Peptide Azacyclols from Linear Precursors

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Formation of tripeptidic azacyclols from Ala-Phe-Pro-ONp·CF_3CO_2H and from phenylacetyl-Ala-Phe-Pro-ONp is reported. Acylation reactions on azacyclols have been investigated. Both *N*-acyl and *O*-acyl derivatives have been isolated.

Peptide azacyclols, a class of stable tetrahedral adducts which we described in an earlier paper,[1] are the subject of increasing interest. The main reason for this is their potential for the synthesis of nine-membered cyclotripeptides containing secondary amide bonds (CO–NH) in the ring,[2] to which they are related by the tautomeric equilibrium shown in the Scheme.

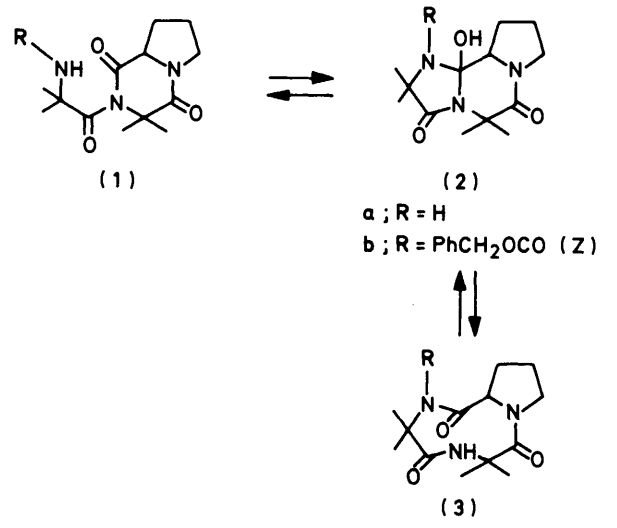
As previous studies have indicated, the stability of three-heteroatom tetrahedral intermediates is strongly dependent on steric and electronic factors.[3] In particular, azacyclols such as (2a) containing a free NH group, exhibit an additional degree of instability as compared with the corresponding *N*-substituted derivatives or with oxacyclols or thiacyclols. Thus, they may undergo easy dehydration to give the corresponding acylamidines;[2c] because of this the only representatives of this class of cyclic peptides known until recently were azacyclols containing a urethane protecting group on the nitrogen (Z-azacyclols) (2b).[1a] It is only very recently that azacyclols containing a free NH group have been isolated and fully characterized. They were obtained by Rothe *et al.*[4] by deprotecting under mild conditions the above mentioned Z-azacyclols.

We report here results we have obtained concerning (i) the isolation of a tripeptide azacyclol containing a free NH group by direct cyclization of a linear peptide and (ii) the first example of a stable tetrahedral intermediate tautomeric with an α-amidoacyl-dioxopiperazine.

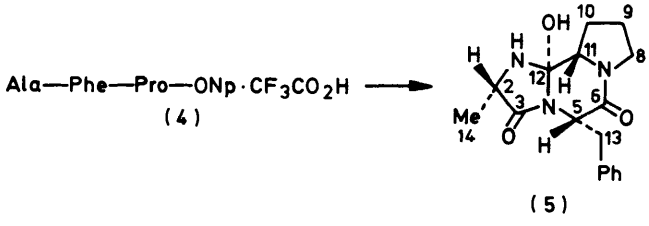
In order to obtain a free azacyclol by direct cyclization, the activated linear precursor Ala-Phe-Pro-ONp·CF_3CO_2H (4) was synthesized and subjected to different cyclizing conditions. By operating in non-aqueous media (*e.g. N,N*-dimethylformamide, *N,N*-di-isopropylethylamine, 12 h at room temperature) complex mixtures, with predominant formation of dehydration products and oligomers, were obtained. On the other hand, treatment of compound (4) at room temperature in mild alkaline aqueous medium (dioxan–water; 0.1M-sodium hydrogen carbonate; 0.1M-sodium carbonate), followed by careful elimination of the solvents, gave in good yield the azacyclol (5), practically pure. The same compound was obtained by hydrogenolysis in methanol of Z-azacyclol (6) [1a] under conditions analogous to those adopted by Rothe.[4] Azacyclol (5) is a fairly stable compound which can be purified by crystallization and stored for several weeks at room temperature.

