Pergamon

0031-9422(94)00924-4

Phytochemistry, Vol. 39, No. 2, pp. 431–434, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0031-9422/95 $9.50 + 0.00

CYCLOPEPTIDE ALKALOIDS OF *DISCARIA FEBRIFUGA* (RHAMNACEAE)

ADEMIR FARIAS MOREL,* EMÍLIA CAROLINA MACHADO and LUDGER A. WESSJOHANN†

Departamento de Química, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil; †Institut für Organische Chemie, Universität München (LMU), Karlstraße 23, 80333 München, Germany

(*Received* 4 *October* 1994)

Key Word Index—*Discaria febrifuga*; Rhamnaceae; peptide alkaloids; discarine-L; spectroscopic structure elucidation.

Abstract—From the methanol extract of the root bark of *Discaria febrifuga* Mart., in addition to the already described alkaloid, a new peptide alkaloid discarine-L has been isolated and its structure elucidated. This alkaloid differs from most of the known 14-membered peptide alkaloids by a 2-hydroxy-2-phenethylamine residue.

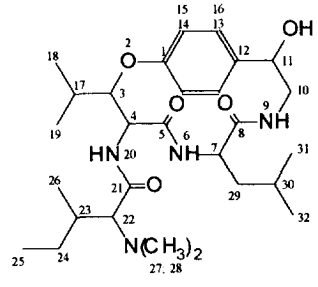
INTRODUCTION

Discaria febrifuga Mart. is a small shrub in the Rhamnaceae found in Uruguay, Paraguay, Argentine and Southern Brazil [1]. Its root bark is employed in folk medicine as a potent antithermic agent. In previous studies several peptide alkaloids were reported from this plant [2–7]. Here we report on the structure of a new peptide alkaloid, discarine-L (**1**), isolated from the root bark of this plant.

RESULTS AND DISCUSSION

From the methanolic extract of the root bark of *D. febrifuga*, a new peptide alkaloid, **1**, was isolated as presented in the Experimental section.

