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FOUR CYCLOPEPTIDE ALKALOIDS FROM *DISCARIA LONGISPINA*

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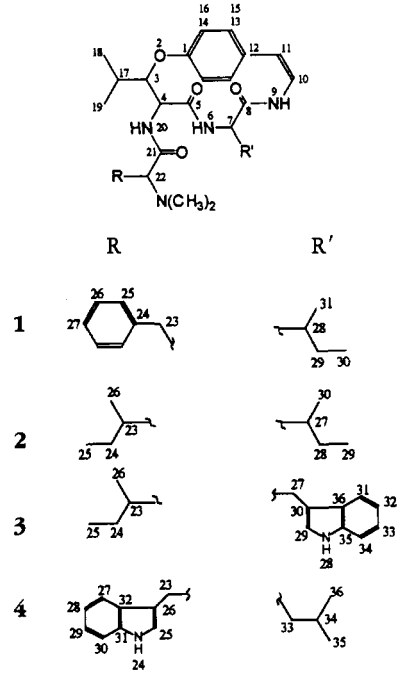
ABSTRACT.—The isolation of the cyclopeptide alkaloids, adoutine-Y′, discarine-B, discarine-E, and discarine-X, a new 14-membered cyclopeptide alkaloid from *D. longispina*, are reported. The structure of the new alkaloid was elucidated by spectroscopic methods and by chemical degradation.

Discaria longispina Miers (Rhamnaceae), usually known as "quina-do-campo," is a small shrub found in southern Brazil and Argentina (1). The root bark is employed in folk medicine as a tonic and agent for the treatment of fevers (1).

Several cyclopeptide alkaloids have been isolated previously from the approximately eight species of the genus *Discaria* (1). In the present paper, we report the isolation and characterization of four peptide alkaloids from *D. longispina*; these are adoutine-Y′ [**1**] (2), discarine-E [**2**] (3), discarine-B [**3**] (4), and discarine-X [**4**]; the last is a novel 14-membered cyclopeptide alkaloid.

RESULTS AND DISCUSSION

The absorption bands of the ir and uv spectra of **1** and **2** were very similar. Their ir spectra were typical of peptide alkaloids and exhibited bands for -NH, -NHCO-, a