It is likely that the tautomeric equilibrium which relates open-chain α-aminoacyl-dioxopiperazines to azacyclols and cyclic tripeptides is influenced by the nucleophilicity of the nitrogen, which depends on the *N*-substituents. It was therefore of interest to examine the nature of the compounds formed by introducing different acyl groups onto the NH of the azacyclol (5).

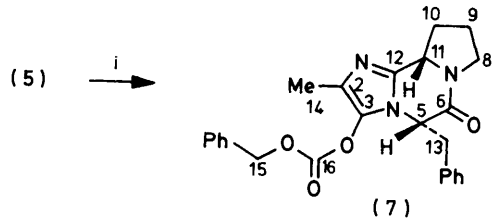
Reactivity toward acylating agents of the NH group in (5) was initially tested by using benzyloxycarbonyl chloride in aqueous sodium hydrogen carbonate (Scheme 2). Although



Scheme 1.

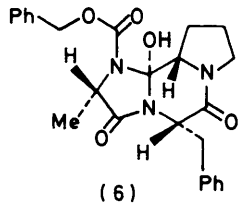


under these conditions acylation takes place neatly, the benzyloxycarbonyl derivative formed was not the expected Z-azacyclol (6). The ^1H n.m.r. spectrum of the new compound (7) shows the methyl group as a singlet shifted to low field (δ 2.1); α-H Pro appears at δ 4.15, long-range coupled to α-H Phe, and no exchangeable protons are present. In the ^{13}C n.m.r. spectrum only two carbonyl singlets are observed (δ 162.4 and 152.3) together with three singlets attributable to aromatic carbons and the signals of the two phenyl groups. The mass spectrum shows a weak molecular ion at *m/e* 431 and significant peaks at *m/e* 387 ($M - CO_2$) and *m/e* 296 ($M - CO_2 - CH_2Ph$). The i.r. spectrum ($CHCl_3$) shows two strong absorptions centred at 1 775 and 1 650 cm^{-1} and no bands in the range 1 600—1 450 cm^{-1} (amide II band). On the basis of these data, an imidazolol-carbonate structure was assigned to the compound (7). The formation of (7) indicates that, under the adopted conditions, the azacyclol (5) undergoes a dehydration reaction to an intermediate acylamidine (imidazolinone) which is in turn *O*-acylated to give the compound (7). It is worth noting that the Z-azacyclol (6) is recovered unchanged when treated with benzyloxy-

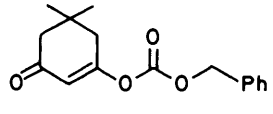


(7)

Scheme 2. *Reagents*: i, $PhCH_2OCOCl$, $NaHCO_3$–H_2O



(6)



(8)

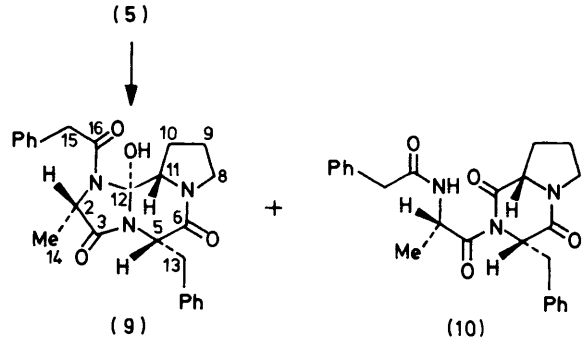
carbonyl chloride in aqueous sodium hydrogen carbonate; under the same conditions, on the other hand, 5,5-dimethylcyclohexane-1,3-dione (dimedone) affords the corresponding enol carbonate (8) in good yield.

