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FOUR CYCLOPEPTIDE ALKALOIDS FROM *ZIZYPHUS LOTUS*

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Key Word Index—*Zizyphus lotus*; Rhamnaceae; root bark; cyclopeptide alkaloids; lotusines B, C, E and F.

Abstract—Four new cyclopeptide alkaloids, lotusines B, C, E and F, were isolated from the root bark of *Zizyphus lotus*. Their structures were elucidated mainly by homo- and heteronuclear NMR techniques.

INTRODUCTION

Several species of the genus *Zizyphus* contain cyclopeptide alkaloids, which are particularly common in the Rhamnaceae [1]. In the course of our investigations on Tunisian medicinal plants, we have studied the root bark of *Z. lotus*, which is used for its antidiabetic properties in traditional medicine. An alkaloid mixture of this species showed significant antibacterial activity [2]. From this extract, we have isolated a series of six novel cyclopeptide alkaloids. The structure elucidation of two of them, lotusines A and D, was published in a previous paper [3]. We report herein on the structures of lotusines F (**1**), E (**2**), C (**3**) and B (**4**), which were established mainly by homo- and heteronuclear NMR techniques.

RESULTS AND DISCUSSION

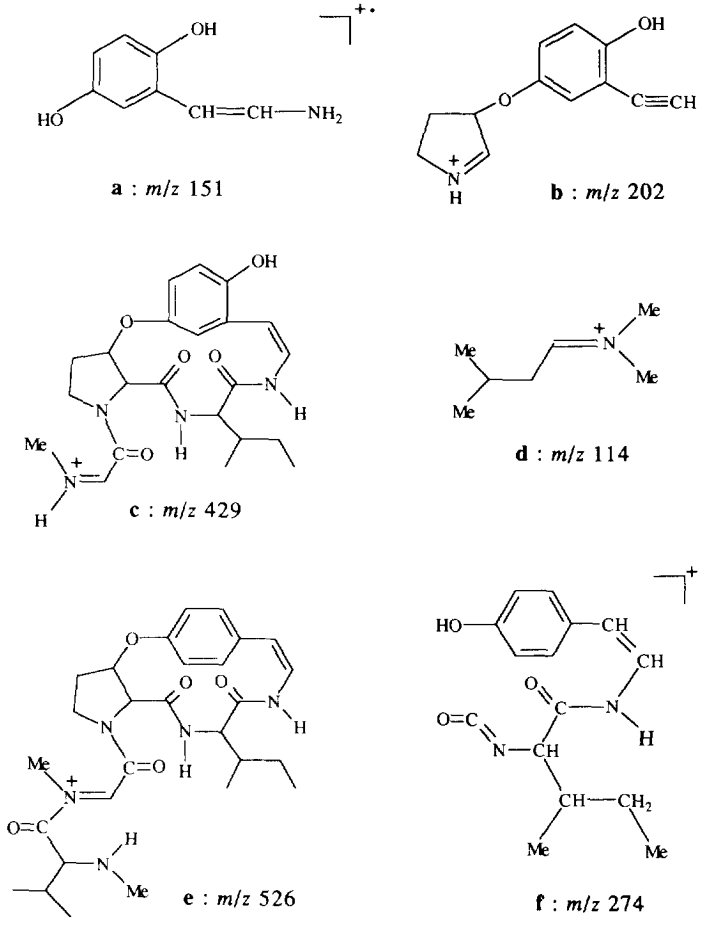
Extraction of the alkaloid mixture is reported elsewhere [3]. The isolation of pure compounds was performed by means of centrifugal TLC on silica gel, followed by preparative TLC. Besides lotusines A and D [3], four new cyclopeptides were obtained in the following order of increasing polarity, lotusines B, C, E and F.

An additional compound was isolated during extraction and was purified by recrystallization from methanol. It was identified as betulinic acid by comparison of its spectral data ($[\alpha]_D$, mass spectrum, ^1H and ^{13}C NMR) with those in the literature [4].

