-phenylalanine derivative VIc to yield VIIIb or during the cleavage of VIIIb to yield IXb, the extent of it was not sufficient to cause any difficulty in purifying the hydrobromide of IXb to give a specific rotation very close to the value recorded in the literature for that substance. The literature concerning other amino protecting groups removable under basic conditions has been reviewed recently by Carpino and Han.[4] Applications in protein modification for NOPY ethyl ether (IV) may perhaps be foreseen in view of results reported with the somewhat analogous reversible blocking reagent, 2-methoxy-5-nitrotropone.[5]

Chart II



The nitro enol ether IV underwent hydrolysis readily when treated with aqueous sodium hydroxide to yield a stable and easily purified sodium salt (XI) of the corresponding nitro enol. The sharply contrasting behavior of enol ethers III and IV toward sodium hydroxide is testimony to the strong electronic effect of the nitro group in this system.



Experimental[6] Section

1-Cyclohexyl-3-ethoxy-2-oxo-3-pyrroline-4-carboxylic Acid (V). A. Ethylation of II with Triethyloxonium Fluoroborate. Triethyloxonium fluoroborate was prepared by the method of Meerwein[7] and stored under anhydrous ethyl ether at 5°. Just prior to use, it was collected by filtration, washed with anhydrous ethyl ether, and dried on the filter under an atmosphere of dry nitrogen. 4-Carbethoxy-1-cyclohexyl-2,3-dioxopyrrolidine (II) was prepared by the method of Southwick, *et al.*,[3] and dried overnight in a vacuum desiccator over Drierite. Triethylamine was distilled and dried over potassium hydroxide pellets. A solution of 48.6 g (0.192 mol) of II and 20.2 g (0.20 mol) of triethylamine in 350 ml of methylene chloride was blanketed with dry nitrogen gas and cooled to −5° in an ice–salt bath. To this solution was added rapidly 76.0 g (0.40 mol) of the white crystalline triethyloxonium fluoroborate. After the pale-yellow solution had been stirred for 45 min at ice-bath temperature, it was extracted with two 75-ml portions of 5% aqueous sodium hydroxide, then with pH 7 phosphate buffer solution until the extracts remained neutral. The methylene chloride solution was dried ($MgSO_4$), filtered, and concentrated in a rotary evaporator over a steam bath. The crude product, 4-carbethoxy-1-cyclohexyl-3-ethoxy-2-oxo-3-pyrroline (III), was obtained in a yield of 51.5 g (95%) as a pale-yellow oil which showed no tendency to crystallize. Spectra: ir (liquid film) 3.33, 3.38, and 3.48 (cyclohexyl C–H), 5.82 (ester C=O), 5.91 (lactam C=O), 6.09 (C=C), 6.89, 7.10, 7.22, 7.40, 8.13 (broad), 9.52 (broad), 11.21, 13.10 μ (broad); nmr ($CDCl_3$) τ 5.14 (q, 2, ethyl $-CH_2-$), 5.63 (q, 2, ethyl $-CH_2-$), 5.50–6.40 (broad, 1, cyclohexyl C–H), 5.89 (s, 2, C-5 $-CH_2-$), 7.90–9.20 (m, broad, 10, cyclohexyl $(CH_2)_5$), 8.58 (t, 3, ethyl CH_3), 8.62 (t, 3, ethyl CH_3).

B. Ethylation of II with Ethyl Orthoformate. Compound II (126.7 g, 0.50 mol) with dissolved in 106 ml (94.5 g, 0.64 mol) of triethyl orthoformate and 70 ml of dimethylformamide containing a few crystals of *p*-toluenesulfonic acid. The mixture was heated and stirred for 16 hr in a distillation apparatus having a 12-in. column packed with steel helixes. During the reaction period heat input was adjusted so that ethyl orthoformate refluxing from the mixture condensed just within a Claisen distillation head fitted above the column while ethyl formate and ethanol distilled. The mixture was then concentrated under reduced pressure (aspirator) in a rotary evaporator over a steam bath, and the residue (136.4 g) was distilled. The product (yield 109 g (77.6%)) was a straw-colored oil which distilled between 170 and 175° at *ca.* 7 mm of pressure.

