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Dipeptide from the Roots of *Zeyhera digitalis*

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Do extrato hexânico das raízes de um espécimen de *Zeyhera digitalis* foi isolado o dipeptídeo benzoato de N(N'-benzoil-S*-fenilalaninil)-S*-fenilalaninol (1). A estrutura deste dipeptídeo foi deduzida com base na análise de dados espectrais.

From the hexane extract from the roots of a specimen of *Zeyhera digitalis* the dipeptide N(N'-benzoyl-*S**-phenylalaninyl)-*S**-phenylalaninol benzoate (1) was isolated. The structure of this compound was established on the basis of its spectral data.

Keywords: Zeyhera digitalis, *Bignoniaceae, dipeptide*

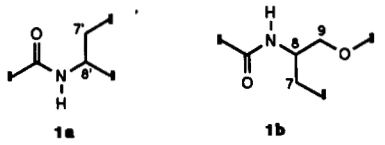
Introduction

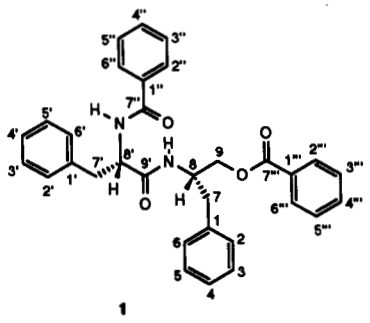
Continuing our chemical investigation of Brazilian plants we describe the isolation of a new dipeptide N(N'-benzoylalaninyl)phenylalaninol benzoate (**1**), from the roots of *Zeyhera digitalis*, family Bignoniaceae. This plant, commonly called "bolsa de pastor", "chapéu de frade" or "mandioquinha do campo", is typical of Brazilian savannah ("cerrado") vegetation and can be collected in the States of Paraná, São Paulo, Minas Gerais and Mato Grosso. From the stem wood of this species were isolated D-glucose, vanillic acid, veratric acid, lapachol and a natural dilignol-type compound[1].

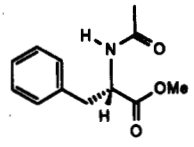
Results and Discussion

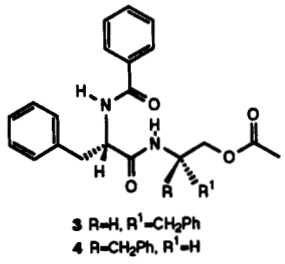
The dipeptide **1** was isolated from the *n*-hexane extract from roots by column chromatography followed by recrystallization from methanol (see "Experimental").

The IR spectrum of **1** revealed the presence of an amide function (3410, 1635 cm^{-1}) and an aromatic ring (1600, 1580, 1490, 700 cm^{-1}). Its molecular formula was determined as $C_{32}H_{30}O_4N_2$ (molecular weight 506) from the mass spectrum taken under an FAB-positive condition which showed an ion peak at m/z 507 (M + H) in combination with the ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) data. The presence of moieties **1a** [δ_H 6.55 (d,J = 7.6 Hz, NH),









4.92 (ddd,J = 7.6, 7.1 and 6.6 Hz, H-8'), 3.30 (dd,J = 13.8 and 6.6 Hz, H-7'a) and 3.21 (dd,J = 13.8 and 7.1 Hz, H-7'b)] and **1b** [δ_H 6.66 (d,J = 8.3 Hz, NH), 4.68-4.58 (m, H-8), 4.55 (dd,J = 11.2 and 3.4 Hz, H-9a), 4.04 (dd,J = 11.2 and 4.6 Hz, H-9b), 3.01 (dd,J = 13.7 and 6.6 Hz, H-7a) and 2.89 (dd,J = 13.7 and 8.3 Hz, H-7b)] was recognized by the homonuclear shift-correlated 2D ^{1}H-NMR (^{1}Hx^{1}H-COSY) spectrum. The ^{13}C-NMR chemical shifts (Table 2) at δ_C 65.51 (C-9), 54.47 (C-8'), 50.33 (C-8), 37.64 and 37.34 (C-7 and C-7') were comparable to the values described in the literature for N-acetylphenylalanine methyl ester (**2**)[2], N(N'-benzoyl-*S*-phenylalaninyl)-*S*-phenylalaninol acetate (**3**)[3,4] and N(N'benzoyl-*S*-phenylalaninyl)-*R*-phenylalaninol acetate (**4**)[3], confirming the presence of the partial structures **1a** and **1b**, and revealing the closely similar structures of **1**, **3** and **4** after additional analysis of the ^{1}H (Table 1) and ^{13}C-NMR (Table 2) spectra. In fact, the major difference observed in the comparative analysis of the ^{1}H and ^{13}C-NMR spectral data (Tables 1 and 2) of these dipeptides (**1**, **3** and **4**) was the presence of a benzoate group at C-9 in the natural product **1** ($C_{32}H_{30}O_4N_2$, molecular weight 506) and an acetate function in **3** and **4** ($C_{27}H_{28}O_4N_2$, molecular weight 444)[3,4]. The existence of two benzoyl groups in structure **1** was clearly defined by

Table 1. ^{1}H-NMR spectral data for dipeptide **1** (270 MHz) compared with the model compounds **3** and **4** (200 MHz[3] and 500 MHz[4]) in $CDCl_3$*.

