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# SYNTHESIS AND PROPOSED CONFORMATIONS OF L-PROLYL-L-LEUCYL-β-ALANINE ALKYLAMIDES[1]

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**Abstract**—The synthesis of H-Pro-Leu-β-Ala-$NH_2$, H-Pro-Leu-β-Ala-$NHCH_3$ and H-Pro-Leu-β-Ala-$N(CH_3)_2$ is described. On the basis of IR and $^1H$ NMR spectral data, a 7-membered ring including the NH of β-alanine with the C=O of proline should be assigned for the H-Pro-Leu-β-Ala-$N(CH_3)_2$. Consequently, the plausible conformations for H-Pro-Leu-β-Ala-$NH_2$ and H-Pro-Leu-β-Ala-$NHCH_3$ derive from the formation of an 11-membered ring, between the trans amide proton and the C=O of Pro, or from the formation of an 8-membered ring, between this carboxamide proton and the C=O of Leu, plus the aforementioned 7-membered ring.

It has been shown that [9-β-alanine]oxytocin has low oxytocic and vasodepressor activities, but similar intrinsic activity to that of oxytocin.[2]

As a first effort to explore the conformational effect(s) of replacing $Gly^9$ with $\beta\text{-}Ala^9$ in oxytocin and oxypressin, we have now synthesised H-Pro-Leu-β-Ala-$NH_2$, H-Pro-Leu-β-Ala-$NHCH_3$ and H-Pro-Leu-β-Ala-$N(CH_3)_2$ and we wish to propose their preferred conformations in connection to that proposed by Walter *et al.*[3] for the H-Pro-Leu-Gly-$NH_2$.

## RESULTS AND DISCUSSION

The desired tripeptide amide, H-Pro-Leu-β-Ala-$NH_2$, was built up in a step-wise manner using conventional methods. Thus, N-carbobenzoxy-L-leucine was coupled with β-alanine ethyl ester hydrochloride[4] by the mixed-anhydride method[5] to give N-carbobenzoxy-L-leucyl-β-alanine ethyl ester (**1**) in good yield. The N-protecting group was removed by hydrogenolysis and the resulting dipeptide ester, H-Leu-β-Ala-$OC_2H_5$, was condensed with N-carbobenzoxy-L-proline either by the N,N'-dicyclohexylcarbodiimide[6] or the mixed-anhydride method.[5] The tripeptide N-carbobenzoxy-L-prolyl-L-leucyl-β-alanine ethyl ester (**2**) was ammonolysed to afford N-carbobenzoxy-L-prolyl-L-leucyl-β-alanine amide (**3**) in crystalline form and high yield. It should be noted that ammonolysis of **2** in $MeOH/NH_3$ solution proceeds considerably faster than in $EtOH/NH_3$. Deprotection of **3** by catalytic hydrogenolysis afforded H-Pro-Leu-β-Ala-$NH_2$ (**4**), which was crystallised from ethyl acetate–petroleum ether.

On the other hand, N-Z-Pro-Leu-β-Ala-$OC_2H_5$ (**2**) was saponified to produce N-Z-Pro-Leu-β-Ala-OH (**5**), which in turn was condensed with methylamine and dimethylamine *via* the mixed carbonic-carboxylic anhydride method, as recently described.[7] While the N-Z-Pro-Leu-β-Ala-$NHCH_3$ (**6**) has a salt like high m.p. 217°, the corresponding N-Z-Pro-Leu-β-Ala-$N(CH_3)_2$ (**7**) is an oil. The homogeneity of the latter was proved by spectral data and tlc. The crystalline product **6** and the oily **7** were subjected to catalytic hydrogenolysis over palladium black. The tripeptide methylamide, H-Pro-Leu-β-Ala-$NHCH_3$ (**8**) was obtained in crystalline form, while the dimethylamido derivative, H-Pro-Leu-β-Ala-$N(CH_3)_2$ (**9**), failed to solidify after several trials. Its homogeneity was secured by chromatographic purification on silica gel column.