Lotusines F (**1**) and E (**2**) had similar spectral properties. Their IR spectra showed bands for cyclopeptide alkaloids, viz., NH and/or –OH, –NH–CO, phenol ether and styryl double bond. The UV spectra exhibited typical absorption bands for 2,5-dioxystyrylamine derivatives, as found in 13-membered ring alkaloids [5]. Furthermore, a bathochromic shift (from 323 to 353 nm, by addition of an alkaline solution) indicated the presence of a phenolic hydroxyl substituted styrylamine. In the mass spectra, the occurrence of ions **a** and **b** indicated that **1** and **2** belonged to the Zizyphine sub-group [6–8]. As reported in the case of lotusines A and D, a phenylalanine unit was detected by the presence of ions at *m/z* 134 and 148 in **1** and **2**, respectively [3, 8].

Lotusine F (**1**) exhibited an intense $[M + H]^+$ at *m/z* 521 in its FAB mass spectrum suggesting the molecular formula $C_{29}H_{36}N_4O_5$, which was supported by ^1H and ^{13}C NMR spectra. The basic structure of this compound was evident from the appearance of the ion at *m/z* 429 for which the structure **c** ($[M - C_7H_7]^+$) was proposed (Scheme 1). The ^1H, COSY and ^{13}C NMR (Tables 1 and 2) spectra showed characteristic signals of the amino acids isoleucine, hydroxyproline and a *N*-methylphenylalanine, as described in the case of lotusine D [3]. Analysis of these spectra evidenced the substitution of the styrylamine unit by two oxygens in *ortho*- (C-14 at δ148.1) and *meta*-positions (C-11 at δ150.7), as found in 13-membered ring cyclopeptides [9]. The HMQC [10] and HMBC [11] spectra confirmed the assignment of ^1H and ^{13}C data. The identification of C-4, C-7 and C-21 as carbonyl resonances was in agreement with $^2J_{C-H}$ intraresidue correlations: C-4/H-5, C-7/H-8 and C-21/H-22 (Fig. 1). The linkage between the various parts of the molecule was deduced from the most significant $^2J_{C-H}$ and $^3J_{C-H}$ interresidue correlations H-2/C-4, H-3/C-4, H-6/C-7, H-9/C-11 and H-8/C-21. Moreover, the presence of C-11/H-9, C-11/H-12, C-11 H12′, C-14/H-1 and C-14/H-13′ correlations confirmed the substitution of the styrylamine moiety, namely the positioning of the hydroxyl group on C-14. The combined HMQC and HMBC data also permitted the attribution of the eight aromatic protons and provided definitive proof of the structure **1**.

Lotusine E (**2**) gave a weak $[M]^+$ at *m/z* 647 in its EI-mass spectrum, suggesting the molecular formula $C_{36}H_{49}N_5O_6$. Similar signals were discernible in the ^1H



Scheme 1. Most significant mass spectral fragments of **1**–**4**.

and COSY spectra of **1** and **2** which indicated that these two compounds contain common amino acid residues. In **2** (Table 1), the presence of *N*,*N*-dimethylleucine as an additional amino acid was supported by the appearance of an amide proton resonance (H-28, *d*, δ7.59, which displayed a correlation with H-22) and by supplementary signals corresponding to four methyl groups: Me-33 and Me-34 (*d* at δ0.88 and 0.89, respectively) and Me-35 and Me-35' (6H, *br s* at δ2.21). In the mass spectrum, this unit gave an intense peak at *m/z* 114 (**d**) [7]. The proton sequences of the constitutive amino acids of the molecule were ascertained by correlations observed in the HOHAHA spectrum of **2**. The ^{13}C NMR spectrum (Table 2) was in agreement with the nature of constitutive parts of the molecule. It was similar to that of **1**, except for the change of C-22 (δ62.7 in **2**, instead of 51.2 in **1**) and the lack of the Me-28 resonance frequency, suggesting a structural modification in the region of *N*-methylphenylalanine. Furthermore, eight supplementary carbon resonances (one of which corresponded to a carbonyl group, C-29 at δ173.8) provided strong evidence for the presence of *N*,*N*-dimethylleucine as the additional amino acid residue. As in **1**, the combined analysis of the HMQC and HMBC data (Fig. 1) ascertained the points of attachment between the constitutive parts of the molecule, particularly, the terminal position of *N*,*N*-dimethylleucine.

