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A METHOD FOR THE DETERMINATION OF THE CONFIGURATION OF THE L-PRO-L,D-PHE PSEUDODIPEPTIDE ISOMERS CONTAINING A TRANS-ETHYLENE ISOSTERIC REPLACEMENT OF THE PEPTIDE BOND

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Abstract - In order to determine the configuration of the diastereoisomers of the dipeptide analogues L-Pro ψ(CH=CH, E) L,D-Phe, a method is described involving reduction of the ethylenic bond and cyclization to the lactams. The ^{1}H NMR spectra of the separated isomers allow the assignment of the S,S or R,S configuration.

In a recent paper ([1]) the synthesis of the L-Pro-L,D-Phe pseudodipeptide 2, containing a trans-ethylenic double bond as an isosteric replacement of the peptide bond has been reported. We have independently prepared 2a by alkylation of the trimethylsilyl ester 1c or of the 2-tetrahydropyranyl ester 1d (Scheme 1) ([2]) instead of the methyl ester 1b as reported, because of the ease of their hydrolysis to the free acid 2a. The resulting pseudodipeptide 2a is a mixture of diastereomers, which could not be separated by crystallization or by HPLC. Sammes et al ([1]) proposed a method involving ozonolysis of the double bond to prove that the alkylation has not resulted in any asymmetric induction. We now report an indirect method allowing the determination of the configuration of the newly introduced asymetric centre in 1a.
Catalytic reduction of the double bond in 2a results in the formation of the

SCHEME 1

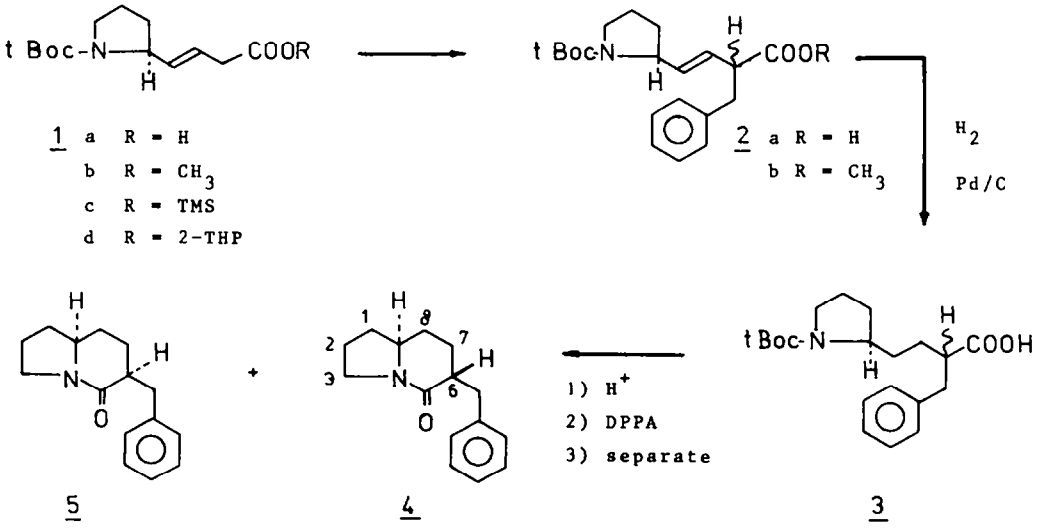
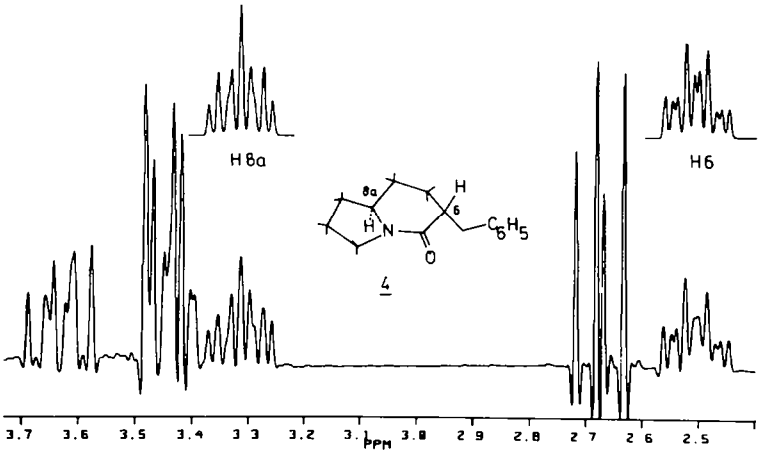
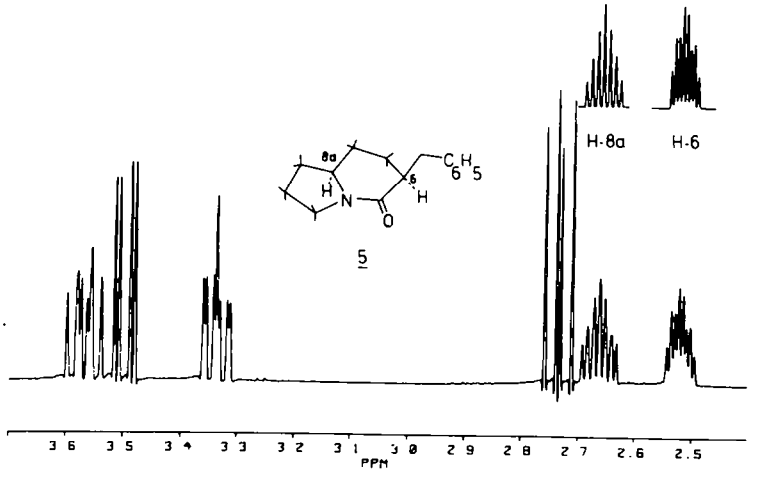