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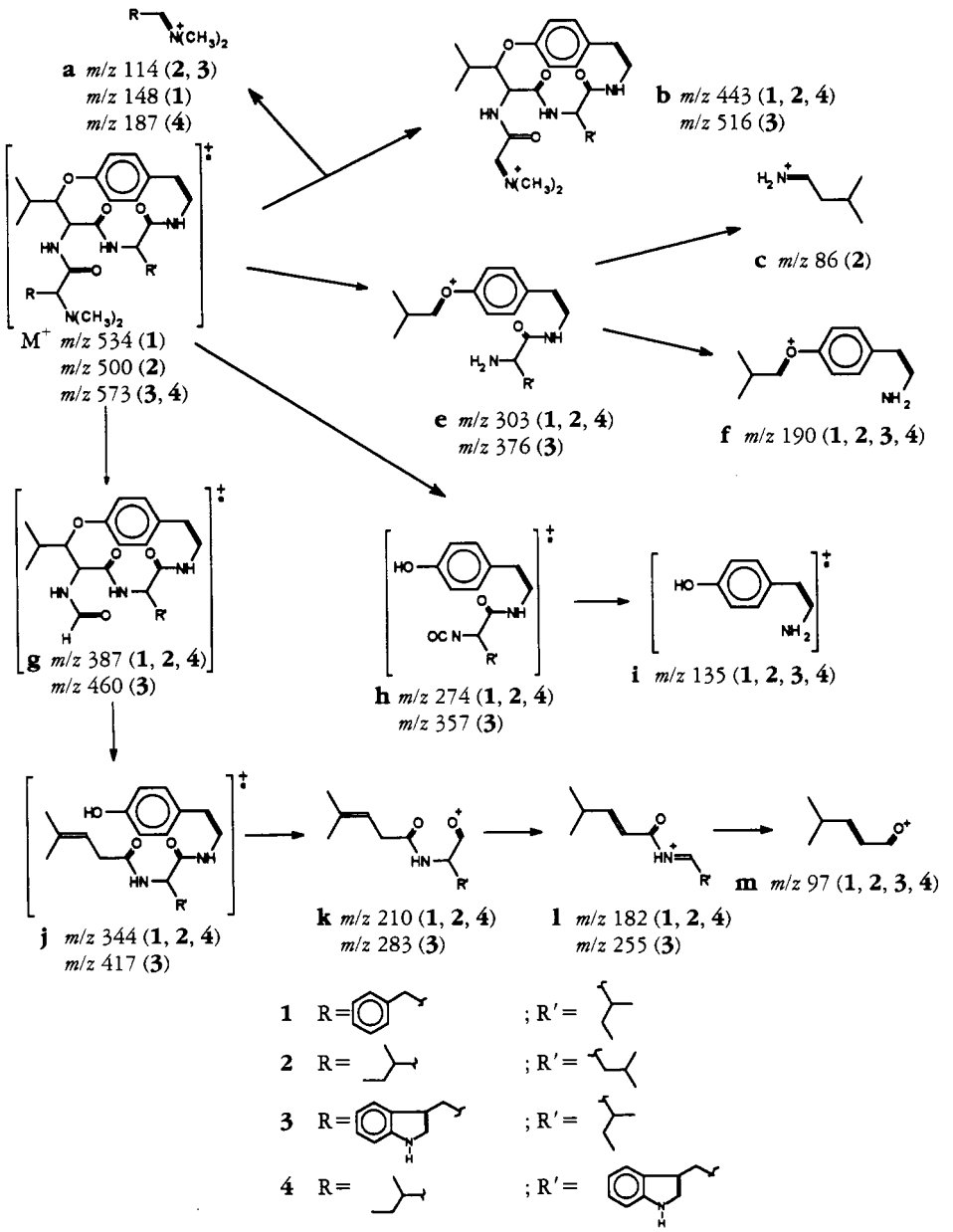
conjugated cis-1,2-disubstituted C=C double bond, and Ar-O-X. The uv spectra of **1** and **2** displayed only strong end absorption at 218 and 222 nm, respectively, and suggested the presence of a *p*-hydroxystyrylamine chromophore as found in certain 14-membered cyclopeptide alkaloids (5). The molecular formula of **1** was derived from eims and elemental analysis as $C_{31}H_{42}N_4O_4$. The eims (see Scheme 1) showed a small $[M]^+$ peak at *m/z* 534, and the ms fragmentation indicated that this cyclopeptide alkaloid belongs to the frangulanine sub-group (5), because the ions **i** at *m/z* 135, **f** at *m/z* 190, **e** at *m/z* 303, and **b** at *m/z* 443 are typical of *p*-hydroxystyrylamine and hydroxyleucine units. The ion **a** at *m/z* 148 showed that the *N,N*-dimethylphenylalanine group was the



SCHEME 1. Mass spectral fragmentations of compounds **1–4**.

terminal amino acid. The ^{1}H-nmr spectrum of **1** (Table 1) showed characteristic signals of the amino acid isoleucine from the presence of an ethyl group (t at δ 0.75, J=6.5 Hz and d at δ 0.40, J=6.5 Hz), and of a β-hydroxyleucine unit, from the occurrence of typical signals of an *i*-propyl unit (d at δ 1.05 and 1.30; J=6.5 Hz). Two olefinic protons, H-10 (dd at δ 6.45; J=7.5 and 9.5 Hz) as well as H-9 (d at δ 6.70; J=9.5 Hz) indicated the presence of a styrylamine moiety. Furthermore, a broad singlet (6H at δ 2.30) and a multiplet (integration for 9 protons) in the aromatic region suggested the presence of a *N,N*-dimethylphenylalanine group. In accordance with the data mentioned above, and after acidic hydrolysis, **1** was identified as adoutine-Y′, which was previously isolated from *Melochia corchorifolia* (2) and *Myrianthus arboreus* (6).

