Cyclopeptides from the Bark of *Discaria americana*

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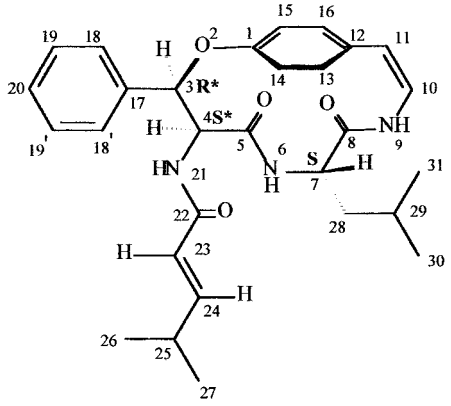
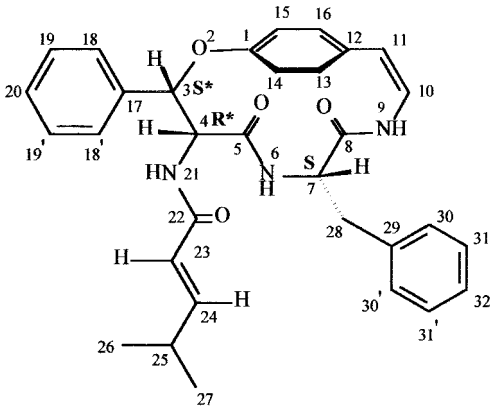
Two new cyclopeptides, named discarene C (**1**) and discarene D (**2**), have been isolated from the bark of *Discaria americana*, along with seven known cyclopeptide alkaloids. The structures of the new compounds were determined by spectroscopic methods, mainly NMR. The stereochemistry of the ring amino acid residues have been assigned by gas chromatography employing modified cyclodextrins as chiral stationary phases.

Discaria americana Gill & Hook (Rhamnaceae), locally called "quina do campo", is a small shrub found in Southern Brazil, Uruguay, Paraguay, and Argentina. Its root bark is used in traditional folk medicine for the treatment of diabetes, stomach disorders, and as a fever-lowering agent.[1,2] In continuation of our studies of Rhamnaceous plants,[3−6] we now report the isolation and structural elucidation of two new cyclopeptides (**1** and **2**), neutral compounds structurally related to cyclopeptide alkaloids, found together with seven known cyclopeptide alkaloids: franganine,[6] adouetine Y′ (= myrianthine B),[7] myrianthine A,[7] discarine B,[8] discarine C,[9] discarine D,[9] and adouetine Y.[10] The absolute configurations of the ring-bound amino acids of **1** and **2** were assigned by enantioselective gas chromatography.



discarene C (**1**)

Compound **1** was obtained as a white powder. Its positive FABMS displayed a prominent $[M + H]^+$ at *m/z* 490, which in combination with the ^{13}C NMR spectrum suggested that **1** had the molecular formula $C_{29}H_{35}N_3O_4$. The ^1H NMR spectrum ($CDCl_3$, 400 MHz) of **1** had four methyl doublets (δ 0.75, 0.64, 0.92, and 0.91). In the $^1H-^1H$ COSY spectrum, the doublets at δ 0.75 (J = 6.5 Hz, CH_3-30) and 0.64 (J = 6.5 Hz, CH_3-31) showed cross-peaks with H-29 at δ 1.18 (1H, m), which in turn showed two cross-peaks with the diasterotopic hydrogens H-28 and H-28′ at δ 1.29/1.79 (2H, m). H-28 and H-28′ also had cross-peaks with H-7 at δ 4.13 (1H, m) and this, with NH-6 at δ 6.38. This spin-system confirmed leucine as the α-amino acid of the ring. The side-chain unit $[(CH_3)_2CHCH{=}CH-]$ was characterized as follows: the signals at δ 0.92 (3H, d, J = 6.7 Hz) and 0.91 (3H, d, J = 6.7 Hz) were assigned to the CH_3-26 and CH_3-27, respectively. These two methyl signals showed cross-peaks with H-25 at δ 2.27 (1H, m), and this with H-24, which resonated at δ 6.36 (1H, d, J = 14 Hz). H-24 had a cross-peak with H-23 at δ 5.23 (1H, d, J = 14 Hz). The β-phenylserine, which is the hydroxylated amino acid of the ring, was identified from cross-peaks between H-3, H-4 and NH-21. H-3 resonated at δ 6.14 and showed a cross-peak with H-4 at δ 4.86, and this with NH-21 at δ 5.12 (1H, d). The hydrogenated carbons were assigned from the HMQC experiment, and the non-hydrogenated carbons were assigned with the aid of the HMBC spectrum. The NMR data are in agreement with related structures previously studied.[5,6,9]



discarene D (**2**)