FIGURE 1



270 MHz ^{1}H NMR spectrum of 4 (2.4-3.7 ppm), with simulated signals of H8a and H6 in insets ($CDCl_3$).

FIGURE 2



500 MHz ^{1}H NMR spectrum of 5 (2.4-3.7 ppm), with simulated signals of H8a and H6 in insets (C_6D_6).

TABLE 1

^{1}H NMR parameters of 4 and 5

	4	5	
		$CDCl_3$	C_6D_6
δ(H6)	= 2.51 ppm	2.65 ppm	2.52 ppm
δ(H8a)	= 3.32 ppm	3.38 ppm	2.66 ppm
J(H6, $HBzl_1$)	= 10.35 Hz	-	10.15 Hz
J(H6, $HBzl_2$)	= 4.07 Hz	-	3.67 Hz
J(H6, H7)	= 10.70 Hz	-	6.70 Hz
J(H6, H7')	= 6.34 Hz	-	3.54 Hz
J(H8a,H8)	= 10.92 Hz	10.38 Hz	10.37 Hz
J(H8a,H8')	= 4.44 Hz	5.02 Hz	4.89 Hz
J(H8a,H1)	= 10.92 Hz	10.38 Hz	10.58 Hz
J(H8a,H1')	= 4.44 Hz	5.02 Hz	4.68 Hz

saturated analogue 3 (63%), corresponding to the L-Pro $\psi(CH_2-CH_2)$ D,L-Phe pseudodipeptide. The t-butyloxycarbonyl group was removed with gaseous hydrochloric acid and the crude aminoacid was cyclized to the lactams 4 and 5 as a 0.35 mM solution in dimethylformamide using diphenylphosporylazide ([3]) as cyclizing agent. A total yield of 70% was obtained. Both isomers were separated by HPLC on silica gel, giving 37% of 4 as the faster eluting component and 63% of 5 as the second component. The mass spectra of both isomers are identical, but the ^1H NMR spectra in $CDCl_3$ solution showed marked differences. Isomer 4 shows well resolved signals in the 270 MHz spectrum (Fig. 1). A 2D COSY experiment allowed a complete signal assignment, except for the orientation of the ring protons. Thus starting from the easily recognizable multiplicity of the benzylic protons, H6 can be assigned, and using the correlations over the C7 and C8 protons, also H8a was assigned unambiguously. The multiplicity of the H6 and H8a signals could be interpreted by first order analysis, and the coupling constants were refined by spectral simulations (Table 1). These values show that both protons are axially oriented, and since carbon 8a has the S-configuration (coming from L-Pro), this allows us to assign the R-configuration to carbon 6.
Isomer 5, which must have (6S,8aS) configuration shows a very complex spectrum at 270 MHz in $CDCl_3$ solution. Signal assignment could be done as for 4, using 2D COSY at 500 MHz. The H8a signal was analysed (Table 1), but even at this high field, the two C7 protons at δ = 2.66 ppm and H6 and one of the benzylic protons at δ = 2.65 ppm are isochronous, thus preventing an analysis of this spin system. However changing the solvent to benzene-d_6 caused sufficient shifts of the signals to yield well resolved multiplets (Fig. 2). The spectral parameters obtained after simulation of the H6 and H8a multiplets are shown in Table 1. The coupling constants show that H8a is axially oriented towards both rings, whereas H6 shows two smaller couplings consistent with a pseudo-equatorial orientation. This confirms the S configuration of carbon 6.
As a result of the procedures described here, the analytical data of 4 and 5 are known, and the method can be applied on an analytical scale once the procedures for the separation of the pseudodipeptides 2 are worked out. In principle the method can be generalized to double bond isosteres containing other amino acid residues. Although the proline ring fixes the six-membered ring into the same conformation in both isomers, in cases where another amino acid residue is present, the (R,S) isomer corresponding to 4, will have both sidechains in the equatorial orientation. The occurence of strong solvent shifts assures that the relevant signals can be separated from overlapping multiplets for analysis.