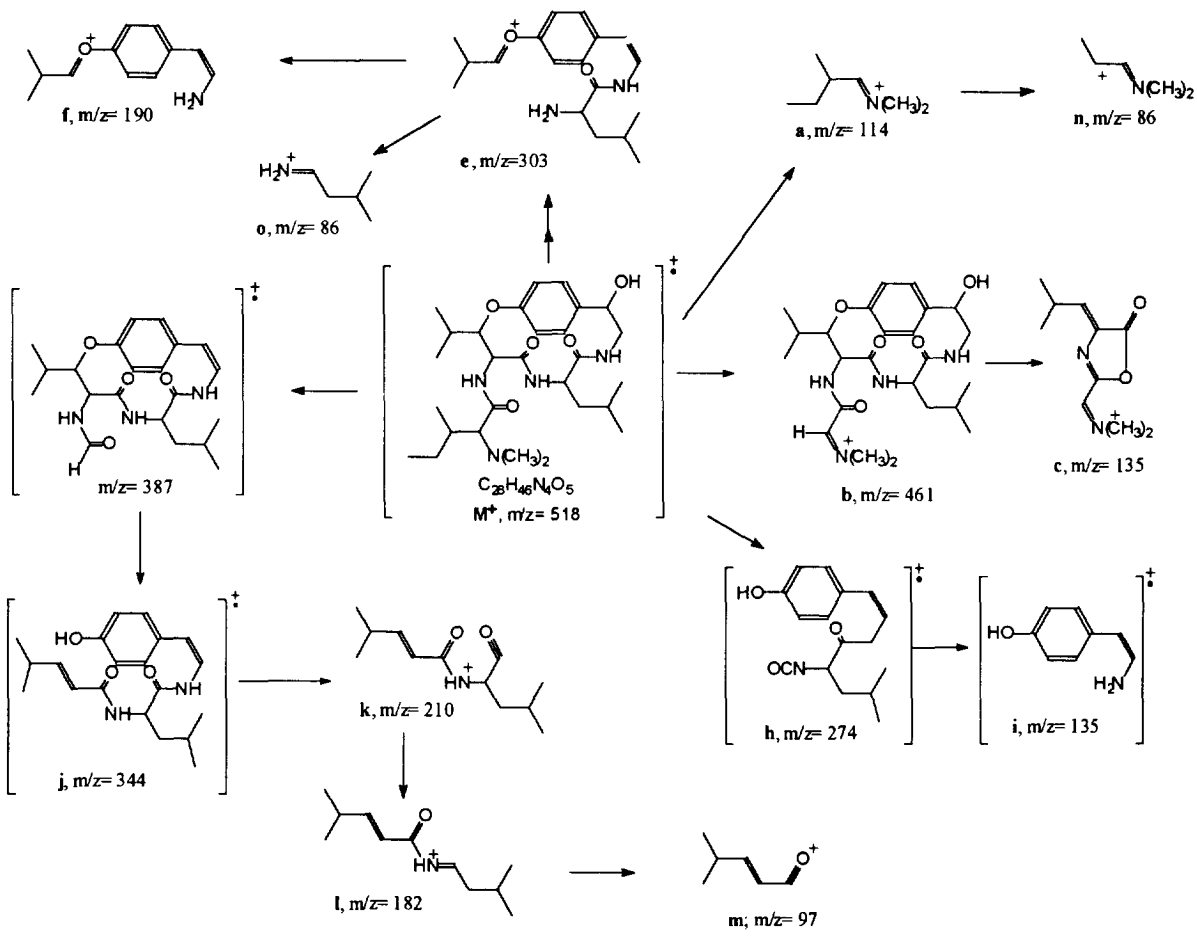
Compound **1** is amorphous. Its IR spectrum shows bands characteristic for the NH-vibration of peptide alkaloids: NMe_2; conjugated *cis*-1,2-disubstituted C=C double bond; Ar–O–C and OH. Microanalysis gives C_4; 35%, H_8; 83% and N_{10}; 54%, corresponding to the molecular formula $C_{28}H_{46}N_4O_5$. The mass spectrum of discarin-L (see Scheme 1) shows the molecular ion peak at $m/z = 518$. The base peak appears at $m/z = 114$, corresponding to $C_7H_{16}N$ (ion **a**), indicating the presence of a *N*,*N*-dimethylisoleucine unit in discarine-L. Another peak appears at $m/z = 86$ and can be attributed to $C_5H_{12}N$ (ion **o**), indicative for leucine as the ring bonded amino acid. A major peak at m/z 461 appears due to the loss of C_4H_9 and can be attributed to $C_{24}H_{37}N_4O_5$ (ion **b**). The peak at $m/z = 97$ likely belongs to C_6H_9N (ion **m**), showing the presence of hydroxyleucine in the peptide. The peak at $m/z = 135$ (ion **i**) corresponding to the styrylamine unit. Thus the new cyclopeptide belongs to the pandamine sub-group [8].



1

The ^{1}H NMR spectrum combined with the double resonance data confirms the proposed structure **1**. In addition to these techniques the assignments were confirmed by comparison with reference compounds [6, 7] and by 2D-NMR (COSY-45°). The data are compiled in Table 1 and were assigned as follows: the complex methyl region ($\delta = 0.71$–1.1 ppm) establishes the side chain identity of the amino acids present in the alkaloids. The β-hydroxyleucine moiety is easily recognizable in the two three-proton doublets at $\delta = 0.89$ and 1.02 ppm ($J = 7$ Hz). The first doublet at $\delta = 0.68$ ppm and a second doublet at $\delta = 0.71$ ppm, with $J = 7.0$ Hz, are characteristic for leucine as ring-bonded amino acid. Furthermore, the *N*,*N*-dimethyl amino group is confirmed to be part of a methylated isoleucine by the presence of a doublet at $\delta = 0.60$ ppm and a triplet at $\delta = 0.78$ ppm with $J = 7.0$ Hz. In the 3.8 to 5.4 ppm region the identification of the α- and β-proton of the β-hydroxyleucine and 2-hydroxy-2-phenethylamine components can be assigned by comparison with similar compounds, as were the remaining signals of the ^{1}H NMR spectrum, additionally confirmed by double resonance experiments. One methylenic proton at C-10 appears as a multiplet at $\delta = 4.02$, in superposition with the signal of the methine proton at C-17. The second proton at C-10 is identified as a multiplet at $\delta = 2.80$ ppm by double resonance experiments. The α-proton (C-4) of the hydroxy leucine unit is observed at $\delta = 4.35$ ppm as

*Author to whom correspondence should be addressed.