The eims of **2** showed a weak $[M]^+$ ion at *m/z* 500 (34 mass units fewer than that of **1**) and suggested, together with the elemental analysis, the molecular formula $C_{28}H_{44}N_4O_4$. The principal fragment ions **i**, **f**, **e**, and **b** were exactly the same as those observed for **1**. This indicated that the cyclopeptide alkaloid **2** bears a leucine or isoleucine unit, a β-hydroxyleucine unit, and a *p*-hydroxystyrylamine group as constituent amino acids of the macrocycle. Moreover, the presence of a *N,N*-dimethylisoleucine or leucine unit as

TABLE 1. ^{1}H-Nmr Spectral Data of Compounds **1–4**.

Proton(s)	Compound			
	1[a]	**2**[a]	**3**[b]	**4**[b]
3	5.10 (dd)	4.80 (dd)	4.90 (dd)	4.80 (dd)
4	4.55 (dd)	4.40 (dd)	4.52 (dd)	4.44 (dd)
6 (NH)	5.80 (dd)	7.25 (d)	6.50 (d)	7.38 (d)
7	3.91 (dd)	3.90 (dd)	4.20 (m)	3.70 (dd)
9 (NH)	6.70 (d)	7.65 (d)	6.85 (d)	7.70 (d)
10	6.45 (dd)	6.20 (dd)	6.20 (dd)	6.15 (dd)
11	6.60 (d)	6.65 (d)	6.40 (d)	6.64 (dd)
13–16	7.08–7.30 (m)	6.80–7.00 (dd)	6.80–7.45 (m)	7.05–7.50 (m)
17	1.80 (m)	2.25 (m)	2.10 (m), 1.25 (d)	2.20 (m)
18	1.30 (d)	0.90 (d)		1.10 (d)
19	1.05 (d)	1.10 (d)	1.05 (d)	1.25 (d)
20 (NH)	7.80 (d)	8.10 (d)	7.80 (d)	8.24 (d)
22	3.15 (dd)	2.75 (d)	2.63 (d)	3.40 (dd)
23	2.05 (dd)	1.70 (d)	1.80 (d)	2.95 (d)
24	6.80–7.40 (m)	1.50, 1.60 (m)	1.20, 1.55 (m)	10.78 (NH)
25	6.80–7.40 (m)	0.80 (t)	0.90 (t)	6.80–7.30 (m)
26	6.80–7.40 (m)	0.65 (d)	0.75 (d)	6.80–7.30 (m)
27	6.80–7.40 (m)	1.28 (m)	2.90, 3.10 (m)	6.80–7.30 (m)
28	1.30 (m)	1.40 (m)	10.25 (s)	6.80–7.30 (m)
29	1.55 (m)	0.75 (t)	7.14 (d)	6.80–7.30 (m)
30	0.75 (t)	0.70 (d)		6.80–7.30 (m)
31	0.40 (d)		7.50 (d)	6.80–7.30 (m)
32			7.28 (t)	2.15 (s)
33			7.05 (t)	1.30, 1.74 (m)
34			7.40 (d)	1.80 (m)
35				0.60 (d)
36				0.62 (d)
N,N-dimethyl	2.30 (s)	2.20 (s)	2.30 (s)	2.20 (s)

[a]Spectra were run in $CDCl_3$ and chemical shifts (δ in ppm) are given downfield from TMS. The spectral assignments of **2** and **3** were made with the aid of the ^{13}C-nmr, DEPT, spin decoupling, and 2D-shift-correlated [^{1}H×^{1}H COSY and ^{1}H×^{13}C-HETCOSY (optimized for $^1J_{CH}$)] nmr. The spectral data of **2** and **3** were used as references for the assignment of **1** and **4**.

[b]Spectra were run in DMSO-d_6.

the terminal amino acid was deduced from the occurrence of an intense peak **a** at *m/z* 114 and the $[M]^+$ ion at *m/z* 500 (Scheme 1). The most significant difference between **1** and **2** is in their *N,N*-dimethyl amino acid component (leucine or isoleucine in **2** and phenylalanine in **1**).