Subsequently, acylation of compound (5) with a carboxylic acid was attempted. No reaction occurred when (5) was treated with phenylacetic acid and dicyclohexylcarbodi-imide as condensing agent. Use of phenylacetyl chloride in aqueous sodium hydrogen carbonate gave, on the other hand, a complex mixture whose resolution proved to be difficult owing, at least in part, to the chemical lability of the products involved. Finally, treatment of the azacyclol (5) in tetrahydrofuran with phenylacetic acid, isobutyl chloroformate, and *N*-methylmorpholine, afforded two main products which could be separated and characterized as the two isomeric *N*-phenylacetyl derivatives (9) and (10). The two compounds exhibit very similar chromatographic mobilities and give *cyclo*-(Phe-Pro) when treated with hydrazine hydrate in methanol.[5]

The ^{13}C n.m.r. spectrum of the less polar isomer shows only three carbonyl signals in addition to a singlet at δ 96.3, consistent with the presence of a non-protonated carbon bonded to three heteroatoms. In the ^1H n.m.r. spectrum no coupling is observed between the exchangeable proton and the α-H of the Ala or Phe residues. α-H Pro resonates at δ 3.55 in accordance with its *trans*-arrangement with the benzylic side-chain.[6] The i.r. spectrum ($CHCl_3$) shows a large absorption centred at 3 400 cm^{-1} and no bands between 1 650 and 1 450 cm^{-1}. On the basis of these properties, structure (9), corresponding to *N*-phenylacetyl-azacyclol, was assigned to this isomer.

In the ^{13}C n.m.r. spectrum of the more polar isomer, four carbonyl signals are present and there are no peaks between the signals of the proline α-carbon (δ 60.6) and those of the phenyl carbon atoms (centred at δ 128). In the ^1H n.m.r. spectrum, α-H Ala and α-H Phe appear shifted downfield compared with the values found for the corresponding protons in compounds (5) and (9) (Table); this effect is generally observed for *N*-acyl-dioxopiperazines.[7] The α-H Pro, normally located at δ 3.8, does not suffer ring-current shielding by the benzylic side-chain, in accordance with a relative *trans*-arrangement.[6] The exchangeable proton appears as a doublet coupled to the α-H Ala. The i.r. spectrum ($CHCl_3$) shows two sharp bands at 3 440 and 1 500 cm^{-1} (NH and amide II band respectively). On the basis of these spectroscopic properties, structure (10) was assigned to this isomer.

Although in the solid state *N*-phenylacetyl-azacyclol (9) is



(9) (10)

Table. ^{13}C N.m.r. data [a] (δ/p.p.m. from tetramethylsilane) for compounds (5), (7), (9), (10), and (12)

Carbon atom	(5) [b]	(7) [c]	(9) [c]	(10) [c]	(12) [c]
2	53.6 [d]	124.6	54.4 [e]	50.8	51.4
3	171.6	129.5 [f]	172.9	170.2 [g]	170.7 [h]
5	54.5 [d]	55.8 [i]	55.4 [e]	59.7 [j]	58.8 [k]
6	164.6	162.4	164.6 [i]	163.0	164.1
8	45.9	44.3	45.5	44.3	44.8
9	21.6	21.8	22.2	20.9	21.9
10	26.5	30.5	31.0	28.0	29.1
11	64.5	57.6 [i]	68.0	60.6 [j]	60.8 [k]
12	94.3	131.3 [f]	96.3	174.6 [g]	175.4 [h]
13	39.1	38.7	39.2	38.6	37.6
14	19.2	11.7	20.9	17.9	17.7
15		71.7	42.7	43.6	43.5
16		152.3	167.2 [i]	167.7 [g]	168.8 [h]

[a] See structures for numbering scheme. The assignments for proton-bearing carbons were confirmed by observing the multiplet structures in off-resonance decoupled spectra. [b] Solvent $(CD_3)_2SO$. [c] Solvent $CDCl_3$. [d–l] Assignments may be interchanged.

stable enough to be stored at room temperature for weeks, in chloroform solution it undergoes a slow conversion into the *N*-(*N*-phenylacetyl-Ala)-*cyclo*-(Phe-Pro) (10). The isomerization, monitored by i.r. spectroscopy and t.l.c., is practically complete in a week at room temperature; an analogous isomerization is not observed in the case of Z-azacyclol (6).