Scheme 1. Mass fragmentation of discarin-L.

a double doublet with $J = 9$ and 10 Hz, while the β-proton (C-3) appears at $\delta = 4.69$ ppm as a double doublet with $J = 2$ and 9 Hz. The chemical shift at $\delta = 4.90$ ppm is assigned to the carbinolic proton at C-11, as doublet with $J = 4$ Hz. Furthermore, the signal of the hydroxylic proton is observed as a singlet at $\delta = 5.3$ ppm, to conclude the characterization of 2-hydroxy-2-phenethylamine. The presence of four aromatic protons is evidenced by four double doublets at $\delta = 6.6$ and 7.3 ppm, with long range coupling constant of 2.0 Hz to the *meta* proton and vicinal coupling of 8.0 Hz to *ortho* protons, a typical coupling in aromatic *para*-substituted benzoid systems. Moreover, the NMR spectrum permits the assignment of all amide protons: $\delta = 6.72$(NH-6); 7.54 (NH-9) and 8.17 ppm (NH-20), as doublets with $J = 9$, 10 and 10 Hz, respectively. Further characterization of discarin-L (1) was obtained by ^{13}C NMR spectroscopy. The data (Table 1) were interpreted on the basis of previous assignments [6, 9] and spin-echo experiments and allowed the assignment of all carbons present.

EXPERIMENTAL

General. Mps: (uncorr.), determined by means of Kofler hot plate coupled to a Reichert microscope. IR spectra: measured on a Perkin Elmer 599B spectrometer using KBr disks. The low resolution mass spectra were obtained on a GC/MS Hewlett Packard HP5980/5988A. The high resolution mass spectra were obtained on a V.G. Analytical 70-250S instrument operating at 70 eV. The ^{1}H and ^{13}C NMR spectra were registered on a Varian VXR 400 (400 MHz), in 5 mm sample tubes.

Plant material. The bark of *Discaria febrifuga* Mart. was collected in October 1990 in the vicinity of the town of Santana do Livramento, in the state of Rio Grande do Sul, Brazil, and identified by Prof. Dr Adelino A. Filho of the Department of Botany. Voucher specimens are kept in the herbarium of the Department of Botany, Universidade Federal de Santa Maria, Santa Maria, RS.

Extraction. Dried ground root bark (10 kg) of *Discaria febrifuga* Mart. was extracted exhaustively with hot MeOH as described before [5], yielding after removal of the solvent, a mixt. of alkaloids (10 g). The alkaloid mixt. was fractionated on a SiO_2 chromatographic column using mixts of $CHCl_3$–MeOH as solvent and on PLC as described before, when further purification was required. Discarin-L was eluted at $R_f = 0.45$ with $CHCl_3$: MeOH = 95 : 5.

Isolation of discarin-L. Yield 10 mg amorphous powder, $[\alpha]_D$ −30° (MeOH; $c = 0.5$). IR ν_{max} cm^{-1}:

Table 1. ^{1}H and ^{13}C NMR of discarin-L (in DMSO-d_6, 400 MHz)