C. Saponification of III. A solution in 250 ml of absolute ethanol of 32 g (0.114 mol) of the ethylation product III from either procedure A or B was concentrated to a total volume of 125 ml by distillation under a nitrogen atmosphere in order to remove dissolved oxygen. In a similar manner, a 10% solution of sodium hydroxide in 80 ml of water was concentrated under nitrogen to one-half the original volume. The deoxygenated solutions were combined and stirred overnight at room temperature while blanketed with a nitrogen atmosphere. The deep-red reaction mixture which resulted was diluted to 800 ml with water and extracted twice with 75-ml portions of methylene chloride. The aqueous solution was treated with activated carbon (Norit) and filtered through a Celite filter cake. The gold-colored filtrate was made strongly acidic with 20% aqueous hydrochloric acid to precipitate the product. The suspension was stored at 5° until the precipitate had settled. The solid was collected by filtration and recrystallized from 95% ethanol to yield 22.8 g (79%) of V; mp 154–156°. A second recrystallization from 95% ethanol provided V as white prisms; mp 155–156°. Spectra: ir (Nujol) 5.81 (carboxyl C=O), 5.97 (lactam C=O), 6.11 (C=C), 6.75, 7.24, 8.04, 8.38, 8.65, 9.02, 9.36, 12.98, 13.10, 14.24 μ; nmr ($CDCl_3$) τ 6.05 (q, 2, ethyl $-CH_2-$), 5.60–6.10 (broad, 1, cyclohexyl C–H), 5.84 (s, 2, C-5 $-CH_2-$), 7.80–9.20 (m, broad, 10, cyclohexyl $(CH_2)_5$), 8.54 (t, 3, ethyl CH_3); uv (95% EtOH) 246 nm (ϵ 10,900).

Anal. Calcd for $C_{13}H_{19}O_4N$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.53; H, 7.62; N, 5.35.

4-Nitro-1-cyclohexyl-3-ethoxy-2-oxo-3-pyrroline (IV). A solution of 12.0 g (0.048 mol) of 1-cyclohexyl-3-ethoxy-2-oxo-3-pyrroline-4-carboxylic acid (V) in 150 ml of concentrated sulfuric

acid was cooled to −5° in an ice–salt bath. A solution of 10 ml of white fuming nitric acid (90%) and 10 ml of concentrated sulfuric acid was added from a dropping funnel to the reaction mixture at a rate such that the temperature did not exceed 0°. The clear, yellow-colored solution was stirred for 4 hr at −5°. After 15 min gas evolution from the stirred solution became evident. The rate of evolution gradually increased to a maximum after *ca.* 1 hr and then slowly subsided to a virtual halt by the end of the 4-hr reaction period. The solution was slowly poured into 2 l. of ice–water with vigorous stirring. The suspension which resulted was stored overnight at 5° to allow the precipitate to settle. The pale-yellow solid was collected by filtration, washed with water to remove acid, and dried on the filter. Recrystallization from 95% ethanol yielded 9.60 g (80%) of IV as yellow needles; mp 88–90°. An analytical sample of IV, mp 89–90°, was prepared by a second recrystallization from 95% ethanol. Spectra: ir (Nujol) 5.92 (lactam C=O), 6.17 (C=C), 6.70 (conj NO_2), 7.13, 7.25, 7.35, 7.72, 8.01, 8.29, 8.40, 11.31 μ; nmr ($CDCl_3$) τ 4.83 (q, 2, ethyl $-CH_2-$), 5.57 (s, 2, C-5 $-CH_2-$), 5.65–6.10 (broad, 1, cyclohexyl C–H), 7.50–9.00 (m, 10, cyclohexyl $(CH_2)_5$), 8.47 (t, 3, ethyl CH_3); uv (95% EtOH) 297 nm (ϵ 8800).