Isolation of *N*-phenylacetyl-azacyclol (9) represents the first chemical proof of the tautomeric equilibrium between *N*-(α-amidoacyl)-dioxopiperazines and *N*-acyl-azacyclols. Previously known azacyclols are in fact of type (2a) or (2b) and are tautomeric with *N*-(α-aminoacyl)- or with *N*-(α-benzyloxycarbonylaminoacyl)dioxopiperazines (1a) and (1b) (see Scheme).

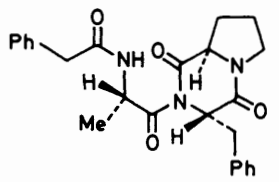
Both free and Z-protected azacyclols (2) can be obtained by cyclizing *C*-activated linear precursors in which the amino-group of the *N*-terminal residue is free or protected with urethane groups. No data are available, however, concerning formation of azacyclols during cyclization of tripeptides in which all nitrogen atoms are engaged in amide bonds. Since this case seem relevant for the chemistry of peptides, we synthesized and subjected to cyclization conditions *N*-(phenylacetyl)-Ala-Phe-Pro-ONp (11), a suitable linear precursor for phenylacetyl-azacyclol (9).

Treatment of compound (11) for 2.5 h at 0 °C with sodium hydride in dry *N*,*N*-dimethylformamide (conditions analogous to those adopted to obtain oxacyclols from the corresponding α-hydroxyacylpeptides [9]) afforded a mixture of two isomeric cyclic compounds.

Structure (12) was assigned to the main component. In the ^1H n.m.r. spectrum, α-H Pro appears at δ 2.4, 1.4 p.p.m.

Ph—CH_2—CO—Ala—Phe—Pro—ONp

(11)



(12) + (9)

upfield of the corresponding proton in (10); both α-H Ala and α-H Phe resonate at low field, as expected for *N*-acyldioxopiperazines,[7] and α-H Ala appears as a multiplet coupled to both β-H_3 Ala and NH. The i.r. mass, and ^{13}C n.m.r. spectra of compound (12) are quite similar to those obtained from its stereoisomer (10). The minor component was identified as *N*-phenylacetyl-azacyclol (9).

Attempted cyclization of compound (11), using weaker bases such as *N,N*-di-isopropylethylamine in *N,N*-dimethylformamide, left the starting material practically unchanged. Use of alkaline buffers in aqueous dioxan, under conditions analogous to those adopted for cyclization of Z-protected tripeptide *p*-nitrophenyl esters,[1a] led to isolation of compound (12) together with *cyclo*-(Phe-D-Pro) and hydrolysis products; no significant amounts of *N*-phenylacetyl-azacyclol (9) were found.

Experimental

M.p.s are uncorrected. I.r. spectra were recorded with a Perkin-Elmer 521 spectrophotometer. ^1H N.m.r. spectra were recorded with a Varian EM 390 spectrometer for compounds (5), (7), (8), (10), and (12) and with a Bruker WP 200 for compound (9). ^{13}C N.m.r. spectra were recorded with a Bruker WH 90 (22.63 MHz) instrument for compounds (5), (8), (9), (10), and (12) and with a Bruker WP 200 (50.28 MHz) instrument for compound (7). Low- and high-resolution mass spectra were obtained with Hewlett-Packard 5980 A and VG Micromass ZAB-2F instruments operating at 70 eV. Optical rotations were taken at 20 °C with a Schmidt-Haensch 16 065 polarimeter. Ether refers to diethyl ether.