Compound **2** was obtained as an amorphous powder. Its FABMS exhibited a molecular ion peak at *m/z* 524 [$(M + H)^+$ 34 amu more than that of **1**], which allowed us to deduce a molecular formula of $C_{32}H_{33}N_3O_4$, with the support of ^{13}C NMR (DEPT, HMQC, and HMBC). The ^1H NMR spectrum of **2** closely resembled that of **1** with the exception of H-3, which was shifted upfield from δ 6.14 to δ 5.80, and the resonances of two methyl groups were absent. From the ^1H NMR spectrum of **1** it was obvious that compound **2** was similar in structure. The difference was that the ring-bonded α-amino acid in **1** was leucine and in **2** it was phenylalanine. H-7 of the phenylalanine appeared as a multiplet at δ 4.06, whereas the diaste-

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reotopic hydrogens bound to C-28 appeared as two double doublets at δ 3.01 (1H, $J_{28,7} = 2.8$; $J_{28,28'} = 14.0$ Hz) and 2.60 (1H, $J_{28',7} = 6.4$; $J_{28,28'} = 14.0$ Hz). Unambiguous assignments of all hydrogens and carbons of **2** were made from a series of 2D NMR experiments (^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC).

To determine whether compounds **1** and **2** were artifacts from the extraction and isolation procedure, the same plant material was extracted with MeOH at room temperature, followed by extractions under neutral conditions. From this procedure we found that compounds **1** and **2** were both still present. This observation eliminated the possibility that compounds **1** and **2** were artifacts derived from the corresponding cyclopeptide alkaloids.

The absolute stereochemistry of the ring-bonded α-amino acids, leucine in compound **1** and phenylalanine in compound **2**, was determined by chiral phase gas chromatography (CPGC) using 2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin (2,6-Me-3-Pe-β-CD)[11] as the stationary phase. The *N*-trifluoroacetylated methyl ester of the amino acids leucine and phenylalanine in the enantiomerically pure L-form and racemic D,L-mixture were used as CPGC standards. By comparison of the t_R's of these standards with those of the corresponding amino acid derivatives, obtained from hydrolysates of **1** and **2**, it was possible to assign the absolute configurations of these two amino acid residues. In discarene C (**1**), leucine has the L(*S*) configuration, whereas in discarene D (**2**) the phenylalanine is in the L(*S*)-form.

The relative stereochemistry of the β-phenylserine unit (not found in the hydrolyzed compounds) was deduced by analysis of the NOESY spectrum. Since the stereochemistry of C-7 is absolute, the spatial position of H-7 (plane β) was the starting point for the assignment of the relative stereochemistry of C-3 and C-4. In the NOESY spectrum of **1**, H-7 does not show a cross-peak with H-6 (NH). In its turn, H-6 exhibited a NOE cross-peak with H-4, and this with H-3. This indicates that H-4 and H-3 are oriented on the same face of the cyclopeptide plane (α). In the NOESY spectrum of discarene D (**2**), H-3 showed a cross-peak with H-4, and this with NH-6. The latter showed a cross-peak with H-7, indicating that H-3, H-4, and H-7 are in the same plane (β). This evidence suggests that phenylserine has the L-*threo* ($3R^*/4S^*$) configuration in **1** and D-*threo* ($3S^*/4R^*$) configuration in **2**.

Experimental Section

General Experimental Procedures. Melting points were determined in a MQAPF-301 melting point apparatus and are uncorrected. IR spectra were recorded in KBr pellets on a Bruker IFS 28 spectrophotometer. Optical rotations were taken on a Perkin-Elmer 341 digital polarimeter. FABMS were obteined on a VG Analytical 70-150-S mass spectrometer equipped with a FAB ion source from a 3-nitrobenzylalcool matrix. NMR spectra were acquired on a Bruker DPX-400 operating at 400 and 100 MHz, for ^{1}H and ^{13}C, respectively. Chemical shifts are given in δ (ppm) using TMS as internal standard.