Experimental Section

NMR spectra were obtained on a Bruker AM 270 or AM 500 spectrometer, using a 0.1 mM concentration in $CDCl_3$. Spectral simulations were performed with the Bruker PANIC iterative program. Mass spectra were recorded on a AEI MS 902 S spectrometer using the FAB mode. HPLC separations were performed on a Du Pont 830 liquid chromatograph, using a LiChrosorb Si 60 column (I.D. = 22.7 mm, l = 25 cm). Analytical TLC was carried out on SiO_2 (5x10 cm, Merck 5724), with UV or I_2 detection.

(2RS; 2'S)-2-Benzyl-4-(1-t-butoxycarbonylpyrrolidin-2-yl)butyric acid 3.
A solution of 800 mg 2a in 50 ml methanol is shaken overnight in a Parr apparatus in the presence of 10% Pd/C under 4 atm. H_2 pressure. After filtration of the catalyst, the solvent is evaporated and the residue is passed through a SiO_2 column using chloroform as eluant. A colourless oil (500 mg, 63%) is obtained. R_f ($CHCl_3/CH_3OH$, 97/3) = 0.45; MS (m/z, %RI) : 348 (2), 292 (5),

248(10), 230(27), 114(18); ^{1}H NMR($CDCl_3$) : ethylenic signals of 2a at δ = 5.4 have disappeared.

(2RS; 2'S)-2-Benzyl-4-(pyrrolidin-2-yl)butyric acid.HCl 6

A vigorous stream of dry HCl gas is led through a solution of 500 mg 3 in 20 ml CH_2Cl_2 during 5 min. The solution is stirred at room temperature for one hour, the solvent is evaporated, leaving a white foam. MS(m/z, %RI) : 248(100), ^{1}H NMR (DMSO) : tBoc-signal at δ = 1.42 has disappeared.

(6RS, 8aS)-6-benzylperhydroindolizin-5-on 4,5

A solution of 400 mg (1.4 mmol) of 6 hydrochloride in 10 ml dry, amine-free DMF is cooled to -10°C and 2.2 g (8 mmol) DPPA are added. After stirring for 3 h, the solution is further cooled to -20°C, DMF is added to a total volume of 250 ml and 650 µl (6 mmol) N-methylmorpholine are added. The solution is left for 4 days at 4°C, the solvent is evaporated and the residue is dissolved in ethylacetate, and washed successively with a 5% HCl solution, $NaHCO_3$ and water. After drying and evaporating the solvent, the residue is passed through a SiO_2(0.040-0.063) column using chloroform as eluant. The two isomers are further separated by HPLC on a SiO_2 column, using a CH_2Cl_2/iPrOH/AcOH, 2000/100/5 mixture. Fraction 1 contains 85 mg (26%) of the (6R,8aS) isomer 4 as a white semi-solid, α_D^{22} = +54.9° (c = 1.074 in $CHCl_3$). R_f ($CHCl_3$/CH_3OH, 97/3) = 0.67; MS(m/z, %RA) : 230(M^++1, 100), 138(28), 91(40); ^{1}H NMR ($CDCl_3$, 270 MHz, assigned by 2D COSY) : 7.25(5H arom), 3.61(H3), 3.45(H Bzl 1), 3.44(H3'), 3.32(H8a), 2.68(H Bzl 2), 2.51(H6), 2.03(H1), 2.00(H2), 1.97(H2'), 1.75(H7), 1.72(H8), 1.43(H1'), 1.42(H7'), 1.20(H8').

Fraction 2 contains 140 mg (44%) of the (6S,8a) isomer 5 as a white solid, which could be crystallized from ether/petroleumether : m.p. 107-108°C, α_D^{22}= -62.4° (c = 1.294 in $CHCl_3$). R_f($CHCl_3$/CH_3OH, 97/3) = 0.56; MS(m/z, %RA) : 230(M^++1, 100), 138(35), 91(47); ^{1}H NMR ($CDCl_3$, 500 MHz, assigned by 2D COSY) : 7.28(3H arom), 7.21(2H arom), 3.60 (H3), 3.48(H3'), 3.38(H8a), 3.31(H Bzl 1), 2.65(H Bzl 2, H6), 2.07(H1), 1.94(H2), 1.87(H8), 1.75(H2), 1.66(H7, H7'), 1.39(H1'), 1.26(H8').

Both isomers could also be separated by gas chromatography on a Sil 5 CB capillary column (25 m, I.D. = 0.33 mm, 0.12 micron), using a 50° to 200° gradient (8°/min).

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