Tripeptide Azacyclol (5).—(*a*) *Starting from* Ala-Phe-Pro-ONp·CF_3CO_2H (4). Compound (4) was prepared starting from Boc-Ala-Phe-Pro-OMe [9] by following standard procedures. To a solution of (4) (0.5 g) in dioxan (90 ml), aqueous 0.1M-sodium hydrogen carbonate (45 ml) and aqueous 0.1M-sodium carbonate (45 ml) were added. After 1.5 h at room temperature the reaction mixture was evaporated under reduced pressure, bath temperature below 50 °C. The residue was taken up in chloroform (100 ml) and washed with cold water (3 × 10 ml). Drying and evaporation of the solvent afforded *5-benzyl-10b-hydroxy-2-methyl-1,8,9,10,10a,10b-hexahydroimidazo*[1,2-a]*pyrrolo*[2,1-c]*pyrazine-3,6(2H,5H)-dione* * (5) (230 mg) as a solid which was crystallized from methanol–ether, m.p. 143—144 °C, $[\alpha]_D$ −20° (*c*, 1.00 in MeOH), $\nu_{max.}$ (KBr) 3 310, 3 290, 3 190, 1 700, and 1 625 cm^{-1}; δ([$^2H_6$]DMSO) 1.10 (3 H, d, *J* 6.8 Hz, Me), 1.6—2.2 (4 H, m, β-H_2 and γ-H_2 Pro), 2.9 and 3.3 (2 H, AB part of ABX system, J_{gem} 13.5 Hz, J_{vic} 9.5 and 4.0 Hz, β-H_2 Phe), 3.47 (1 H, m, α-H Ala), 3.5 (2 H, m, δ-H_2 Pro), 3.6 (1 H, m, α-H Pro), 3.85 (1 H, d, *J* 7.2 Hz, NH), 4.40 (1 H, X part of ABX system, α-H Phe), 6.65 (1 H, s, OH), and 7.2—7.5 (5 H, m, Ph); on exchange with D_2O, the NH doublet and OH singlet disappeared and α-H Ala multiplet was changed into a quartet coupled (*J* 6.8 Hz) to the methyl doublet; *m/e* 297 (M^+ − 18, 29%), 206 (297 − CH_2Ph, 68), 178 (100), 91 (39), and 70 (12) (Found: C, 64.7; H, 6.8; N, 13.3. $C_{17}H_{21}N_3O_3$ requires C, 64.7; H, 6.7; N, 13.3%).

(*b*) *Starting from Z-azacyclol* (6). A solution of 5-benzyl-1-benzyloxycarbonyl-10b-hydroxy-2-methyl-1,8,9,10,10a,-10b-hexahydroimidazo[1,2-*a*]pyrrolo[2,1-*c*]pyrazine-3,6-(2*H*,5*H*)-dione (6) (1.0 g) in dry methanol (200 ml) was hydrogenated in the presence of 5% Pd-on-alumina (0.35 g) within 2.5 h at room temperature. The catalyst was filtered off and the solution evaporated under reduced pressure, bath temperature below 50 °C, to give compound (5) (0.67 g) as a solid which was further purified by crystallization from methanol–ether.

Reaction of Azacyclol (5) *with Benzyl Chloroformate*.—To a stirred, ice-cold suspension of benzyl chloroformate (0.4 g) in saturated aqueous sodium hydrogen carbonate (10 ml), azacyclol (5) (0.25 g) was added. Stirring was continued for 1 h at toom temperature, then the reaction mixture was diluted with saturated aqueous sodium hydrogen carbonate and extracted with chloroform. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and water, dried and evaporated to give an oily residue (0.5 g). Column chromatography (silica gel; benzene–ethyl acetate as eluant) afforded *5-benzyl-3-benzyloxycarbonyloxy-2-methyl-8,9,10,10a-tetrahydroimidazo*[1,2-a]*pyrrolo*[2,1-c]-*pyrazin-6*(5H)-*one* (7) (110 mg) as an oil, $[\alpha]_D$ +69.0° (*c* 1.00 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1 775, 1 650, and 1 450 cm^{-1}; δ($CDCl_3$) 1.4—2.2 (4 H, m, β-H_2 and γ-H_2 Pro), 2.12 (3 H, s, Me), 3.15 and 3.45 (2 H, AB part of ABX system, J_{gem} 14.0 Hz, J_{vic} 4.5 and 3.3 Hz, β-H_2 Phe), 3.0—3.6 (2 H, m, δ-H_2 Pro), 4.15 (1 H, m, α-H Pro), 4.83 (1 H, X part of ABX system, α-H Phe), 5.33 (2 H, s, OCH_2), and 6.75—7.40 (10 H, m, aromatics); *m/e* 431 (M^+, 0.5%), 387 ($M - CO_2$, 8), 297 (14), 296 (12), 295 (10), 268 (22), 178 (14), 176 (16), 123 (16), and 91 (100) (Found: M^+, 431.1850. $C_{25}H_{25}N_3O_4$ requires *M*, 431.1845).