Compounds **3** and **4** gave the expected IR and UV spectra for 14-membered cyclopeptides, as found in lotusines A and D [3]. Their mass spectra displayed fragments characteristic of amphibine-type compounds [6–8]: *m/z* at 135 for a styrylamine, *m/z* 96 for a proline, *m/z* 86 for a leucine or an isoleucine, *m/z* 91 and *m/z* 134 for a phenylalanine [8].

Compound **3** was assigned the molecular formula $C_{35}H_{47}N_5O_5$ based on the presence of a weak $[M]^+$ at *m/z* 617 (EI-mass spectrum) and on NMR data. The ^{1}H, COSY and ^{13}C spectra (Tables 1 and 2) revealed the characteristic signals attributable to an isoleucine, a hydroxyproline and a phenylalanine as described in the case of lotusine D [3]. Another amino acid unit was deduced from the occurrence of a fourth carbonyl resonance (C-29 at δ172.4), as well as from the appearance of five supplementary carbon peaks corresponding to three methyl groups (C-32, C-33 and C-34 at δ18.4, 19.3 and 42.3, respectively) and two methines (C-30 and C-31 at δ54.9 and 31.0, respectively). These data suggested the

Table 1. ^{1}H NMR spectral data for compounds **1**–**4** [$CDCl_3$, 300 MHz, δppm, J (Hz)]

H	**1**			**2**			**3**			**4**		
1	5.85	*d*	(9.0)	5.87	*d*	(9.0)	6.32	*d*	(7.7)	6.32	*d*	(7.7)
2	6.96	*dd*	(11.3, 9.0)	6.94	*dd*	(11.2, 9.0)	6.74	*dd*	(10.1, 7.7)	6.75	*dd*	(10.5, 7.7)
3	8.47	*d*	(11.3)	8.46	*d*	(11.2)	6.47	*br d*	(10.1)	6.50	*br d*	(10.5)
5	4.36	*dd*	(4.7, 4.5)	4.35	*dd*	(5.0, 4.5)	4.17	*dd*	(8.6, 3.0)	4.20	*dd*	(8.6, 3.0)
6	7.39	*d*	(5.1)	7.27	*m*	—	6.46	*br d*	(8.6)	6.56	*br d*	(8.6)
8	4.41	*d*	(2.8)	4.40	*d*	(2.9)	4.23	*d*	(5.4)	4.29	*d*	(5.7)
9	5.33	*dt*	(7.0, 2.8)	5.34	*dt*	(7.0, 2.9)	5.55	*m*	—	5.54	*m*	—
12	6.57	*d*	(2.9)	6.58	*d*	(2.8)	7.14	*m*	—	7.12	*m*	—
12′	6.68	*dd*	(8.8, 2.9)	6.67	*dd*	(8.8, 2.8)	7.28	*m*	—	7.28	*m*	—
13	—	—	—	—	—	—	7.09	*m*	—	7.10	*m*	—
13′	6.80	*d*	(8.8)	6.80	*d*	(8.8)	7.12	*m*	—	7.07	*m*	—
15	2.16–2.06	*m*	—	2.09	*m*	—	2.20–2.10	*m*	—	2.30–2.17	*m*	
16	1.44	*ddd*	(13.2, 7.3, 3.8)	1.39	*m*	—	1.29–1.19	*m*	—	1.35–1.20	*m*	—
16′	1.28–1.15	*m*	—	1.20	*m*	—	1.13–1.01	*m*	—	1.20–1.05	*m*	—
17	0.92	*t*	(7.3)	0.92	*t*	(7.0)	0.86	*t*	(6.5)	0.91	*t*	(7.2)
18	1.06	*d*	(6.9)	1.03	*d*	(6.9)	0.75	*d*	(6.9)	0.82	*d*	(6.9)
19	2.40–2.31	*m*	—	2.42	*m*	—	2.61–2.53	*m*		2.60–2.45	*m*	—
19′	2.16–2.06	*m*	—	2.20	*m*	—	2.34–2.13	*m*	—	2.25–2.10	*m*	—
20	3.67	*m*		4.03	*ddd*	(11.1, 8.4, 3.0)	4.32	*dd*	(10.6, 8.3)	4.20–4.18	*m*	
20′	2.51	*dt*	(6.5, 8.5)	2.88	*m*	—	3.43	*m*	—	3.05–2.90	*m*	—
22	3.60	*dd*	(9.3, 4.9)	4.96	*q*	(7.7)	3.36	*t*	(6.4)	4.89	*q*	(7.0)
23	3.05	*dd*	(13.0, 4.9)	2.96	*br d*	(7.4)	3.21	*dd*	(14.0, 6.4)	2.86	*br d*	(7.0)
23′	2.74	*dd*	(13.0, 9.3)	2.96	*br d*	(7.4)	2.92	*dd*	(14.0, 6.4)	2.86	*br d*	(7.0)
25, 25′	7.02 (2H)	*dd*	(7.7, 2.0)	7.08 (2H)	*dd*	(7.6, 1.9)	7.26 (2H)	*m*		7.22 (2H)	*m*	—
26, 26′	7.25 (2H)	*m*	—	7.26 (2H)	*m*	—	7.23 (2H)	*m*	—	7.15 (2H)	*m*	—
27	7.21	*m*		7.25	*m*	—	7.22	*m*	—	7.19	*m*	
28	2.38 (3H)	*br s*		7.59	*br d*	(7.7)	2.36	*br s*	—	n.d.	—	
30	—		—	2.90	*m*	—	4.43	*t*	(8.5)	n.d.	—	—
31	—	—	—	1.48	*m*		2.01–1.81	*m*	—	n.d.	—	—
31′	—	—	—	1.48	*m*			—	—	n.d.	—	—
32	—	—	—	1.61	*m*	—	0.93–0.79	*m*		n.d.	—	—
33	—	—		0.88	*d*	(6.4)	0.93–0.79	*m*	—	0.87	*d*	(6.4)
34	—	—		0.89	*d*	(6.4)	2.34 (3H)	*br s*	—	0.85	*d*	(6.4)
35	—	—	—	2.21	*br s*	—	7.36	*d*	(8.5)	2.22	*br s*	
35′	—		—	2.21	*br s*	—	—	—	—	2.22	*br s*	—