Anal. Calcd for $C_{12}H_{18}O_4N_2$: C, 56.68; H, 7.14; N, 11.02. Found: C, 56.85; H, 7.02; N, 11.05.

Sodium 4-Nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-oxide (XI). A suspension of 1.00 g (3.9 mmol) of IV in 20 ml of 20% aqueous sodium hydroxide solution was stirred at room temperature overnight. The starting material slowly dissolved to give a bright yellow solution and then the product began to precipitate as a fluffy tan solid. The suspension was filtered to collect the precipitate, which was dried on the filter and recrystallized from water to give 0.84 g (86%) of XI as pale-yellow plates. Two additional recrystallizations from water provided an analytical sample of XI; mp 163° (softens to give a gel). The product gave a weak ferric chloride test (brown) in aqueous solution. Spectra: ir (Nujol) 5.85 (lactam C=O), 6.04 (C=C), 7.10 (conj NO_2), 7.60, 7.90, 8.40, 9.56, 10.00, 10.63 μ; nmr (DMSO-d_6) τ 5.92 (s, 2, C-5 $-CH_2-$), 5.80–6.35 (broad, 1, cyclohexyl C–H), 8.00–9.30 (m, 10, cyclohexyl $(CH_2)_5$); uv (H_2O) 363 nm (ϵ 17,300).

Anal. Calcd for $C_{10}H_{13}O_4N_2Na$: C, 48.41; H, 5.24; N, 11.29. Found: C, 48.59; H, 5.48; N, 11.09.

***N*-(4-Nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-amino Acid Derivatives (VI). General Procedure.** To a solution of 6.0 mmol of the amino acid in 40 ml of a pH 10.0 borate buffer solution (VWR Scientific) was added 1.02 g (4.0 mmol) of the nitro enol ether IV and 8 ml of acetronitrile. The suspension was stirred at room temperature until the starting material had dissolved to give a clear bright-yellow solution (*ca.* 30 min usually sufficed). An aliquot was then withdrawn from the solution, diluted with 95% ethanol, and its uv spectrum recorded. Completion of the reaction was indicated by the appearance of an absorption maximum at *ca.* 380 nm and the disappearance of that at 290 nm (due to unchanged IV). After a total period of 1 hr at room temperature, the reaction mixture was diluted to 100 ml with water. The aqueous solution was made strongly acidic (pH <2) with 20% aqueous hydrochloric acid, the resulting suspension was stirred vigorously at room temperature until the precipitated material solidified, and the mixture was then stored at 5° until the solid had settled. The precipitate was collected by filtration, recrystallized, and characterized as indicated for the following individual examples.

***N*-(4-Nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-glycine (VIa).** The crude product obtained from the reaction of glycine with 1.02 g (4.0 mmol) of IV was recrystallized from 95% ethanol to give 1.07 g (94%) of VIa as fine pale-yellow needles, mp 180–183°. A second recrystallization from 95% ethanol provided an analytical sample of VIa, mp 185–186° dec. Spectra: ir (Nujol) 2.98 (enamine N–H), 5.69 (acid C=O), 5.90 (lactam C=O), 6.01 (C=C), 6.80 (conj NO_2), 7.22 (broad), 7.79, 7.92, 8.03, 8.30 (broad), 8.97, 13.24 μ; nmr ($CDCl_3/CF_3CO_2H$) τ 1.20–1.80 (broad, 1, enamine N–H), 5.21 (d, J = 7 Hz, 2, glycine $-CH_2-$), 5.68 (s, 2, C-5 $-CH_2-$), 5.70–6.35 (broad, 1, cyclohexyl C–H), 8.75–9.05 (m, 10, cyclohexyl $(CH_2)_5$); uv (95% EtOH) 370 nm (ϵ 16,600).

Anal. Calcd for $C_{12}H_{17}O_5N_3$: C, 50.88; H, 6.05; N, 14.83. Found: C, 50.60; H, 5.99; N, 14.79.