3-Benzyloxycarbonyloxy-5,5-dimethylcyclohex-2-en-1-one (8).—Dimedone (5.0 g) was stirred for 4 h at room temperature with benzyl chloroformate (18.5 g) in saturated aqueous sodium hydrogen carbonate (450 ml). After work-up analogous to that reported for compound (7), the reaction mixture was purified by column chromatography (silica gel; benzene–ethyl acetate 9 : 1 as eluant) affording *compound* (8) (7.0 g) as an oil, $\nu_{max.}$ ($CHCl_3$) 1 770 and 1 670 cm^{-1}; δ_H($CDCl_3$) 1.08 (6 H, s, 2 × Me), 2.20 (2 H, s, CH_2), 2.32 (2 H, s, CH_2), 4.90 (2 H, s, CH_2Ph), 5.50 (1 H, s, CH=C), and 7.40 (5 H m, Ph); δ_C($CDCl_3$) 28.0 (2 × Me), 32.8 (*C*Me), 41.6 (CH_2), 50.6 (CH_2), 70.6 (CH_2O), 115.2 (*C*H=C–O), 150.8 (O–CO–O), 167.1 (*C*=C–CO), and 192.7 (C–*C*O–C); *m/e* 230 ($M^+ - CO_2$, 0.5%) and 91 (100) (Found: C, 69.9; H, 6.6. $C_{16}H_{18}O_4$ requires C, 70.05; H, 6.6%).

Reaction of Azacyclol (5) *with Phenylacetic Acid*.—To a solution of phenylacetic acid (0.46 g) and *N*-methylmorpholine (0.5 ml) in tetrahydrofuran (44 ml) cooled to −10 °C, isobutyl chloroformate (0.44 ml) was added with stirring. Stirring was continued for 10 min and a cold (ice-bath) solution of (5) (1.12 g) in tetrahydrofuran (200 ml) was then added. After 4.5 h at room temperature, the reaction mixture

* Structures (5), (7), and (9) show the n.m.r. numbering scheme, and not the IUPAC systematic numbering used for the names.

was evaporated under reduced pressure; the residue was then taken up in chloroform and washed with 1M-hydrochloric acid, saturated aqueous sodium carbonate, and water. After being dried and evaporated, the residue (1.3 g) was crystallized from ethyl acetate to give *5-benzyl-10b-hydroxy-2-methyl-1-phenylacetyl-1,8,9,10,10a,10b-hexahydroimidazo-[1,2-a]pyrrolo[2,1-c]pyrazine-3,6(-2H,5H)-dione* (9) (0.36 g), m.p. 186—188 °C, $[\alpha]_D$ −30.0° (*c*, 0.5 in $CHCl_3$); $\nu_{max.}$ (KBr) 3 280br, 1 720, 1 665, and 1 615 cm^{-1}; $\nu_{max.}$ ($CHCl_3$) 3 400br, 1 730, and 1 645cm^{-1}; δ($CDCl_3$) 1.37 (3 H, d, *J* 6.5 Hz, Me), 1.6—2.0 (4 H, m, β-H_2 and γ-H_2 Pro), 3.05 and 3.55 (2 H, AB part of ABX system, J_{gem} 13.6 Hz, J_{vic} 12.0 and 4.5 Hz, β-H_2 Phe), 3.4—3.6 (3 H, m, α-H Pro and δ-H_2 Pro), 3.66 (2 H, s, CH_2CO), 4.0 (1 H, q, *J* 6.5 Hz, α-H Ala), 4.8 (1 H, X part of ABX system, α-H Phe), 5.55 (1 H, s, OH), and 7.1—7.4 (10 H, m, aromatics); *m/e* 433 (M^+, 2%), 342 (M − CH_2Ph, 1), 245 (30), 244 (40), 125 (40), 91 (100), and 70 (35) (Found: 69.1; H, 6.3; N, 9.65. $C_{25}H_{27}N_3O_4$ requires C, 69.3; H, 6.3; N, 9.7%).

