Mucronine J, a 14-Membered Cyclopeptide Alkaloid from *Zizyphus mucronata*

Catherine Auvin, Françoise Lezenven, Alain Blond, Isabelle Augeven-Bour, Jean-Louis Pousset, and Bernard Bodo*

Laboratoire de Chimie des Substances Naturelles, URA 401 CNRS, GDR 964 CNRS, Muséum National d'Histoire Naturelle, 63 rue Buffon, 75231 Paris Cedex 05, France

José Camara

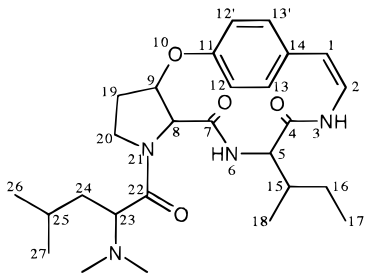
Institut Henri Beaufour, 1 avenue des Tropiques, ZA Courtaboeuf, 91940 Les Ulis, France

Received October 25, 1995[⊗]

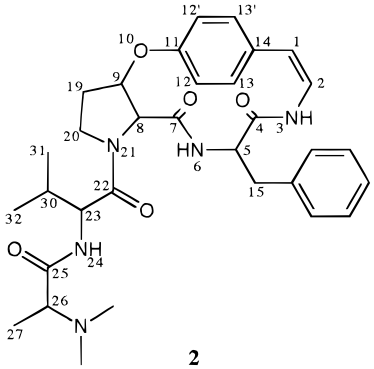
From the CH_2Cl_2 extract of the root bark of *Z. mucronata* (Rhamnaceae), a new cyclopeptide alkaloid, named mucronine J, was isolated together with previously known alkaloids abyssenine A and mucronine D. The structure of mucronine J was elucided by mass spectrometry and 1D and 2D NMR. A solution conformation is proposed on the basis of NOE experiments in combination with MM2 calculations.

Zizyphus mucronata Willd., a Rhamnaceae of central and southern Africa, is employed by the natives in the treatment of diarrhea, dysentery, and skin infections.[1] Previous chemical studies on the bark revealed cyclopeptide alkaloids of the mucronine family.[2−7] All were characterized by the presence of a styrylamine group in a 15-membered cyclopeptide alkaloid, except mucronine D, which is a 13-membered ring system.

From the root bark, a new 14-membered cyclopeptide alkaloid (**1**) together with the known abyssenine A[6] and mucronine D[7] were isolated. In this paper, we report the isolation, structure determination, and molecular modeling of mucronine J (**1**).



1



2

The peptide alkaloid **1** was isolated from the alkaloidal fraction of the CH_2Cl_2 extract of *Z. mucronata* root bark by successive column chromatography and gave a weak positive reaction with Dragendorff's reagent. The molecular formula $C_{27}H_{40}N_4O_4$ was derived from the HR positive ion FABMS where the MH^+ ion was observed at *m/z* 485.3088. The EIMS exhibited the characteristic fragmentations of a 14-membered cyclopeptide alkaloid such as amphibine.[8,9] The presence of an ion at *m/z* 135 was typical of *p*-hydroxystyrylamine. The ions at *m/z* 86 and 72 suggested leucine or isoleucine as a ring-bonded amino acid. Moreover, the presence of an *N,N*-dimethylleucine (or *N,N*-dimethylisoleucine) unit as the terminal residue was deduced from the occurrence of the base peak at *m/z* 114.

The ^1H NMR spectrum of **1** displayed signals for two ethylenic protons, four aromatic protons, and three amino acids, *β*-hydroxyproline, isoleucine, and *N,N*-dimethylleucine, which were identified and assigned with the aid of $^1H-^1H$ COSY experiment (Table 1).