Position	Assignment	δ H[ppm]* J(Hz)	δ C[ppm]†
1	–C(Ar)		155.5
3	–CH–O	4.69 (*dd*) $J_{3,17}=2.0, J_{3,4}=9.0$	79.2
4	–NH–CH–CO	4.35 (*dd*) $J_{4,3}=9.0, J_{4,20}=10.0$	54.6
5	–CO–		169.5
6	–OC–NH–CH–	6.72 (*d*) $J_{6,9}=9.0$	
7	–NH–CH–CO–	4.20 (*m*)	50.4
8	–CO–		169.2
9	–CO–NH–CH–	7.54 (*d*) $J_{9,10}=10.0$	
10, 10′	–NH–CH_2–CHOH–(Ar)	4.02, 2.80 (*m*)	47.3
11	–NH–CH_2–CHOH–(Ar)	4.90 (*d*) $J_{11,10}=4.0$	70.7
12	–C–(Ar)		134.7
13	–CH–(Ar)	6.85 (*dd*) $J_{13,14}=8, J_{13,16}=2$	113.7
14	–CH–(Ar)	7.24 (*dd*) $J_{14,13}=8, J_{14,15}=2$	118.3
15	–CH–(Ar)	6.85 (*dd*) $J_{15,16}=8, J_{15,14}=2$	126.5
16	–CH–(Ar)	6.78 (*dd*) $J_{16,15}=8, J_{16,13}=2$	126.8
17	–CH–	2.20 (*m*)	27.7
18	–Me–CH	1.02 (*d*) $J_{18,17}=7$	14.5
19	–Me–CH	0.89 (*d*) $J_{19,17}=7$	20.1
20	CH–NH–CO–	8.17 (*d*) $J_{20,14}=10$	
21	–CO–		170.3
22	Me_2–N–CH–CO–	2.68 (*d*)	71.0
23	Me–CH–CH_2–	1.71 (*m*)	32.7
24, 24′	Me–CH_2–CH–	1.52, 1.05 (*m*)	24.7
25	Me–CH_2	0.78 (*t*) $J_{25,24}=7$	10.3
26	Me–CH	0.60 (*d*) $J_{26,23}=7$	14.8
27, 28	–NMe_2	2.16 (*s*)	41.2
29, 29′	–CH–CH_2–CH–	1.25, 1.71 (*m*)	42.3
30	–CH_2–CH–	1.24 (*m*)	23.6
31	–CH–Me	0.71 (*d*) $J_{32,30}=7$	21.9
32	–CH–Me	0.68 (*d*) $J_{32,30}=7$	23.6
–OH	(Ar)–CH–OH	5.30 (*l*)	

*These assignments were confirmed by 2D shift correlated ^{1}H–^{1}H COSY and by double resonance data.

†These assignments were confirmed by DEPT and spin ECHO measurements.

3400–3200 (NH), 3040 (CH-arom.), 1680–1630 (C=O), 2980–2880 (NMe), 2800 (C–O–arylether) and 1230 (C–O alcohol). MS: *m/z* 518 $[M]^+$; 114 (**a**) base peak; 461 (**b**); 303 (**e**); 190 (**f**); 274 (**h**); 135 (**i**); 344 (**j**); 210 (**k**); 182 (**l**); 97 (**m**); 86 (**o**). ^{1}H NMR and ^{13}C NMR: see Table 1.

Acknowledgements—The authors thank FAPERGS (Fundação de Amparo a Pesquisa do Rio Grande do Sul), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and GTZ (Deutsche Gesellschaft für Technische Zusammenarbeit) for financial

support. We are grateful to Prof. N. Zanatta for comments on the manuscript.

REFERENCES

1. Reissek, S. in Martius C. F. R. *Flora Brasiliensis* **11**, 100.
2. Digel, M., Morel, A. F., Biermann, J. and Voelter, W. (1983) *Hoppe-Seyler's Z. Physiol. Chem.* **364**, 1641.
3. Morel, A. F., Herzog, R., Biermann, J. and Voelter, W. (1984) *Z. Naturforsch.* **34b**, 1825.
4. Morel, A. F., Herzog, R. and Voelter, W. (1985) *Chimia* **4**, 98.
5. Hennig, P., Morel, A. F. and Voelter, W. (1986) *Z. Naturforsch.* **41b**, 1180.
6. Voelter, W and Morel, A. F. (1987) *Z. Naturforsch.* **42b** 467.
7. Machado, E. C., Zanatta, N. and Morel, A. F. (1983) *Quimica Nova* **16**, 397.
8. Pais, M., Mainil, J. and Goutarel, R. (1964) *Bull. Soc. Chim. Fr.* 817.
9. Pais, M., Jarreau, F. X., Sierra, M. G., Mascaretti, O. A., Ruveda, E. A., Chang, C. J., Hagman, E. W. and Wenkert, E. (1979) *Phytochemistry* **18**, 1869.