n.d.: not detected.

Table. 2. ^{13}C NMR spectral data for compounds **1**–**4** ($CDCl_3$, 75 MHz, δ, ppm)

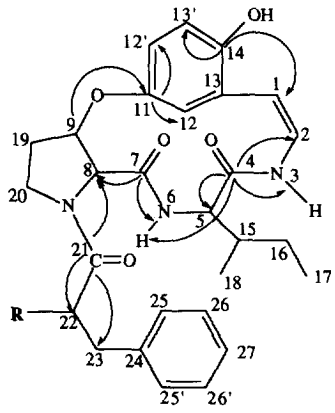
C	Lotusine D [2]	1	2	3	4
1	114.7	106.0	106.3	114.7	114.8
2	126.0	122.1	121.8	125.4	125.5
4	167.5	167.4	167.3	167.0	166.9
5	58.9	60.3	60.2	58.6	59.0
7	171.5	169.7	169.7	170.6	171.3
8	62.8	64.5	64.8	63.9	63.9
9	83.6	76.4	76.5	83.6	83.5
11	157.5	150.7	150.6	157.4	157.4
12	122.6	110.7	110.6	122.7	122.8
12′	122.7	118.1	118.1	122.5	122.8
13	130.1	122.0	121.9	130.1	130.2
13′	132.7	118.6	118.4	132.5	132.5
14	132.7	148.1	148.2	132.5	132.6
15	34.3	35.5	35.5	35.3	35.2
16	23.9	24.6	24.7	23.6	23.6
17	12.2	11.8	11.8	12.1	12.2
18	16.0	16.2	16.1	15.8	16.0
19	31.8	32.2	32.2	31.9	31.9
20	45.8	45.8	46.4	46.8	46.5
21	173.2	173.3	171.1	171.6	170.2
22	63.9	62.7	51.2	70.7	49.2
23	39.7	40.0	39.0	32.6	38.5
24	136.9	136.3	135.3	139.8	135.9
25, 25′	128.6	128.7	128.7	128.3	128.7
26, 26′	129.1	128.9	129.0	129.1	129.1
27	126.8	126.9	127.2	126.1	127.1
28	35.5	34.2	—	42.3	—
29	—	—	173.8	172.4	n.d.
30	—	—	67.1	54.9	67.1
31	—	—	36.6	31.0	36.4
32	—	—	25.7	18.4	25.7
33	—	—	23.2	19.3	23.3
34	—	—	22.0	42.3	21.9
35, 35′	—	—	42.1	—	42.0

n.d.: not detected.