^1H and ^{13}C assignments arose from the analysis of J-modulated ^{13}C, HMQC, and HMBC spectra. The carbonyl resonances at C-4, C-7, and C-22 were assigned from their $^2J_{C-H}$ intraresidue correlations with α-protons at H-5, H-8, and H-23, respectively. HMBC correlations from the oxygenated sp^2 quaternary carbon (C-11) (*δ* 157.4) to H-12, H-12′, H-13, and H-13′ confirmed a *para*-substituted aromatic ring. As previously observed for 14-membered cyclopeptide alkaloids,[10] conformational constraints in the macrocyclic ring system induced the nonequivalence of the protons and carbon atoms of each aromatic methine pair (C-12 and -12′, C-13 and -13′). The observed HMBC correlations from C-13 (*δ* 132.6) to H-12 (*δ* 7.28) and from C-13′ (*δ* 130.2) to H-12′ (*δ* 7.10) allowed their unambiguous identification. The combined analysis of HMBC and HMQC data ascertained the linkages between constitutive parts of **1** and allowed full resonance assignment (Table 1).

The two ethylenic protons at *δ* 6.27 (H-1) and 6.74 (H-2) had a mutual coupling constant value of 7.7 Hz, indicating a *cis* stereochemistry, which was confirmed by their strong mutual NOE effect. NH-3 did not give NOE with these ethylenic protons, but a strong effect with aromatic protons H-13 and H-13′. Moreover, the coupling constant value between H-2 and NH-3 (^3J = 10.8 Hz) indicated they were in a *trans* coplanar position. Strong NOEs were observed between H-9 and both H-19α and H-20α but not between H-19*β* and H-20*β*, indicating that H-9, H-19α, and H-20α were on

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1996.

S0163-3864(96)00197-8 CCC: $12.00

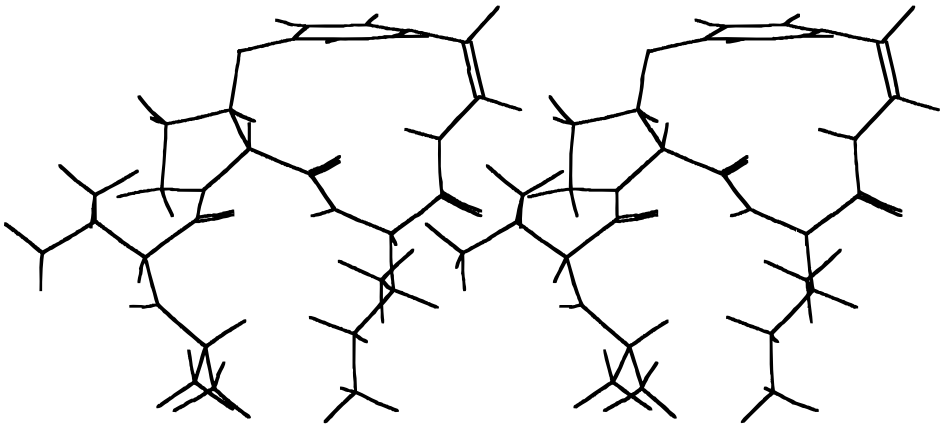


Figure 1. Stereoscopic view of the mucronine J as predicted by NOE experiments and MM2 calculations.

Table 1. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) Data for Mucronine J, **1** ($CDCl_3$, 296 K)

	^{1}H δ (m, *J*, Hz)	^{13}C δ
1	6.27 (d, 7.7)	113.8
2	6.74 (dd, 7.7, 10.8)	125.5
3	6.52 (d, 10.8)	
4		167.0
5	4.16 (dd, 2.9, 8.8)	58.9
6	6.70 (d, 8.8[a])	
7		170.8
8	4.31 (d, 5.3)	63.5
9	5.59 (ddd, 5.3, 7.2, 9.8)	83.7
11		157.4
12	7.28 (dd, 2.6, 9.0)	122.9
12′	7.10 (m)	123.1
13	7.06 (m)	132.6
13′	7.10 (m)	130.2
14		132.6
15	2.18 (m)	35.0
16a	1.05 (m)	23.6
16b	1.20 m	
17	0.82 (t, 7.2)	12.1
18	0.69 (d, 7.0)	15.8
19α	2.55 (ddd, 5.2, 7.2, 12.2)	32.1
19*β*	2.13 m	
20α	4.28 (dd, 8.2, 10.8)	46.0
20*β*	3.32 (ddd, 5.2, 10.8, 13.1)	
22		171.7
23	3.21 (dd, 3.4, 9.4)	63.3
24a	1.67 (dd, 9.1, 9.4)	33.3
24b	1.23 (m)	
25	1.23 (m)	25.0
26	0.83 (d, 6.3[a])	23.4
27	0.79 (d, 6.3[a])	21.9
$N(CH_3)_2$	2.27 (s)	41.3

[a] Coupling constants obtained at 306 K.

the same side of the pyrrolidine ring. No significant NOE was shown between H-9 and H-8, suggesting these protons were *trans*. Finally, strong NOEs from H-9 to H-12 and from H-8 to NH-6 indicated the *trans* configuration of the peptide bond between *β*-hydroxyproline and isoleucine.