***N*-(4-Nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-D,L-phenylalanine (VIb).** Recrystallization of the crude product from an ethanol–water mixture yielded 1.42 g (95%) of VIb as pale-yellow, very fine needles; mp 147–149°. An analytical sample, mp 148–149.5° dec, was obtained by two additional recrystallizations from ethanol–water. Spectra: ir (Nujol) 2.75, 2.89 (enamine N–H and acid O–H), 5.76 (acid C=O), 5.84 (lactam C=O), 6.01 (C=C), 6.86 (conj NO_2), 7.13, 7.39, 7.50, 7.80 (broad), 7.90, 8.44, 9.11, 13.26, 13.80 (broad), 14.13 μ (broad); nmr ($CDCl_3/CF_3CO_2H$) τ 1.36–1.82 (d, broad, 1, enamine N–H), 2.44 (s, 5, C_6H_5), 3.65–4.20 (m, broad, 1, phenylalanine C–H), 5.67 (s, 2, C-5 $-CH_2-$), 5.50–6.20 (broad, 1, cyclohexyl C–H), 6.40–7.00 (m, 2, phenylalanine $-CH_2-$), 7.76–9.30 (m, 10, cyclohexyl $(CH_2)_5$); uv (95% EtOH) 373 nm (ϵ 15,600).

Anal. Calcd for $C_{19}H_{23}O_5N_3$: C, 61.11; H, 6.21; N, 11.25. Found: C, 60.99; H, 5.98; N, 11.16.

***N*-(4-Nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-L-phenylalanine (VIc).** The crude product from the reaction of 2.04 g (8 mmol) of IV with L-phenylalanine was recrystallized from carbon tetrachloride to yield 2.65 g (89%) of VIc as clusters of very fine, pale-yellow needles: mp 153–156°. A second recrystallization from carbon tetrachloride provided an analytical sample of VIc; mp 155–156° dec. Spectra: ir (Nujol) 3.00, 3.27 (broad) (enamine N–H and acid O–H), 5.71 (acid C=O), 5.90 (lactam C=O), 6.03 (C=C), 6.90 (conj NO_2), 7.15 (broad), 7.44, 7.84, 7.93, 8.39, 9.10, 13.29, 14.42 μ (broad); nmr ($CDCl_3$) τ 0.95 (broad, 1, OH), 1.50–2.00 (d, broad, 1, enamine N–H), 2.70 (s, 5, C_6H_5), 3.75–4.30 (m, broad, 1, phenylalanine C–H), 5.88 (s, 2, C-5 $-CH_2-$), 5.70–6.30 (broad, 1, cyclohexyl C–H), 6.40–7.00 (m, 2, phenylalanine $-CH_2-$), 7.60–9.10 (m, 10, cyclohexyl $(CH_2)_5$); uv (95% EtOH) 373 nm (ϵ 15,800); $[\alpha]^{25}D$ −164° (c 2, 95% EtOH).

Anal. Calcd for $C_{19}H_{23}O_5N_3$: C, 61.11; H, 6.21; N, 11.25. Found: C, 60.88; H, 6.35; N, 10.98.

***N*-(4-Nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-L-leucine (VId).** Recrystallization of the crude product from 95% ethanol yielded 1.44 g (93%) of VId as bright green-yellow needles; mp 81–86°. A second recrystallization gave an analytical sample; mp 86–87°. The nmr and analysis both showed the presence of ethanol of crystallization in a ratio of 1 mol of ethanol per mol of VId. Spectra: ir (Nujol) 2.97 (broad) (enamine N–H and acid O–H), 5.81 (acid C=O), 5.91 (lactam C=O), 6.02 (C=C), 6.90 (broad) (conj NO_2), 7.18, 7.22, 7.73, 8.07, 8.25 μ; nmr ($CDCl_3$) τ 1.25–1.86 (d, broad, 1, enamine N–H), 1.95 (s, 2, OH's), 3.83–4.50 (m, broad, leucine C–H), 5.61 (s, 2, C-5 $-CH_2-$), 5.40–6.31 (broad, 1, cyclohexyl C–H), 6.16 (q, 2, ethanol $-CH_2-$), 7.30–9.35 (m, 22, cyclohexyl $(CH_2)_5$, leucine $CH_2-CH(-CH_3)_2$, and ethanol CH_3); uv (95% EtOH) 272 nm (ϵ 7200), 372 nm (ϵ 17,100); $[\alpha]^{25}D$ +22.4° (c 2, 95% EtOH) (based upon molecular weight without ethanol of crystallization).