A 25 m fused silica column with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin[11] diluted with polysiloxane OV 1701 (1:1 w/w) and a column temperature of 85 °C was used in a Varian 3800 gas chromatograph, equipped with FID. TLC was performed on precoated TLC plates (Merck, silica 60 F-254).

Plant Material. The root bark of *D. americana* was collected in Santana do Livramento, Brazil, in January 1997 and identified by Prof. Renato Záchia. A voucher specimen (SMDB: 2829) is deposited in the herbarium of the Department of Botany, Universidade Federal de Santa Maria, RS, Brazil.

Extraction and Isolation. Dried powdered root bark (2.6 kg) was extracted with MeOH under reflux for 6 h and the resulting MeOH solution evaporated in vacuo to give 600 g of extract. This was dissolved in H_2O (80 mL) and acidified to pH 2−3. The acidic solution was extracted with ethyl ether (4 × 80 mL) to yield the acid ether extract (21 g). The aqueous solution was then made basic (pH 9) to yield the basic ether extract (7.0 g). The basic ether extract was submitted to CC on silica gel (700 g), with a gradient of $CHCl_3$−MeOH to afford discarene C (**1**, 43 mg), discarene D (**2**, 30 mg), adouetine Y′ (85 mg), franganine (500 mg), discarine B (13 mg), discarine C (15 mg), discarine D (18 mg), myrianthine A (20 mg), and adouetine Y (10 mg). The known compounds were identified by spectroscopic methods and comparison with reported data.[6−10]

Discarene C (**1**): white powder, mp 297 °C (decomp); $[\alpha]^{20}_D$ −51.7 (*c* 0.2, MeOH−$CHCl_3$, 1:1); TLC 0.28 ($CHCl_3$−MeOH, 98:2); IR (KBr) ν_{max} 3280, 1625, 1234 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.14 (1H, d, J = 6.2 Hz, H-3), 4.86 (1H, dd, J = 6.2, 8.8 Hz, H-4), 6.38 (1H, NH-6), 4.13 (1H, m, H-7), 6.64 (1H, d, J = 10.2 Hz, NH-9), 6.74 (1H, dd, J = 7.6, 10.2 Hz, H-10), 6.37 (1H, d, J = 7.6 Hz, H-11), 7.17 (1H, H-13), 7.33 (1H, H-14), 7.41 (1H, H-15), 7.15 (1H, H-16), 7.52 (2H, H-18, H-18′), 7.47 (2H, H-19, H-19′), 7.43 (1H, H-20), 5.12 (1H, d, J = 8.8 Hz, NH-21), 5.23 (1H, d, J = 14.0 Hz, H-23), 6.36 (1H, dd, J = 6.8, 14.0 Hz, H-24), 2.27 (1H, m, H-25), 0.92 (3H, d, J = 6.7 Hz, CH_3-26), 0.91 (3H, d, J = 6.7 Hz, CH_3-27), 1.79, 1.29 (each 1H, m, H-28, H-28′), 1.18 (1H, m, H-29), 0.75 (3H, d, J = 6.5 Hz, CH_3-30), 0.64 (3H, d, J = 6.5 Hz, CH_3-31); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 155.2 (C-1), 81.9 (C-3), 56.1 (C-4), 171.1 (C-5), 52.5 (C-7), 167.4 (C-8), 125.4 (C-10), 114.8 (C-11), 132.6 (C-12), 130.3 (C-13), 123.6 (C-14), 129.1 (C-15), 132.1 (C-16), 136.5 (C-17), 127.8 (C-18, C-18′), 128.9 (C-19, C-19′), 123.4 (C-20), 165.8 (C-22), 119.1 (C-23), 153.0 (C-24), 30.7 (C-25), 21.2 (C-26), 21.1 (C-27), 39.1 (C-28), 23.1 (C-29), 22.5 (C-30), 20.4 (C-31); FABMS *m*/*z* 490 $[M + H]^+$; *anal*. C 71.30%, H 7.19%, N 8.44%, calcd for $C_{29}H_{35}N_3O_4$, C 71.54%, H 7.21%, N 8.58%.