The relative configuration of **1** was compared to that of mauritine A (**2**), a structurally related 14-membered cyclopeptide alkaloid, the X-ray structure of which was previously described.[11] Mauritine A has a distorted *β*-hydroxy-substituted proline ring with *trans* stereochemistry and the absolute configuration of all chiral carbons is L. Similar NOEs were measured between ring proton systems of mauritine A, isolated from *Z. mauritiana*, and **1**, suggesting the same relative stereochemistry for the two compounds.

The absolute configuration of isoleucine and *N*,*N*-dimethylleucine was determined as L from the acidic chemical degradation of **1**, derivatization, and analysis by GC on a chiral capillary column. Identical absolute configuration of these residues together with the same relative stereochemistry deduced from the NMR data indicate the same absolute stereochemistry for both cyclopeptides.

In order to ascertain the conformation, the structure of **1** was calculated using the MacroModel V3.5 molecular modeling program[12] with the MM2-derived force field.[13] The lowest energy conformation (Figure 1) was in good agreement with the NOE data and exhibited two hydrogen bonds, one between NH-3, CO-7 (2.1 Å) and the other between NH-6, CO-22 (2.6 Å). The benzene ring incorporated in the central ring system was slightly but significantly bent, and the attached atoms O-10 and C-1 were out of the aromatic plane with angles [O-10···C-14] = 169° and [C-11···C-1] = 165°, respectively. The energy minimization positioned the aromatic H-12 in the deshielded region of the carbonyl group at C-7, explaining its lower field chemical shift (δ 7.28), instead of 7.10 for H-12′, -13, and -13′. Molecular modeling exhibited a very constrained ring system, with a highly hydrophobic face bearing *N*,*N*-dimethylleucine and isoleucine residues.

Experimental Section

General Experimental Procedures. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC 300 spectrometer, equipped with an Aspect 3000 computer, at 300.13 MHz and 75.47 MHz, respectively, using $CDCl_3$ as solvent. The resonances of residual $CHCl_3$ at δ_H 7.24 and δ_C 77.0 were used as internal reference. Positive HRFABMS and EIMS were obtained on a ZAB-HF and on a NERMAG R 10-10 mass spectrometers, respectively. The $[\alpha]_D$ value was determined with a Perkin-Elmer Model 243 B polarimeter and infrared spectra registered on a Nicolet Model 205 FT-IR spectrometer. TLC and column chromatography were carried out on Merck precoated Si gel F_{254} plates and on Si gel 60 (Merck 230−400 mesh).

Reference Mauritine A (2). Mauritine A (110 mg) was isolated from dried ground root bark (1.2 kg) of *Z. mauritiana* and identified by comparision of its physical properties with those reported in the literature.[11]

Plant Material. Root bark of *Z. mucronata* Willd. was collected in August 1994 in the region of Dakar (Senegal). A voucher specimen is on deposit in the

herbarium of the Institut Fondamental d'Afrique noire, University of Dakar (Senegal).

Extraction and Isolation. Dried ground root bark (1.5 kg) of *Z. mucronata* was moistened with 50% NH_4OH (24 h) and percolated with 10 L of CH_2Cl_2. The organic layer was evaporated *in vacuo* to give 10.2 g of residue. The crude extract was loaded onto a silica gel column and eluted with increasingly polar CH_2Cl_2–MeOH mixtures. The first fraction, 1.8 g, which gave a slight yellow color with Dragendorff's reagent, was chromatographed over silica gel (EtOAc) to give **1** (29 mg).