H	1	3[a]	4[a]
2-6	7.54-7.18(m)	7.21-7.05(m); 7.78-7.12(m)	7.23-7.08(m); 7.72-7.10(m)
7a	3.01(dd,J=13.7,6.6)	2.75(ddd,J=13.2,7.3,6.4); 2.74(d,J=7)	2.78(dd,J=13.6,6.0); 2.85(dd,J=13.8,5.6)
b	2.89(dd,J=13.7,8.3)	2.75(ddd,J=13.2,7.3,6.4);	2.61(dd,J=13.6,8.1); 2.79(dd,J=13.8,8.0)
8	4.63(m)	4.35(m); 4.47-4.20(m)	4.34(m); 4.31-4.28(m)
9	4.55(dd,J=11.1,3.4)	3.92(dd,J=11.1,5.1); 3.95(dd,J=11.0,5.0)	3.98(dd,J=11.5,4.3); 3.96(dd,J=11.0,5.5)
	4.04(dd,J=11.1,4.6)	3.84(dd,J=11.1,4.3); 3.84(dd,J=11.0,5.0)	3.94(dd,J=11.5,5.5); 3.90(dd,J=11.0,6.5)
2'-6'	7.54-7.18(m)	7.30-7.21(m); 7.78-7.12(m)	7.32-7.24(m); 7.72-7.10(m)
7'	3.30(dd,J=13.8,6.6)	3.22(dd,J=13.7,6.0); 3.22(dd,J=14.5,6.0)	3.22(dd,J=13.7,6.0); 3.12(dd,J=13.6,6.5)
	3.21(dd,J=13.8,7.1)	3.07(dd,J=13.7,8.5); 3.07(dd,J=14.5,8.0)	3.07(dd,J=13.7,8.1); 2.99(dd,J=13.6,9.0)
8'	4.92(ddd,J=6.6,7.1,7.6)	4.78(ddd,J=8.5,7.7,6.0); 4.78(m)	4.79(ddd,J=8.1,7.3,6.0); 4.81(m)
2'',6''	7.72-7.63(m)	7.70(dd,J=7.3,1.2); 7.78-7.12(m)	7.70(dd,J=7.7,1.3); 7.72-7.10(m)
3'',5''	7.54-7.18(m)	7.42(t,J=7.3); 7.78-7.12(m)	7.42(t,J=7.7); 7.72-7.10(m)
4''	7.54-7.18(m)	7.51(dt,J=7.3,1.2); 7.78-7.12(m)	7.51(dt,J=7.7,1.3);7.72-7.10(m)
2''',6'''	7.72-7.63(m)	-	-
3''',5'''	7.54-7.18(m)	-	-
4'''	7.54-7.18(m)	-	-
HN-8	6.66(d,J=8.3)	6.10(d,J=8.5); 6.10(d,J=7.5)	6.74(d,J=7.3); 6.90(d,J=7.5)
HN-8'	6.55(d,J=7.6)	6.73(d,J=7.7); 6.85(d,J=7.5)	6.03(d,J=8.1); 6.31(d,J=8.0)
OAc	-	2.02(s); 2.03(s)	1.96(s); 2.00(s)

* Chemical shifts in δ (ppm) and coupling constants (J) in Hz. Homonuclear ^{1}Hx^{1}H-COSY 2D NMR spectra were also used for these assignments.
[a] The data of Ref. 3 (200 MHz) are described after those of 4 (500 MHz).

signals observed at δ_H 7.72-7.63 (m, 4H) in the ^{1}H-NMR (Table 1) spectrum, corresponding to four aromatic protons (H-2'', 6'' and H-2''', 6''') deshielded by anisotropic (prevailing) and mesomeric effects of an *orto*-carbonyl function, and at δ_C 131.39 and 132.00 (C-4'' and C-4''') in the ^{13}C-NMR spectrum (Table 2), consistent with two aromatic carbon atoms conjugated with a *para*-carbonyl group (mesomeric effect). The remaining signals revealed by the ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) are also consistent with structure **1** or its epimer at C-8.