presence of a methylvaline unit [12] which appeared in ion **e** at *m/z* 526 (Scheme 1). The shift of C-22, C-23, C-24 and C-28 resonance frequencies was in agreement with the terminal position of the latter amino acid residue. The combined analysis of HMBC and HMQC spectra of **3** allowed an unambiguous identification of all proton and carbon resonances. Moreover, the connectivities between the constitutive parts of the molecule were ascertained by intra- and interresidue heteronuclear correlations as shown in Fig. 2; the structure of lotusine C (**3**) was thus established. As previously observed in 14-membered cyclopeptide alkaloids [12], conformational constraints in the macrocycle could induce the non-equivalence of protons and carbons of each aromatic methine pair (CH-12 and CH-12′, CH-13 and CH-13′). As shown in Fig. 2, the observed correlations permitted the linkage of C-13 (δ130.1) and C-13′ (δ132.5) to C-12 (δ122.7) and C-12′ (δ122.5) which, respectively, bore the protons H-12 (δ7.14) and H-12′ (δ7.28).

Lotusine B (**4**) was isolated in a small amount. For this reason, some signals were not detected in NMR spectra. The mass spectrum showed a $[M]^+$ at *m/z* 631 corresponding to the molecular formula $C_{36}H_{49}N_5O_5$ and an ion at *m/z* 274 for which the structure **d** was earlier assigned [5]. The ^1H NMR, COSY and ^{13}C spectra of **4** were very similar to those of **3**, suggesting the same basic skeleton. The main differences observed between these two compounds consisted in the nature of the terminal amino acid and the lack, in **4**, of an *N*-methyl group (Me-28) linked to the phenylalanine unit (as found in **3**). This change induced a strong upfield shift of C-22 from δ49.2, in **4**, to δ70.7 in **3**. The assignment of *N*,*N*-dimethylleucine as the terminal amino acid was deduced, as found in **2**, from the appearance of four methyl groups in NMR spectra and from ion **d** at *m/z* 114 (base peak) in the mass spectrum. All these NMR data suggested the structure **4** for lotusine B (Fig. 3).

The striking feature in this study was the isolation of two novel 13-membered cyclopeptide alkaloids with a phenolic group on styrylamine. To our knowledge, only



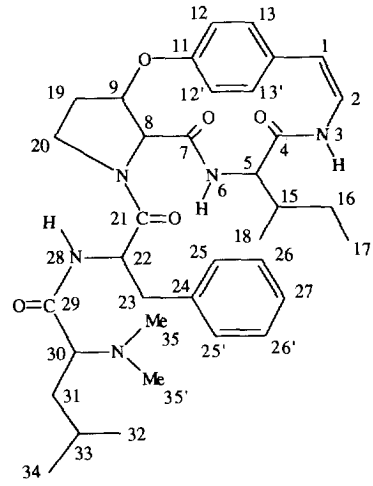
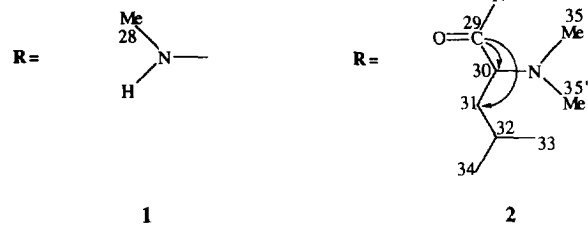


Fig. 3. Structure of **4**.



1 **2**

Fig. 1. Most significant correlations observed in the HMBC spectra of **1** and **2**.