Anal. Calcd for $C_{16}H_{25}O_5H_3 \cdot C_2H_5OH$: C, 56.09; H, 8.11; N, 10.90. Found: C, 56.00; H, 7.98; N, 11.08.

Ethyl *N*-(4-Nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-glycyl-D,L-phenylalaninate (VIIIa). To a suspension of 1.12 g (4.0 mmol) of *N*-(4-nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-glycine (VIa) in 40 ml of methylene chloride was added 0.76 g (4.0 mmol) of ethyl D,L-phenylalaninate.[8] The starting materials dissolved to give a clear yellow solution. The solution was cooled to 0° in an ice bath and 1.70 g (4.0 mmol) of 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (VII)[9] was added. The mixture was stirred at 0° for 40 min while the by-product urea began to precipitate. The reaction mixture was then allowed to warm slowly to room temperature and stirred overnight. After 18 hr at room temperature the reaction mixture was filtered, and the filtrate was extracted with two 10-ml portions of 1% aqueous hydrochloric acid and two 25-ml portions of water to remove the urea by-product. The methylene chloride solution was dried ($MgSO_4$), filtered, and concentrated in a rotary evaporator over a steam cone. The evaporation was continued at room temperature until the residue solidified. The solid was recrystallized from 95% ethanol to yield 1.38 g (76%) of VIIIa as pale-yellow, very fine crystals; mp 123–128°. A second recrystallization from 95% ethanol provided an analytical sample of VIIIa; mp 129–130°. Spectra: ir (Nujol) 2.99 (NH), 5.77 (ester C=O), 5.86 and 5.99 (amide C=O), 6.03 (C=C), 6.50 (amide), 6.91 (conj NO_2), 7.17, 7.71, 7.90, 8.28, 8.89, 9.12, 9.65, 10.34, 13.22, 14.30 μ; nmr ($CDCl_3$) τ 0.80–1.35 (broad, 1, enamine N–H), 2.45 (s, 5, C_6H_5), 2.50–3.00 (m, 1, amide N–H), 4.60–5.10 (m, 1, phenylalanine C–H), 5.13 (d, J = 7 Hz, 2, glycine $-CH_2-$), 5.34–6.10 (m, 5, C-5 $-CH_2-$, ethyl ester $-CH_2-$, and cyclohexyl C–H), 6.72 (d, J = 6 Hz, 2, phenylalanine $-CH_2-$), 7.40–8.90 (m, 10, cyclohexyl $(CH_2)_5$), 8.70 (t, 3, ethyl ester CH_3); uv (95% EtOH) 370 nm (ϵ 17,000).

Anal. Calcd for $C_{23}H_{30}N_4O_6$: C, 60.25; H, 6.60; N, 12.22. Found: C, 60.01; H, 6.63; N, 12.10.

Ethyl *N*-(4-Nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-L-phenylalanylglycinate (VIIIb). To a solution of 1.49 g (4.0 mmol) of *N*-(4-nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-L-phenylalanine in 40 ml of methylene chloride, maintained at 0° by cooling in an ice bath, was added 0.44 g (4.2 mmol) of ethyl glycinate.[8] A bulky precipitate (probably the carboxylate salt) formed