The $^1H$ NMR spectrum of H-Pro-Leu-β-Ala-$NH_2$ in DMSO-$d_6$† shows conspicuously large difference in chemical shift between the resonances of the *trans* and *cis* carboxamide protons of the β-alanine amide moiety (δ 7.3 and 6.8). This could be attributed to a H-bonding either between the *trans* amide proton and the C=O of proline to form an 11-membered ring, or with the C=O of leucine to form an 8-membered ring.

Actually, constructing conformational models, we assumed that the formation of an 8-membered ring would allow also the formation of an additional 7-membered ring between the NH of β-alanine and the C=O of proline. The latter hypothesis is in line with the results of Ovchinnikov for systems with two peptide bonds.[8,9]

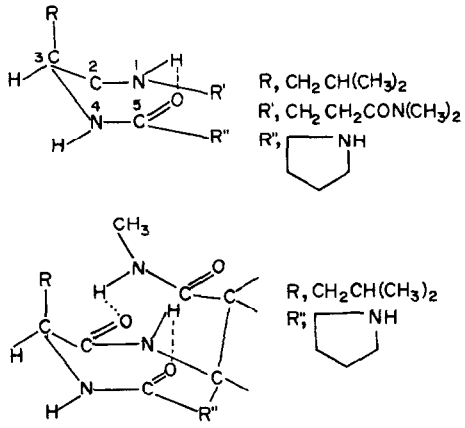
Study of the $^1H$ NMR spectra of H-Pro-Leu-β-Ala-NHMe and H-Pro-Leu-β-Ala-$NMe_2$ in $CDCl_3$ solution revealed a large coupling constant $J = 7.8$ Hz for the $N_4H\text{–}C_3H$ fragment and a smaller constant of the order $J = 6.0\text{–}6.5$ Hz for the protons of the other NH groups. The large value of J indicates that 7-membered rings tend to form in the above tripeptide derivatives by intramolecular H-bonding between the NH of residue i + 2 and the C=O of residue i, which correspond to the NH of β-alanine and the C=O of proline.[8]

Indeed the molecular models of these peptides show that intramolecular H-bonding, which provides stability to the folded form, occurs without difficulty when the planes $HN_4C_3$ and $N_4C_3H$ form a dihedral angle $\theta < 20°$. This value is compatible with the large constant $J = 7.8$ Hz observed for the methyl and dimethylamide derivatives of H-Pro-Leu-β-Ala-$NH_2$. As the 7-membered H-bonded ring of the assigned conformation is not planar, a weak character of that particular bond should be expected.[8,10] Exchange experiments with $D_2O$ differentiate, but not markedly, the NH protons. However, after 20 min all disappeared completely in a solution of $CDCl_3 + D_2O$ (15:1).

The postulated 7-membered ring was further supported by IR data. Thus the IR spectrum ($CHCl_3$) of H-Pro-Leu-β-Ala-$NMe_2$ shows, as expected, reduced absorption in comparison to the methylamide derivative at the region

†The tripeptide amide has very low solubility in $CDCl_3$.

3320 $cm^{-1}$. At very low concentrations to eliminate intermolecular association, this absorption has the same integral intensity with the free NH stretching band at 3440 $cm^{-1}$. The low band at 3320 $cm^{-1}$ is attributed to a bonded NH stretching frequency and in particular to an intramolecular H-bond between the NH of β-alanine and the C=O of proline to form a 7-membered ring.[11] In contrast, the IR spectrum ($CHCl_3$) of H-Pro-Leu-β-Ala-NHMe displays a strong broad band at 3340 $cm^{-1}$ characteristic for H-bonded NH groups and a weak one at 3440 $cm^{-1}$ for free NH groups.[12]




Therefore, the NH proton of the methylamide moiety of H-Pro-Leu-β-Ala-$NHCH_3$ participates in a H-bond, which could be attributed to a bonding between the $NHCH_3$ of the β-alanine moiety and the C=O of leucine to form an 8-membered ring, in addition to the above mentioned 7-membered ring.