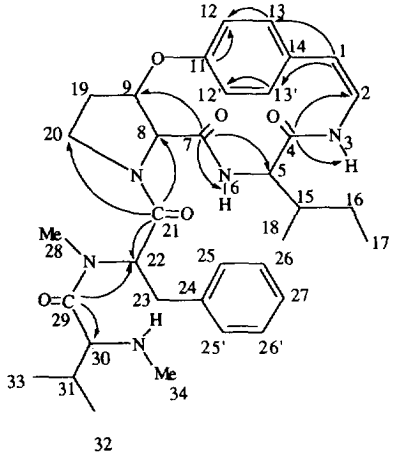


Fig. 2. Most significant correlations observed in the HMBC spectrum of **3**.

three compounds belonging to this sub-group have been previously reported: zizyphine F [5], daechucyclopeptide 1 [13] and compound 3 from *Z. mucronata* [9].

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR were recorded in $CDCl_3$ at 300 and 75 MHz, respectively. Chemical shifts are given on the δ (ppm) scale with TMS as int. standard. EI-MS were obtained by direct probe insert. FAB-MS were measured in the positive FAB mode with glycerin as matrix. TLC was performed on silica gel K6F Whatman and centrifugal TLC was carried out on a Chromatotron apparatus (Harrison Research) on silica gel 60 PF254 (Merck). Alkaloids were detected by spraying with Dragendorff's reagent.

Plant material. Collected in March 1992 in the region of Monastir (locality of Cherahil, Tunisia) and identified by Pr M. A. Nabli, University of Tunis. Voucher specimens are kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Monastir.

Extraction. This was conducted as described in ref. [3]. The alkaloid mixt. (AM) yield was 1.33 g kg^{-1}.

Isolation. The AM was fractionated by centrifugal TLC (CTLC) on a 4 mm layer of silica gel and eluted in 30 ml frs with $CHCl_3$–MeOH (49:1) (frs 1–8) and $CHCl_3$–MeOH (19:1) (frs 9–12). Alkaloid **1** was in fr. 11 (111 mg), **2** in fr. 5 (96 mg), and **3** and **4** in frs 2–3 (230 mg).

Purification. Compound **1** was isolated from fr. 11 by CTLC on a 1 mm layer of silica gel and elution in 10 ml frs with $CHCl_3$–MeOH (99:1) (frs I–III) and $CHCl_3$–MeOH (49:1) (frs IV–XI). Alkaloids **1** and **2** were purified by prep. TLC from frs VII–IX and 5, respectively, using $CHCl_3$–MeOH (93:7) as eluent. Compounds **3** and **4** were isolated from frs 2–3 by CTLC on a 2 mm layer of silica gel eluted in 10 ml frs with $CHCl_3$–MeOH (99:1) (frs I–XI) and $CHCl_3$–MeOH (49:1) (frs XII–XIV). Alkaloid **3** was purified from fr. VII by CTLC [(1 mm; 5 ml frs; $CHCl_3$–MeOH (49:1)]. Compound **4** was purified by prep. TLC from fr. V using $CHCl_3$–MeOH (19:1) as eluent.

Lotusine F (**1**). $[\alpha]_D$ −244° ($CHCl_3$; *c* 0.5). UV λ_{max}^{MeOH} nm: 210, 268, 323; $\lambda_{max}^{MeOH+NaOH}$ nm 210, 263, 353. FT-IR ν_{max}^{Br} cm^{-1}: 3272, 2970, 1692, 1211. Positive FAB-MS *m/z* (rel. int.): 521 ($[M + H]^+$) (28), $[M]^+$ for $C_{29}H_{36}N_4O_5$), 460 (12), 429 (6), 391 (13), 307 (64), 289 (29), 273 (8), 202 (17), 181 (10), 167 (10), 154 (100), 151 (13), 138 (76), 134

(29), 124 (24), 107 (44), 96 (8), 91 (20), 86 (6), 89 (31), 77 (29), 65 (13). ^{1}H and ^{13}C NMR in Tables 1 and 2.

Lotusine E (**2**). $[\alpha]_D$ − 106° ($CHCl_3$; *c* 1.0). UV λ_{max}^{MeOH} nm: 206, 268, 323. $\lambda_{max}^{MeOH+NaOH}$ nm: 212, 268, 353, FT-IR ν_{max}^{KBr} cm^{-1}: 3257, 2954, 1684, 1645, 1219. EI-MS *m/z* (rel. int.): 647 $[M]^+$ (0.5), 590 (1), 442 (5), 359 (6), 289 (0.5), 269 (0.7), 261 (0.4), 245 (0.8), 243 (0.6), 227 (1), 209 (0.7), 202 (3), 190 (1), 185 (1), 181 (1), 177 (1), 165 (0.4), 161 (0.5), 151 (7), 148 (100), 134 (3), 131 (3), 120 (6), 114 (100), 98 (7), 96 (4), 91 (6), 86 (10), 84 (10), 72 (26), 69 (21). ^{1}H and ^{13}C NMR in Tables 1 and 2.