Preparative t.l.c. of the mother liquors from the crystallization of (9) (silica gel; chloroform–ether 1 : 1 as eluant) afforded N-(N-*phenylacetyl-Ala*)-cyclo-(*Phe-Pro*) (10) (0.15 g) as a foam, pure by t.l.c. analysis, $[\alpha]_D$ +72° (*c*, 1.00 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3 440, 1 720, 1 655, and 1 500 cm^{-1}; δ($CDCl_3$) 1.20 (3 H, d, *J* 7.0 Hz, Me), 1.3—2.2 (4 H, m, β-H_2 and γ-H_2 Pro), 3.15 and 3.38 (2 H, AB part of ABX system, J_{gem} 14.0 Hz, J_{vic} 4.3 and 3.5 Hz, β-H_2 Phe), 3.6 (4 H, m, δ-H_2 Pro and CH_2CO), 3.8 (1 H, m, α-H Pro), 5.15 (1 H, X part of ABX system, α-H Phe), 5.75 (1 H, m, α-H Ala), 6.25 (1 H, d, *J* 7.5 Hz, NH Ala), and 7.1—7.5 (10 H, m, aromatics) [addition of D_2O caused the signal at 6.25 to disappear and that at 5.75 to collapse to a quartet (*J* 7.0 Hz)]; *m/e* 433 (M^+, 1%), 298 (2), 244 (21), 125 (31), 91 (100), and 70 (32) (Found: C, 69.2; H, 6.3; N, 9.7. $C_{25}H_{27}N_3O_4$ requires C, 69.3; H, 6.3; N, 9.7%). Treatment of compound (10) with methanolic hydrazine [5] gave *cyclo*-(Phe-Pro), $[\alpha]_D$ −89° (*c*, 0.5 in H_2O).[10]

Cyclization of Phenylacetyl-L-alanyl-L-phenylalanyl-L-proline p-Nitrophenyl Ester (11).—To a solution cooled at −10 °C of (11) [11] (1.5 g) in dry *N,N*-dimethylformamide (30 ml), was added sodium hydride (50% in white oil; 150 mg) with stirring. After 2 h at 0 °C, ice-cold aqueous sodium hydrogen carbonate and then methylene dichloride were added. The organic layer was washed with 0.5M-sodium carbonate and water. Drying and evaporation afforded a residue (1.05 g) that was crystallized from ethyl acetate to give the azacyclol (9) (60 mg).

Column chromatography (silica gel; ethyl acetate–methanol 97 : 3 as eluant) of the mother liquors from the crystallization of (9), followed by crystallization from ethyl acetate of collected fractions, gave N-(N-*phenylacetyl-Ala*)-cyclo-(*Phe-D-Pro*) (12) (0.35 g), m.p. 181—182 °C, $[\alpha]_D$ +90° (*c*, 1.00 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 3 440, 1 720, 1 670, and 1 505 cm^{-1}; δ($CDCl_3$) 1.30 (3 H, d, *J* 7.0 Hz, Me), 1.4—2.2 (4 H, m, β-H_2 and γ-H_2 Pro), 2.40 (1 H, m, α-H Pro), 3.2 (2 H, m, β-H_2 Phe), 3.5 (2 H, m, δ-H_2 Pro), 3.65 (2 H, s, CH_2CO), 5.3 (1 H, apparent t, α-H Phe), 5.65 (1 H, m, α-H Ala), 6.4 (1 H, d, *J* 7.5 Hz, NH Ala), and 7.4 (10 H, m, aromatics) [addition of D_2O caused the signal at 6.4 to disappear and that at 5.65 to collapse to a quartet (*J* 7.0 Hz)]; *m/e* 433 (M^+, 3%), 298 (2), 244 (25), 125 (27), 91 (100), and 70 (34) (Found: C, 69.15; H, 6.3; N, 9.7. $C_{25}H_{27}N_3O_4$ requires C, 69.3; H, 6.3; N, 9.7%). Treatment of compound (12) with methanolic hydrazine [5] gave *cyclo*-(Phe-D-Pro), $[\alpha]_D$ +90° (*c*, 0.5 in H_2O).[10]

References

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