## EXPERIMENTAL

M.ps were taken on a Buchi SMP-20 capillary m.p. apparatus and are uncorrected. Optical rotations were determined with a Carl Zeiss precision polarimeter (0.005°). IR spectra were taken on a Hitachi Perkin–Elmer 457 Grating Infrared spectrophotometer. $^1H$ NMR spectra were obtained on a Hitachi Perkin–Elmer R-24 (60-MHz) spectrometer in different solvents as indicated, with TMS as internal reference, and are expressed as δ values. TLC was carried out on silica gel SiF chromatogram sheets with the solvent system I (BAWP), 1-butanol–acetic acid–water–pyridine (15:3:12:10) and II (BE), benzene–ethanol (8:2) and visualised by UV, ninhydrin and chlorine tolidine reagent. Elemental analyses were performed at the National Hellenic Research Foundation, Athens, Greece.

*Carbobenzoxy-L-leucyl-β-alanine ethyl ester* (**1**). Coupling of Z-L-leucine (7.95 g, 30 mmol) with 4.68 g (30 mmol) of β-alanine ethyl ester hydrochloride[4] via the mixed anhydride procedure,[5] provided 6 g (55%) of product; m.p. 72–73° (recrystallised from EtOAc–petroleum ether); $[a]_D^{24}$ −20.2° (*c*, 2, EtOH). (Found: C, 62.42; H, 7.87; N, 7.52). Calc. for $C_{19}H_{28}N_2O_5$: C, 62.63; H, 7.69; N, 7.69%).

*Carbobenzoxy-L-prolyl-L-leucyl-β-alanine ethyl ester* (**2**). Catalytic hydrogenolysis of 5.46 g (15 mmol) of **1** produced 3.3 g of oily L-leucyl-β-alanine ethyl ester, which was condensed with Z-L-proline (3.45 g, 15 mmol) by the mixed anhydride method;[5] yield 4.3 g (60%); m.p. 125–126°; $[a]_D^{24}$ −78.3° (*c*, 2, EtOH). (Found: C, 62.58; H, 7.68; N, 8.93. Calc. for $C_{24}H_{35}N_3O_6$: C, 62.47; H, 7.59; N, 9.11%).

*Carbobenzoxy-L-prolyl-L-leucyl-β-alanine amide* (**3**). A methanolic soln (200 ml) of 3.7 g (8 mmol) of **2** saturated with dry ammonia, was permitted to remain for 3 days at room temp. Then the solvent was removed *in vacuo* and the resulting product solidified with ether: yield 3.2 g (92%); m.p. 185–188°. Recrystallisation from EtOH raised the m.p. up to 190–191°; $[a]_D^{24}$ −60° (*c*, 1, DMF), −76.36° (*c*, 1.25 EtOH); reported[13] m.p. 176°; $[a]_D^{31}$ −46° (*c*, 1.25, EtOH). The $^1H$ NMR spectrum (DMSO-$d_6$) shows, as expected, no absorption for $COOCH_2CH_3$ protons, δ 0.85 (br, d, 6H, $(CH_3)_2C$), 5.1 (s, 2H, $ArCH_2$), 7.4 (s, 5H, $C_6H_5$).

*L-Prolyl-L-leucyl-β-alanine amide* (**4**). A soln of 3.9 g (7 mmol) of **3** in MeOH (200 ml) was subjected to catalytic hydrogenolysis over PdO (450 mg) for 6 hr. The solvent was removed *in vacuo* and the resulting oily product was crystallised from EtOAc–petroleum ether; yield 1.25 g (60%); m.p. 116–118°; $[a]_D^{25}$ −50.4° (*c*, 1 DMF). The $^1H$ NMR spectrum in DMSO-$d_6$ shows, as expected, no absorption for aromatic protons, δ 0.85 (br d, 6H, $(CH_3)_2C$), 2.25 (t, J ~ 6 Hz, 2H, $CH_2CO$), 6.8 (s, 1H, *cis* $CONH_2$), 7.3 (s, 1H, *trans* $CONH_2$). (Found: C, 56.00; H, 8.85; N, 18.53. Calc. for $C_{14}H_{26}O_3N_4$: C, 56.37; H, 8.72; N, 18.79%).