The comparison of all spectral data of **1** with those described in the literature for the model dipeptides **3** and **4**, isolated from the red alga *Acantophora spicifera*[3] and (**3**) from *Hypericum japonicum* (family Guttiferae)[4], along with **2** (Table 2) allowed to establish the structure N(N'-benzoyl-*S*-phenylalaninyl)-*S*-phenylalaninol benzoate (**1**), or its respective enantiomer, for the dipeptide isolated from *Zeyhera digitalis*, a biodimer derivative involving two molecules of α-amino acid phenylalanine (one with the carboxyl group reduced to an alcohol function forming phenylalaninol) modified by a bioreaction of benzoylation at nitrogen (8'-NHCOC$_6$H$_5$) and oxygen (9-O-COC$_6$H$_5$) atoms. From the red alga *Acantophora spicifera*[3] the analogous dipeptide **4** was isolated (a minor constituent) containing the monomer D-amino acid phenylalaninol [(*R*)-phenyllalaninol], along with the epimer **3** (15.6 mg) present as the major constituent. Almost all naturally occurring amino acids have the *L* configuration at the α-carbon, with the exception of glycine (achiral molecule) and some *D*-amino acids obtained from the material comprising the cell walls of bacteria[5], along with the (*R*)-phenylalaninol moiety of **4**[3]. The ^{1}H-NMR spectra were used to distinguish the two natural stereoisomers **3** and **4** on the basis of a significant difference only between the multiplicity and coupling constant of the signal corresponding to the benzylic methylene protons at C-7 (numbering adopted for this paper): δ_H 2.74 (d,J = 7.0 Hz) in **3**, and δ_H 2.79 (dd,J = 14.0, 8.0 Hz) and 2.85 (dd,J = 13.8, 5.6 Hz) in **4**[3]. The four stereoisomeric structures (two pairs of enantiomers) were synthesized and the ^{1}H-NMR (200 and 500 MHz) spectral difference indicating that the diasteroisomers differ at C-4 was confirmed (Table 1)[3,4]. The ^{1}H-NMR (270 MHz) spectra (one- and two-dimensional ^{1}Hx^{1}H-COSY) of **1** clearly showed two signals [δ_H 3.01 (dd,J = 13.7 and 6.6 Hz) and 2.89 (dd,J = 13.7 and 8.3 Hz)] for the same benzylic methylene hydrogens at C-7, but in this structure (**1**) one benzoate group is sustained by C-9. The additional anisotropic effect attributed to the aromatic ring (phenyl group) of this benzoate group can be used to justify the different chemical surroundings for the two methylene hydrogens at C-7 of **1**. The comparative analysis of the chemical shifts of the hydrogen atoms bound to sp^3 carbons C-7, C-8 and C-9 of **1** and **3** allowed the conclusion that the major

Table 2. ^{13}C-NMR spectral data for dipeptide **1** (67.5 MHz) compared with the model compounds **2** (20 MHz)[2] and **3** (125 MHz)[4] in $CDCl_3$*.

C	1	3	2
1	**	136.67	
2,6	129.21	129.15	
3,5	128.72	128.65	
4	126.84	126.77	
7	37.34	37.49	
8	50.33	49.51	
9	65.51	64.61	
1'	**	136.72	136.30
2',6'	129.32	129.31	129.10
3',5'	128.81	128.72	128.40
4'	127.41	127.17	126.80
7'	37.64	38.43	37.70
8'	54.47	55.03	53.50
9'	**	170.25	
1''	**	133.75	
2'',6''	127.13	127.06	
3'',5''	128.69	128.61	
4''	131.32	131.91	
7''	**	167.11	
1'''	**	-	
2''',6'''	128.89	-	
3''',5'''	128.45	-	
4'''	132.00	-	
7'''	**	-	

* Chemical shifts in δ (ppm)

** Signals were not observed because of the small amount of sample used and the reduced scan number.

downfield shifts correspond to one of the 2H-7 [$\Delta\delta_H$ = 3.01 (**1**)-2.75 (**3**) = 0.26 ppm; $\Delta\delta_H$ = 2.89 (**1**)- 2.75 (**3**) = 0.14 ppm], H-8 [$\Delta\delta_H$ = 4.63 (**1**)-4.35 (**3**) = 0.28 ppm], and one of the 2H-9 [$\Delta\delta_H$ = 4.55 (**1**)-3.92 (**3**) = 0.63 ppm; $\Delta\delta_H$ = 4.04 (**1**)-3.84 (**3**) = 0.20 ppm] hydrogen atoms (Table 1). An analogous comparison of the spectral data of **3** and **4** [$\Delta\delta_H$ = 2.75 (**3**)-2.78 (**4**) = -0.03 ppm and $\Delta\delta_H$ = 2.75 (**3**)-2.61 (**4**) = 0.14 ppm, 2H-7; $\Delta\delta_H$ = 4.35 (**3**)-4.34 (**4**) = 0.01 ppm, H-8; $\Delta\delta_H$ = 3.92 (**3**)-3.98 (**4**) = -0.06 ppm and $\Delta\delta_H$ = 3.84 (**3**)-3.94 (**4**) = -0.10 ppm, 2H-9] revealed significant modification in the chemical shifts for only one of the signals for the hydrogen atoms of the methylene groups CH_2-7 ($\Delta\delta_H$ = 0.14 ppm, downfield shift) and CH_2-9 ($\Delta\delta_H$ = -0.10 ppm, upfield shift), whereas the signals of H-8 ($\Delta\delta_H$ = 0.01 ppm) continued practically unaltered. The