The ^{1}H-nmr spectrum of **2** (Table 1) confirmed the structure proposed. On analysis of the 2D COSY nmr spectrum (7), the assignment of all protons, including those of the aromatic region, was possible. The protons of each amino acid, namely, β-hydroxyleucine (d at δ 0.90 and 1.10; J=6.6 Hz), isoleucine (t at δ 0.75 and d at δ 0.70; J=6.6 Hz), and a substituted *N,N*-dimethylisoleucine (d at δ 0.65 and t at δ 0.80; J=6.6 Hz) could be assigned. Furthermore, the two olefinic protons, H-10 (dd at δ 6.20; J=5.5 and 7.5 Hz) and H-11 (d at δ 6.65; J=7.5 Hz), and the four aromatic protons (δ 6.80–7.10) observed indicated the presence of a styrylamine moiety. Based on these data, **2** could be identified as discarine-E, previously isolated from *Discaria febrifuga* (3).

The uv and ir spectra of the alkaloids **3** and **4** were also very similar. Each uv spectrum showed end absorption due to an aromatic chromophore (220 nm) and bands indicative of an indole group (288, 278, and 267 nm). These are basically the only differences between compounds **3** and **4** and compounds **1** and **2**. The eims of **3** (see Scheme 1) showed a weak $[M]^+$ at *m/z* 573 suggesting, together with the elemental analysis, the molecular formula $C_{33}H_{43}N_5O_4$. In the ms fragmentation pattern (Scheme 1) the ions **i** (*m/z* 135) and **f** (*m/z* 190) could be observed which characterize the attachment of β-hydroxyisoleucine bound to the *p*-position of the styrylamine. Furthermore, the ions **a** (*m/z* 114, 100%) and **h** (*m/z* 357) are indicative of leucine or isoleucine and tryptophan units as the *N,N*-dimethylated and ring-bonded amino acids, respectively. The ^{1}H-nmr spectrum of **3** (Table 1) exhibited characteristic signals of the constituent amino acid *N,N*-dimethylisoleucine by the presence of an ethyl group (t at δ 0.90; J=6.5 Hz and d at δ 0.75; J=6.5 Hz). A complex broad signal in the aromatic region with 10 protons and a singlet at δ 10.25 (indole NH) suggested the presence of tryptophan. The COSY and DEPT nmr spectra then permitted identification of this alkaloid as discarine-B [**3**], previously isolated from *Discaria americana* (8).

As mentioned above, the last compound [**4**] exhibited essentially the same uv and ir spectra as reported for discarine-B [**3**], thereby indicating the presence of an indole group. The eims (see Scheme 1) showed a $[M]^+$ at *m/z* 573, suggesting that **4** has the same molecular formula ($C_{33}H_{43}N_5O_4$) as **3**. The styrylamine unit was deduced from the occurrence of peaks **i** (*m/z* 135), **f** (*m/z* 190), and **e** (*m/z* 303) (Scheme 1). The fragment ion **c** (*m/z* 86) indicated the presence of leucine as the ring-bonded amino acid. A tryptophan residue was determined to be *N,N*-dimethyltryptophan by the occurrence of an intense peak **a** at *m/z* 187 (100%) (Scheme 1). The ^{1}H-nmr spectrum (Table 1) of **4**, combined with the analysis of the COSY spectrum and literature data (10,11), provided further information. The four diastereotopic methyl groups appeared as sharp doublets (J=6.7 Hz) at δ 0.60 (C-35), 0.62 (C-36), 1.10 (C-18), and 1.25 (C-19) ppm. The first pair of doublets is typical of leucine as the ring-bonded amino acid, which could be confirmed by acid hydrolysis (9). The β-hydroxyleucine unit was characterized by the occurrence of doublets in expected positions (11), at δ 1.10 and 1.25 (J=6.7 Hz). A complex broad signal in the aromatic region with nine protons and a broad singlet at δ 10.78 ppm (NH-24) suggested the presence of an *N,N*-dimethyltryptophan unit and a styrylamine moiety. Furthermore, the two olefinic protons, H-10 and H-11, could be assigned to the signals at δ 6.15 (dd, J=4.3 and 7.4 Hz) and δ 6.64 (d, J=7.4 Hz), respectively. The interpretation of all protons (including those of the aromatic region), as well as the bonding sites between the styrylamine moiety, the β-hydroxyleucine, leucine, and *N,N*-dimethyltryptophan units, permitted elucidation of the structure as a new peptide alkaloid, named discarine-X [**4**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-Nmr: $CDCl_3$ and DMSO at 80 MHz (Bruker AC 80), and 300 MHz (Varian Gem 300). Chemical shifts are given in δ (ppm) using TMS as internal reference; eims: direct probe insert, at 70 eV (gc/ms Hewlett-Packard HP 5980/5988 A); tlc: Si gel plates 60 PF_{254} (Merck), cellulose plates and Whatman paper No. 1. The alkaloids were detected by spraying tlc plates with Dragendorff's reagent. The amino acids were detected by spraying with ninhydrin reagent.