almost immediately. To the cold suspension was added 1.70 g (4.0 mmol) of 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate. The starting materials first dissolved and then the by-product urea began to precipitate. The reaction mixture was stirred overnight while allowing it to warm slowly to room temperature. After a total period of 20 hr the suspension was filtered and extracted with three 100-ml portions of water. The methylene chloride solution was dried ($MgSO_4$), filtered, and concentrated in a rotary evaporator over a steam cone. The evaporation was continued at room temperature until the residue solidified. The residue was recrystallized from carbon tetrachloride to give 1.49 g (82%) of VIIIb; mp 61–65° (softens to a gel). Two additional recrystallizations from carbon tetrachloride provided an analytical sample of VIIIb; mp 62–63° (softens). Spectra: ir (Nujol) 2.99 (NH), 5.70 (ester C=O), 5.95 (amide C=O), 6.04 (C=C), 6.47 (amide), 6.85 (broad) (conj NO_2), 7.25 7.62, 7.82 (broad, 8.30 (broad), 9.65 μ (broad); nmr ($CDCl_3$) τ 1.40–1.85 (broad, 1, enamine N–H), 2.74 (s, 5, C_6H_5), 2.95–3.35 (broad, 1, amide N–H), 3.80–4.40 (m, 1, phenylalanine C–H), 5.52–6.30 (m, 7, glycine $-CH_2CH-$, C-5 $-CH_2-$, ethyl ester $-CH_2-$, and cyclohexyl C–H), 6.50–7.00 (m, 2, phenylalanine $-CH_2-$), 7.60–9.00 (m, 10, cyclohexyl $(CH_2)_5$), 8.73 (t, 3, ethyl ester CH_3); uv (95% EtOH) 373 nm (ϵ 15,900); $[\alpha]^{25}D$ −164° (*c* 2, 95% EtOHz.

Anal. Calcd for $C_{23}H_{30}O_6N_4$: C, 60.25; H, 6.60; N, 12.22. Found: C, 60.04; H, 6.46; N, 12.06.

Ammonia Cleavage of Ethyl *N*-(4-Nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-glycyl-D,L-phenylalaninate (VIIIa). Preparation of Ethyl Glycyl-D,L-phenylalaninate Hydrobromide (IXa). Anhydrous ammonia gas was bubbled slowly with stirring into a solution of 0.92 g (2.0 mmol) of ethyl *N*-(4-nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-glycyl-D,L-phenylalaninate (VIIIa) in 20 ml of acetonitrile. After 2 hr, the addition of ammonia was discontinued, the reaction flask was stoppered loosely, and the solution was stirred at room temperature. The progress of the reaction was monitored by withdrawing aliquots of the solution periodically and determining the wavelength of maximum absorptivity in the uv. Over a 24-hr period the absorption maximum gradually shifted from 370 nm (due to the starting material VIIIa) to 352 nm (due to the by-product 4-nitro-1-cyclohexyl-3-amino-2-oxo-3-pyrroline (X)). After 24 hr the acetonitrile solution was poured slowly into 200 ml of dilute hydrochloric acid and crushed ice to give an acidic suspension (pH *ca.* 2) from which most of the by-product X precipitated. The solid was removed by filtration in a yield of 0.41 g (91%) of crude 4-nitro-1-cyclohexyl-3-amino-2-oxo-3-pyrroline (X); mp 181–190° (*vide infra*). After the precipitated X had been filtered out, the acidic aqueous filtrate was extracted with three 30-ml portions of methylene chloride to remove the last traces of X. The pH of the aqueous solution was adjusted successively to pH 7–8, 8–9, and 9–12 by the stepwise addition of portions of 3% aqueous sodium hydroxide. Following the addition of each increment of sodium hydroxide, the aqueous solution was extracted with two 20-ml portions of methylene chloride. The combined methylene chloride extracts were dried ($MgSO_4$), filtered, and concentrated in a rotary evaporator over a steam bath to give ethyl glycyl-D,L-phenylalaninate (IXa) as a pale-yellow oil. The identification of this product as IXa was supported by its nmr spectrum. Spectra: nmr ($CDCl_3$) τ 1.80–2.20 (d, broad, 1, amide N–H), 2.58 (s, 5, C_6H_5), 4.80–5.30 (m, 1, phenylalanine C–H), 5.75 (q, 2, ethyl ester $-CH_2-$), 6.50–7.10 (m, 4, glycine $-CH_2-$ and phenylalanine $-CH_2-$), 8.00–8.50 (broad, 2, amine NH_2), 8.80 (t, 3, ethyl ester CH_3).