absorption of the H-8 of **1** at δ_H 4.63 ($\Delta\delta_H$ = 0.28 ppm) suggests a deshielding of $\Delta\delta_H$ = 0.28-0.14 ppm [H-8' (*e.g.*): $\Delta\delta_H$ = 4.92 (**1**)-4.78 (**3**) = 0.14 ppm, a value which can be used as a correction factor because this hydrogen atom is located at a position relatively distant from the benzoate groups] by an additional anisotropic effect (along with the carbonyl function) of the aromatic ring of the benzoate group. The observation of this effect at H-8 (downfield shift) of **1** requires a corresponding relative position of the aromatic ring and, consequently, one of the hydrogen atoms of the methylene group CH_2-7 suffers analogous influence [CH_2-7: $\Delta\delta_H$ = 0.26 (**1-3**)-0.14 = 0.12 ppm and $\Delta\delta_H$ = 0.14 (**1-3**)-0.14 = 0 ppm]. The orientation of this same aromatic ring may also justify the different anisotropic effect in the methylenic hydrogens at CH_2-9 [$\Delta\delta_H$ = 0.63 (**1-3**)- 0.14 = 0.49 ppm and $\Delta\delta_H$ = 0.20 (**1-3**)-0.06 ppm]. Thus, the different chemical shifts of signals for the methylenic hydrogens at C-7 of **1** can be assigned to an anisotropic effect of the aromatic ring of the benzoate group.

Experimental

Plant material – A specimen of the *Zeyhera digitalis* (Vell.) Hoehne, family Bignoniaceae, was collected and identified by Professor José Luiz Pedersoli (Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil) on December 8, 1985, in the municipality of Lagoa Santa, in the State of Minas Gerais, Brazil.

Extraction and isolation of **1** – The air-dried roots (0.9 kg) were extracted with *n*-hexane. The extract was evaporated under reduced pressure to give a residue (1.35 g) which was chromatographed on a silica gel column using CH_2Cl_2 and mixed of increasing polarity with an CH_2Cl_2-EtOAc as eluents. 185 fractions, 10 mL each, were collected. Fractions 105-100 eluted with CH_2Cl_2-EtOAc (10:1) furnished a colorless precipitate mixed with an oily material. This precipitate was separated and recrystallized from MeOH to yield the dipeptide **1** (15.0 mg).

N(N'-benzoyl-S-phenylalaninyl)-S*-phenylalaninol benzoate* (**1**). Colorless crystals from MeOH, m.p. 196-198 °C. IR υ_{max} cm^{-1}: 3410 (NH), 1635 (amide carbonyl), 1600, 1580, 1490, 700 (aromatic rings). ^{1}H-NMR (270 MHz, $CDCl_3$): Table 1. ^{13}C-NMR (67.5 MHz, $CDCl_3$): Table 2. FABMS (positive mode) *m/z*: 507 ($[M+H]^+$), 307 ($[M\text{-}Ph\text{-}PhCOOH]^+$), 289 ($[M\text{- }Ph\text{-}PhCOOH\text{-}H_2O]^+$ and/or $[M\text{- }PhCH_2\text{-}PhCH_2\text{-}H_2O\text{- }OH]^+$), 256 ($[M\text{-}MeOCOPh\text{-}PhH\text{- }2H_2O]^+$ and/or $[M\text{-}PhCOOH\text{- }PhCH_3\text{-}2H_2O]^+$), 105 ($[PhCO]^+$).

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References

1. J.C. da Silveira, O.R. Gottlieb and G.G. de Oliveira, *Phytochemistry* **14,** 1829 (1975) and M. Sc. Thesis cited.
2. L.-R.F. Johnson and W.C. Jankowski, *Carbon-13 NMR Spectra: A Collection of Assigned, Coded, and Indexed Spectra* (Robert E. Krieger Publishing Company, New York, 1978), Spectrum 435.
3. S. Wahidulla, L. D'Souza and S.V. Kamat, *Phytochemistry* **30,** 3323 (1991).
4. K. Ishiguro, S. Nagata, H. Fukumoto, M. Yamaki, S. Takagi and K. Isoi, *Phytochemistry* **30,** 3639 (1991).
5. T.W.G. Solomons, *Organic Chemistry,* (Wiley, New York, 5th ed., 1992), p. 1093.