Mucronine J (1): colorless amorphous powder; $[\alpha]^{21}_D$ −236° ($CHCl_3$, $c = 1$); IR νmax cm^{-1} (neat) 3398, 2963, 2931, 2880, 2791, 1658, 1632, 1511, 1460, 1229, 1171, 1106, 1031; ^1H and ^{13}C ($CDCl_3$), see Table 1; HRFABMS MH^+ 485.3088 (calcd for $C_{27}H_{41}N_4O_4$ 485.3128); FABMS *m/z* 485 (MH^+, 59), 355 (9), 341 (7), 327 (8), 281 (20), 267 (10), 221 (22), 207 (22); EIMS *m/z* 427 (8), 229 (0.5), 209 (1), 189 (0.5), 161 (8), 153 (1), 135 (10), 114 (100), 86 (18), 72 (35).

Amino Acid Analysis. Compound **1** (1 mg) was hydrolyzed with 8 N HCl (0.5 mL) in a sealed tube at 110 °C under N_2 for 24 h. After cooling, the residue was dried over potassium hydroxide pellets. The crude residue was dissolved in an anhydrous solution of 3 N HCl in 2-propanol and heated at 100 °C for 20 min. The reagents were evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2 (0.5 mL), and 0.5 mL of trifluoroacetic anhydride was added. The mixture was kept in a screw-capped tube at 100 °C for 5 min. The reagents were evaporated, and the mixture was analyzed on a Chirasil-L-Val (*N*-propionyl-L-valine-*tert*-butylamide polysiloxane) quartz capillary column.

Molecular Modeling. Computer modeling was performed by using the MacroModel V3.5 program[12] running on a Silicon Graphics Indigo 2 computer. The structure was fully minimized using the MM2-derived force field. Chloroform was selected as the solvent. The structure was then subjected to a random conformational search using the *MCrlo* option of the software. A 20 $kJ \cdot mol^{-1}$ energetic window allowed us to retain about 200 conformations. The generated structures were then manually processed to find similar conformations. All of the structures within 4 $kJ \cdot mol^{-1}$ from the lowest energy conformation were almost the same; only slight differences in the side chains were observed.

References and Notes

(1) Joullié, M. M.; Nutt, R. F. In *Alkaloids, Chemical and Biological perspectives*; Pelletier, S. W., Ed.; John Wiley & Sons: New York, 1983; Vol. 3, pp 113−168.
(2) Tschesche, R.; Kaussmann, E. U. In *The Alkaloids;* Manske, R. H. F., Ed.; Academic Press: New York, 1975; Vol. 15, Chapter 4, pp 165-205.
(3) Shah, A. H.; Al-Yahya, M. A.; Devi, S.; Pandey, V. B. *Phytochemistry* **1987**, *26*, 1230−1232.
(4) Barboni, L.; Gariboldi, P.; Torregiani, E.; Verotta, L. *Phytochemistry* **1994**, *35*, 1579−1582.
(5) Fehlhaber, H. W.; Uhlendorf, J.; David, S. T.; Tschesche, R. *Liebigs Ann. Chem.* **1972**, *759*, 195−207.
(6) Tschesche, R.; David, S. T.; Zerbes, R.; von Radloff, M.; Kaussmann, E. U.; Eckhardt, G. *Liebigs Ann. Chem.* **1974**, 1915−1928.
(7) Tschesche, R.; David, S. T.; Uhlendorf, J.; Fehlhaber, H. W. *Chem. Ber.* **1972**, *105*, 3106−3114.
(8) Marchand, J.; Païs, M.; Monseur, X.; Jarreau, F. X. *Tetrahedron* **1969**, *25*, 937−954.
(9) Ghedira, K.; Chemli, R.; Richard, B.; Nuzillard, J. M.; Zeches, M.; Le Men-Olivier, L. *Phytochemistry* **1993**, *32*, 1591−1594.
(10) Ghedira, K.; Chemli, R.; Caron, C.; Nuzillard, J. M.; Zeches, M.; Le Men-Olivier, L. *Phytochemistry* **1995**, *38*, 767−770.
(11) Kirfel, A.; Will, G. *Z. Kristallogr. Bd.* **1975**, *142*, 368−383.
(12) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R. *J. Comput. Chem.* **1990**, *11*, 440−467.
(13) Allinger, N. L.; Yuh, Y. H.; Lii, J. H. *J. Am. Chem. Soc.* **1989**, *111*, 8551−8566.

NP9601972