*Carbobenzoxy-L-prolyl-L-leucyl-β-alanine* (**5**). An 80% methanolic soln (15 ml) of **2** (2.8 g, 6.3 mmol), containing 0.25 g (7 mmol) of NaOH, was stirred for 5 hr at room temp. Then water (100 ml) was added and the soln was extracted twice with EtOAc (100 ml). The water layer was subjected to deairation by rotary evaporator at room temp. and was acidified with 6N HCl. The ppt solidified upon cooling and scratching; yield 1.5 g (55%); m.p. 144–145°; $[a]_D^{20}$ −47.6° (*c*, 1, DMF). (Found: C, 60.58; H, 7.25; N, 9.52. Calc. for $C_{22}H_{31}N_3O_6$: C, 60.97; H, 7.16; N, 9.69%).

*Carbobenzoxy-L-prolyl-L-leucyl-β-alanine methylamide* (**6**). To a soln of **5** (1 g, 2.3 mmol) and triethylamine (0.22 g, 2.3 mmol) in THF (20 ml) cooled to −10°, ethylchlorocarbonate (0.25 g, 2.3 mmol) was added. After 3 min a mixture of methylamine hydrochloride (0.47 g, 6.9 mmol) and triethylamine (0.66 g) in 6 ml THF–$H_2O$ (7:3) was added with shaking and the soln kept for 2 hr at room temp. The solvent was removed *in vacuo* and the remaining residue washed with 5% $NaHCO_3$ and water several times. After recrystallisation from EtOH the product (0.6 g, 60%) had m.p. 217–218°; $[a]_D^{24}$ −54° (*c*, 1, DMF); IR (KBr disk), 1660–1670, 1520 $cm^{-1}$; NMR (pyridine-$d_5$) δ 0.85 (br d, 6H, $(CH_3)_2C$), 2.8 (d, J ~ 6 Hz, 3H, $NCH_3$, on exchange with $D_2O$ it collapses to a singlet), 5.2 (s, 2H, $ArCH_2$). (Found: C, 62.20; H, 7.49; N, 12.37. Calc. for $C_{23}H_{34}N_4O_5$: C, 61.88; H, 7.66; N, 12.56%).

*Carbobenzoxy-L-prolyl-L-leucyl-β-alanine dimethylamide* (**7**). To a soln of **5** (0.8 g, 1.8 mmol) and triethylamine (0.18 g, 1.8 mmol) in THF (20 ml) cooled to −10°, ethylchlorocarbonate (0.2 g, 1.8 mmol) was added. After 3 min a mixture of dimethylamine hydrochloride (0.364 g, 5.4 mmol) and triethylamine (0.54 g, 5.4 mmol) in 5 ml of THF–$H_2O$ (7:3) was added with shaking and the soln kept for 2 hr at room temp. The solvent was evaporated and the residue was taken up in $CH_2Cl_2$ (80 ml). The organic layer was washed with 5% $NaHCO_3$ (50 ml × 2), $H_2O$ (50 ml) and dried ($Na_2SO_4$). The solvent was evaporated and the remaining oily material (0.63 g, 74%) was found to be homogeneous according to tlc (BAWP and BE). Several attempts to crystallise the oily dimethylamide derivative failed; IR ($CHCl_3$), 1640–1670, 1510 $cm^{-1}$; NMR ($CDCl_3$) δ 0.9 (br d, 6H, $(CH_3)_2C$), 2.5 (t, J ~ 6 Hz, 2H, $CH_2CO$), 2.9 (d, 6H, $N(CH_3)_2$), 5.2 (s, 2H, $ArCH_2$), 7.4 (s, 5H, $C_6H_5$).