PLANT MATERIAL.—The plant material was collected in August 1992, in the region of São Martinho, RS, Brazil, and identified by Prof. A. Alvarez Filho, Universidade Federal de Santa Maria. Voucher specimens are kept in the Herbarium of the Department of Botany, Universidad Federal de Santa Maria, RS, Brazil.

EXTRACTION AND ISOLATION.—The dried and ground root bark (1.6 kg) of *Discaria longispina* was extracted with MeOH in a Soxhlet apparatus. After evaporation of the solvent, the crude extract was diluted with H_2O (1:1), acidified with 2% HCl, and extracted with 5 liters of Et_2O. The aqueous phase was basified with NH_4OH to pH 9.0 and extracted with Et_2O until a Dragendorff's test was negative. The Et_2O solution was evaporated *in vacuo* to give 1.9 g of a mixture of alkaloids (1.2 g/kg). This mixture was fractionated by cc with Si gel G 60 and eluted with $CHCl_3$-MeOH (99:1) (fractions I–III) and $CHCl_3$-MeOH (90:10) (fraction IV–VIII). Alkaloids **1** and **2** were found in fraction II, **3** in fraction IV, and **4** in fraction VI. Compound **1** was isolated by cc over Si gel G 60 and eluted with $CHCl_3$. It was purified by recrystallization with MeOH. Compounds **2–4** were isolated and purified by prep. tlc with elution in $CHCl_3$-MeOH (90:5).

Adoutine Y' [**1**].—Mp 296–299°; 80 mg; $[\alpha]^{25}D$ −350° (*c*=0.1, $CHCl_3$); ir ν max (KBr) 3280, 2790, 1680, 1635, 1240 cm^{-1}; ^{1}H nmr data ($CDCl_3$), see Table 1; ^{13}C nmr ($CDCl_3$) δ 172.5 (C-21), 171.5 (C-5), 166.7 (C-8), 155.9 (C-1), 125.5 (C-10), 123.1 (C-11), 81.7 (C-3), 69.1 (C-22), 54.9 (C-4), 54.2 (C-7), 41.7 (-NMe_2), 36.3 (C-23), 34.0 (C-28), 29.2 (C-17), 23.9 (C-29), 20.4 (C-19), 15.1 (C-18), 14.2 (C-31), 10.8 (C-30); eims *m/z* 534 $[M]^+$, 443 (42), 303 (2), 210 (4), 195 (8), 182 (6), 167 (10), 148 (100), 135 (28), 97 (20), 86 (32); *anal.*, found C 69.4, H 8.08, N 10.8, $C_{31}H_{42}N_4O_4$ requires C 69.68, H 7.91, N 10.47.