Lotusine C (**3**). $[\alpha]_D$ − 168° ($CHCl_3$; *c* 0.5). UV λ_{max}^{MeOH} nm: 204. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3310, 2960, 1615, 1220, 1045. EI-MS *m/z* (rel. int.): 617 $[M]^+$ (0.3), 603 (0.8), 544 (2), 542 (2), 528 (5), 526 (11), 513 (4), 427 (0.7), 347 (0.9), 344 (5), 316 (0.9), 314 (0.9), 286 (1), 285 (1), 272 (6), 233 (1), 229 (1), 221 (1), 213 (2), 203 (3), 183 (6), 169 (9), 156 (24), 148 (100), 135 (33), 134 (100), 105 (24), 100 (17), 96 (5), 91 (17), 86 (15), 77 (12), 69 (33), 68 (19), 56 (22). ^{1}H and ^{13}C NMR in Tables 1 and 2.

Lotusine B (**4**). $[\alpha]_D$ ($CHCl_3$; *c* 0.32): − 179°. UV λ_{max}^{MeOH} nm: 207, 245 (sh). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3310 − 2920 − 1655 − 1225. EI-MS *m/z* (rel. int.): 631 $[M]^+$ (0.9), 574 (0.2), 519 (2), 427 (62), 274 (0.3), 243 (0.4), 227 (1), 209 (0.5), 203 (1.6), 200 (1), 190 (4), 148 (100), 135 (11), 134 (12), 114 (100), 96 (4), 91 (9), 86 (9), 84 (11), 72 (27), 68 (17).

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REFERENCES

1. Southon, I. W. and Buckingham, J. (1989) In *Dictionary of Alkaloids* (Cordell, G. A., ed.) Chapman & Hall, London.
2. Ghedira, K., Chemli, R., Caron, C., Nuzillard, J.-M. and Zeches, M. (1994) Poster Communication. Ist International Colloquy: Pharmacopée Arabo-musulmane, hier et aujourd'hui, Rabat, Marocco.
3. Ghedira, K., Chemli, R., Richard, B., Nuzillard, J.-M., Zeches, M. and Le Men-Olivier, L. (1993) *Phytochemistry* **32**, 1591.
4. Kircher, H. W. (1980) *Phytochemistry* **19**, 2707.
5. Tschesche, R., Khokhar, I., Spilles, Ch., Eckhardt, G. and Cassels, B. K. (1974) *Tetrahedron Letters* 2941.
6. Tschesche, R. and Kaußmann, E. U. (1975) in *The Alkaloids* Vol. XV (Manske, R. H. F., ed.), pp. 165–205. Academic Press, New York.
7. Hesse, M. and Bernhard, H. O. (1975) in *Progress in Mass Spectrometry* Vol. III (Budzikiewicz, H. ed.), pp. 335–348. Verlag Chemie, Weinheim.
8. Marchand, J., Pais, M., Monseur, X. and Jarreau, F.-X. (1969) *Tetrahedron* **25**, 937.
9. Barboni, L., Gariboldi, P., Torregiani, E. and Verotta, L. (1994) *Phytochemistry* **35**, 1579.
10. Bax, A. and Subramanian, S. J. (1986) *J. Magn. Reson.* **67**, 565.
11. Bax, A. and Summers, M. F. (1986) *J. Am. Chem. Soc.* **108**, 2093.
12. Pais, M., Jarreau, F.-X., Gonzalez Sierra, M., Mascaretti, O. A., Ruveda, E. A., Chang, C.-J., Hagaman, E. W. and Wenkert, E. (1979) *Phytochemistry* **18**, 1869.
13. Han, B. H. and Park, M. H. (1987) *Pharmacol. Res.* **10**, 108