*L-Prolyl-L-leucyl-β-alanine methylamide* (**8**). A soln of **6** (0.4 g, 0.9 mmol) in EtOH (60 ml) was subjected to hydrogenation over PdO (60 mg). After 6 hr the soln was filtered and the solvent removed *in vacuo* to afford an oily product (280 mg, 98%), which was crystallised from EtOAc–Petroleum ether; yield 150 mg (53%); m.p. 135–136°; $[a]_D^{24}$ −32° (*c*, 1, DMF); IR ($CHCl_3$) 3440, 3340, 2960, 1660, 1515 $cm^{-1}$; NMR ($CDCl_3$) δ 1.0 (br d, 6H, $(CH_3)_2C$), 1.5–2.2 (br signal, 7H, $2CH_2$, $CHCH_2$), 2.5 (t, J = 6 Hz, 2H, $\alpha CH_2$Ala), 2.85 (d, J = 6 Hz, 3H, $CH_3$, on exchange with $D_2O$ it collapses to a singlet), 3.1 (t, J = 6 Hz, 2H, $\beta CH_2$Ala), 7.3 (q, J = 6 Hz, 1H, $CONHCH_3$), 7.8 (t, J ~ 6 Hz, 1H, $CONHCH_2$), 8.2 (d, J = 7.8 Hz, 1H, CONHCHLeu). (Found: C, 57.48; H, 8.89; N, 17.78. Calc. for $C_{15}H_{28}N_4O_3$: C, 57.69; H, 8.97; N, 17.94%).

*L-Prolyl-L-leucyl-β-alanine dimethylamide* (**9**). A soln of **7** (0.7 g, 1.5 mmol) in EtOH (60 ml) was hydrogenolysed over PdO (90 mg). After 6 hr the soln was filtered and the solvent was evaporated to give an oily product (490 mg, 98%) homogeneous according to tlc (BAWP and BE). Its purity was further ascertained by silica gel (50 g) column chromatography (2 × 60 cm)

using benzene as the eluant; IR ($CHCl_3$) 3420, 3320, 2970, 1630–1660, 1550 $cm^{-1}$; NMR ($CDCl_3$) $\delta$ 0.95 (br d, 6H, $(CH_3)_2C$), 2.95 (d, 6H, $N(CH_3)_2$), 7.5 (t, J ~ 6 Hz, 1H, CONH β-Ala), 8 (d, J = 7.8 Hz, 1H, CONHCHLeu).

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## REFERENCES

[1]All optically active amino acids are of the L-configuration. Abbreviations follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature: *J. Biol. Chem.* **247**, 977 (1972).

[2]A. S. Dutta, N. Anand and K. Kar, *Indian J. Chem.* **4**, 488 (1966).

[3]R. Walter, I. Bernal and L. Johnson, In *Chemistry and Biology of Peptides* (Edited by J. Meienhofer), p. 131. Ann Arbor, Michigan (1972).

[4]J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, Vol. 2, p. 926. Wiley, New York (1961).

[5]R. Boissonnas, *Helv. Chim. Acta* **34**, 974 (1951).

[6]J. G. Sheehan and G. P. Hess, *J. Am. Chem. Soc.* **77**, 1067 (1955).

[7]J. Matsoukas, P. Cordopatis and D. Theodoropoulos, *J. Org. Chem.* **42**, 2105 (1977).

[8]V. Bystrov, S. Portnova, V. Tsetlin, V. Ivanov and Y. Ovchinnikov, *Tetrahedron* **25**, 493 (1969).

[9]V. Bystrov, V. Ivanov, S. Portnova, T. Balashova and Y. Ovchinnikov, *Ibid.* **29**, 873 (1973).

[10]A. Tonelli, *J. Am. Chem. Soc.* **94**, 346 (1972).

[11]V. Bystrov, S. Portnova, T. Balashova, V. Tsetlin, V. Ivanov, P. Kostetzky and Y. Ovchinnikov, *Tetrahedron Letters* 5225 (1969).

[12]Y. Ovchinnikov, *The Chemistry of Polypeptides* (Edited by P. G. Katsoyannis), p. 172. Plenum Press, New York (1973).

[13]A. Dutta and A. Anand, *Indian J. Chem.* **3**(5), 232 (1965).