Discarine F [**2**].—Mp 270–273°; 55 mg; $[\alpha]^{25}D$ +236° (*c*=0.5, HOAc); ir ν max (KBr) 3270, 2780, 1680, 1640, 1285 cm^{-1}; ^{1}H-nmr data ($CDCl_3$), see Table 1; ^{13}C nmr ($CDCl_3$) δ 172.8 (C-21), 171.9 (C-5), 168.8 (C-8), 155.9 (C-1), 130.8 (C-12), 129.1 (C-13), 128.6 (C-16), 125.5 (C-14), 121.5 (C-15), 119.5 (C-11), 82.0 (C-3), 62.0 (C-22), 55.9 (C-4), 51.6 (C-7), 41.9 (-NMe_2), 33.9 (C-23), 33.5 (C-27), 26.5 (C-24), 24.0 (C-28), 19.5 (C-19), 14.6 (C-26), 14.5 (C-30), 14.2 (C-18), 11.9 (C-25), 10.6 (C-29); eims *m/z* 500 $[M]^+$, 443 (18), 387 (4), 210 (8), 195 (3), 182 (28), 135 (9), 114 (100), 86 (6), 72 (20); *anal.* found C 67.77, H 9.30, N 11.29, $C_{28}H_{44}N_4O_4$ requires C 67.17, H 8.85, N 11.19.

Discarine B [**3**].—Mp 246–248°; 210 mg; $[\alpha]^{25}D$ −154° (*c*=0.5, $CHCl_3$); uv λ max (EtOH) 229, 270, 278, 289 nm; ir ν max (KBr) 3400, 2800, 1650, 1620, 1230 cm^{-1}; ^{1}H-nmr data ($CDCl_3$), see Table 1; ^{13}C nmr ($CDCl_3$) δ 171.3 (C-21), 171.0 (C-5), 167.6 (C-8), 156.0 (C-1), 135.0 (C-35), 130.0 (C-12), 129.7 (C-13), 128.4 (C-16), 126.1 (C-36), 124.7 (C-10), 122.9 (C-11), 120.2 (C-29), 119.6 (C-14), 119.1 (C-15), 117.5 (C-33), 110.2 (C-34), 107.6 (C-32), 81.4 (C-3), 71.5 (C-22), 54.2 (C-4), 53.0 (C-7), 40.2 (NMe_2), 27.3 (C-17), 26.5 (C-24), 24.8 (C-27), 21.3 (C-19), 13.9 (C-18), 13.8 (C-26), 10.0 (C-25); eims *m/z* 573 $[M]^+$, 516 (20), 460 (8), 361 (10), 304 (18), 195 (10), 190 (5), 170 (58), 135 (70), 130 (78), 117 (100), 97 (38); *anal.*, found C 69.16, H 7.81, N 12.11, $C_{33}H_{43}N_5O$ requires C 69.07, H 7.56, N 12.21.

Discarine X [**4**].—Mp 295–298°; 38 mg; $[\alpha]^{25}D$ −184° (*c*=0.5, MeOH), uv λ max (EtOH) 218, 270, 240, 288 nm; ir ν max (KBr) 3270, 2800, 1670, 1620, 1230 cm^{-1}; ^{1}H-nmr data (DMSO), see Table 1; ^{13}C nmr ($CDCl_3$) δ 172.2 (C-21), 171.0 (C-5), 167.8 (C-8), 156.2 (C-1), 136.2 (C-31), 128.2 (C-32), 125.5 (C-10), 123.5 (C-11), 122.8 (C-25), 120.9 (C-29), 118.4 (C-28), 118.0 (C-27), 112.8 (C-26), 110.8 (C-30), 88.8 (C-22), 81.6 (C-3), 55.0 (C-4), 54.2 (C-7), 41.8 (-NMe_2), 40.2 (C-33), 28.0 (C-17), 26.6 (C-34), 23.0 (C-35), 22.5 (C-36), 21.9 (C-23), 20.9 (C-19), 20.5 (C-36), 14.1 (C-18); eims *m/z* 573 $[M]^+$, 530 (3), 387 (10), 303 (7), 274 (10), 210 (10), 195 (15), 190 (12), 187 (100), 135 (18), 130 (50), 97 (10), 86 (22); *anal.*, found C 69.16, H 7.81, N 12.11, $C_{33}H_{43}N_5O_4$ requires C 69.07, H 7.56, N 12.21.

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