The oil IXa which was isolated from the methylene chloride extractions was immediately dissolved in 20 ml of anhydrous ethyl ether and anhydrous hydrogen bromide was slowly bubbled with stirring into the solution at room temperature. A bulky white precipitate formed immediately. After 5 min, the addition of hydrogen bromide was discontinued, and the reaction mixture was cooled overnight to 5° in a stoppered flask. The solid was collected by filtration, washed with anhydrous ethyl ether, and recrystallized from a mixture of acetic acid and ethyl ether to yield 0.48 g (73%) of the white crystalline hydrobromide salt of IXa; mp 152–154°. A second recrystallization from acetic acid and ethyl ether raised the mp to 154–155° (lit.[10] mp 154–155°).

4-Nitro-1-cyclohexyl-3-amino-2-oxo-3-pyrroline (X). The crude by-product which precipitated upon dilution of the ammonia-cleavage reaction mixture from VIIIa with aqueous acid was recrystallized twice from 95% ethanol to give X as pale tan prisms; mp 191–192°. Spectra: ir (Nujol) 2.90, 3.06 (NH_2), 5.80 (lactam C=O), 5.96 (C=C), 6.95 (conj NO_2), 7.25, 7.60, 7.84, 8.31, 8.43, 10.15, 13.45 (broad), 15.20 μ (broad); nmr ($CDCl_3/CF_3CO_2H$) τ 1.90–2.90 (broad, 2, NH_2), 5.50 (s, 2, C-5 $-CH_2-$), 5.20–6.30 (broad, 1, cyclohexyl C–H), 7.50–9.20 (m, 10, cyclohexyl $(CH_2)_5$); uv (95% EtOH) 353 nm (ϵ 14,700).

Anal. Calcd for $C_{10}H_{15}O_3N_3$: C, 53.32; H, 6.71; N, 18.66. Found: C, 53.30; H, 6.71; N, 18.56.

Ammonia Cleavage of *N*-(4-Nitro-1-cyclohexyl-2-oxo-3-pyrrolin-3-yl)-L-phenylalanylglycinate (VIIIb). Preparation of Ethyl L-Phenylalanylglycinate Hydrobromide (IXb). The procedure employed was essentially identical with that for the conversion of VIIIa to IXa. The hydrobromide of the crude dipeptide ester IXb was collected by filtration, washed with anhydrous ethyl ether, and recrystallized from acetonitrile to yield 0.58 g (88%) of the white crystalline salt IXb; mp 131–136°. The salt was recrystallized a second time from acetonitrile to provide a sharper melting sample; mp 132–134° (lit. mp[11] 134–137°); $[\alpha]^{25}D$ +39.1° (*c*, 2, H_2O) (lit.[11] $[\alpha]^{25}D$ +39.2° (*c*, 2, H_2O)).

Acknowledgment. The authors are much indebted to Merck Sharp and Dohme Research Laboratories for a generous contribution to the support of this work, to Mr. Frank Leskovic for technical assistance, and to Dr. R. Madhav for his contribution of valuable information and advice.

Registry No.—II, 4563-90-0; III, 52555-21-2; IV, 52555-22-3; V, 52555-23-4; VIa, 52555-24-5; VIb, 52555-25-6; VIc, 52555-26-7; VId, 52555-27-8; VII, 2491-17-0; VIIIa, 52555-28-9; VIIIb, 52555-29-0; IXa, 52555-30-3; IXa HBr, 52555-31-4; IXb HBr, 5399-16-2; X, 52555-32-5; XI, 52555-33-6; triethyloxonium fluoroborate, 368-39-8; triethyl orthoformate, 122-51-0; glycine, 56-40-6; D,L-phenylalanine, 150-30-1; L-phenylalanine, 63-91-2; L-leucine, 61-90-5; ethyl D,L-phenylalaninate, 1795-96-6; ethyl glycinate, 459-73-4.

References and Notes

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