Discarene D (**2**): amorphous powder ($CHCl_3$); $[\alpha]^{25}_D$ −176.0 (*c* 0.2, MeOH−$CHCl_3$, 1:1); TLC 0.23 ($CHCl_3$−MeOH, 98:2); IR (KBr) ν_{max} 3260, 1648, 1277, 1261 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.80 (1H, d, J = 7.6 Hz, H-3), 4.88 (1H, dd, J = 7.6, 10.0 Hz, H-4), 8.03 (1H, d, J = 8.2 Hz, NH-6), 4.06 (1H, m, H-7), 6.51 (2H, H-10, H-11), 6.97−7.28 (4H, H-13, H-14, H-15, H-16), 7.42 (2H, H-18, H-18′), 6.97−7.28 (3H, H-19, H-19′, H-20), 7.59 (1H, d, J = 10.0 Hz, NH-21), 5.32 (1H, d, J = 14.0 Hz, H-23), 6.29 (1H, dd, J = 6.6, 14.0 Hz, H-24), 2.25 (1H, m, H-25), 0.90 (3H, d, J = 6.0 Hz, CH_3-26), 0.92 (3H, d, J = 6.0 Hz, CH_3-27), 3.01, 2.60 (each 1H, dd, J = 6.4, 14 Hz, H-28, 28′), 6.67−7.28 (5H, H-30, H-30′, H-31, H-31′, H-32); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 155.1 (C-1), 82.2 (C-3), 55.7 (C-4), 170.5 (C-5), 55.2 (C-7), 167.5 (C-8), 125.8 (C-10), 119.2 (C-11), 131.4 (C-12), 130.7 (C-13), 120.9 (C-14), 122.4 (C-15), 129.9 (C-16), 137.6 (C-17), 128.1 (C-18, C-18′), 127.2 (C-19, C-19′), 127.4 (C-20), 163.5 (C-22), 120.9 (C-23), 148.2 (C-24), 29.8 (C-25), 21.1 (C-26), 21.3 (C-27), 36.1 (C-28), 137.1 (C-29), 128.5 (C-30), 127.9 (C-31), 125.6 (C-32); FABMS *m*/*z* 524 $[M + H]^+$; *anal*. C 73.20%, H 6.34%, N 7.95%, calcd for $C_{32}H_{33}N_3O_4$, C 73.40%, H 6.34%, N 7.95%.

Hydrolysis of Compounds 1 and 2. Total hydrolysis of **1** and **2** was performed by heating them in a sealed tube at 110 °C with 6 N HCl for 15 h. The acidic solutions were concentrated and the residues were treated as described for amino acids.[12]

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References and Notes

(1) Correa, M. P. *Dicionario de Plantas Uteis do Brasil e das exóticas cultivadas*; Imprensa Nacional: Rio de Janeiro, 1978; p 568.
(2) Záchia, N. R. B.; Moraes, D. *Pesquisas Botânicas* **1999**, *49*, 121−142.

(3) Machado, E. C.; Filho, A A.; Morel, A. F.; Monache, F. *J. Nat. Prod.* **1995**, *58*, 548−553.
(4) Morel, A. F.; Bravo, R. V. F.; Reis, F. D. A. M.; Ruveda, E. A. *Phytochemistry* **1979**, *18*, 473−477.
(5) Morel, A. F.; Dessoy, E. S. M.; Moreira, J. J.; Meneses, A. S.; Mostardeiro, M. A.; Zanatta, N.; Wessjohann, L. A. *Phytochemistry* **1998**, *47*, 125−129.
(6) Voelter, W.; Morel A. F. *Z. Naturforsch.* **1987**, *42b*, 467−472.
(7) Marchand, J.; Monseur, X. *Ann Pharm. Fr.* **1968**, *26*, 771−778.
(8) Chang, C.-J.; Hagaman, E. W.; Wenkert, E.; Sierra, M. G.; Mascaretti, O. A.; Merkuza,V. M.; Rúveda, E. A *Phytochemistry* **1974**, *13*, 1273−1279.
(9) Digel, M.; Morel, A. F.; Layer, H.; Biermann, J.; Voelter, W. *Hoppe Seyler's Z. Physiol. Chem.* **1983**, *364*, 1641−1643.
(10) Païs, M.; Marchand, J.; Jarreau, F. X.; Goutarel, R. *Bull. Soc. Chim.* **1968**, *3*, 1145−1148.
(11) König, W. A.; Icheln, D.; Runge, T.; Pforr, I.; Krebs, A. *J. High Resolut. Chromatogr.* **1990**, *13*, 702−703.
(12) Silva, U. F.; Cardoso, C. D.; Zanatta, N.; Morel, A. F.; Icheln, D.; Gehrcke, B. *Phytochem. Anal.* **1996**, *7*, 20−23.

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