Phytochemical Constituents from *Salsola tetrandra*

M. Habib Oueslati,[†] Hichem Ben Jannet,[†] Zine Mighri,[†] J. Chriaa,[§] and Pedro M. Abreu*[,‡]

Laboratoire de Chimie des Substances Naturelles et de Synthèse Organique, Faculté des Sciences de Monastir, 5000 Monastir, Tunisia, Laboratoire de Microbiologie de l'Environnement, Faculté de Farmacie de Monastir, 5019 Monastir, Tunisia, and CQFB/REQUIMTE, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

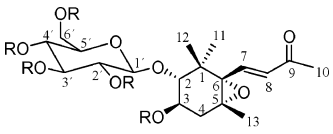
Received May 16, 2006

The new norisoprenoid 3β-hydroxy-5α,6α-epoxy-β-ionone-2α-*O*-β-D-glucopyranoside (**1**) and the long-chain hydroxy fatty acids 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid (**2**) and 9,12,13-trihydroxyoctadeca-10(*E*)-dienoic acid (**3**) were isolated from *Salsola tetrandra* aerial parts, together with 3,4,5-trimethoxyphenyl-β-D-glucopyranoside (**4**), 9-hydroxylinaloyl glucoside (**5**), taxiphyllin (**6**), *trans*-*N*-feruloyltyramine (**7**), and *S*-(−)-*trans*-*N*-feruloyloctopamine (**8**). Their structures were elucidated by extensive spectroscopic analysis and chemical methods. Compounds **6** and **8** displayed mild antibacterial activity against *Staphylococcus aureus*, whereas compound **6** showed the highest activity in the *Artemia salina* bioassay.

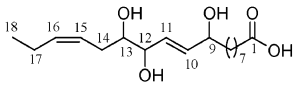
The genus *Salsola* (Chenopodiaceae) consists of over 100 species found in the dry regions of Asia, Europe, and Africa, some of which are utilized in local traditional medicine.[1] As part of our research on plants from Tunisia,[2−6] we have investigated *Salsola tetrandra* Forak., a shrub growing in North Africa, Palestine, and Saudi Arabia.[7,8] In the scant phytochemical work reported for this species, a coumarinolignan, triacetoamine, betaine, estrone, and cholesterol have been identified.[5,9,10] In this communication, we report the characterization of the new norisoprenoid 3β-hydroxy-5α,6α-epoxy-β-ionone-2α-*O*-β-D-glucopyranoside (**1**) and the long-chain hydroxy fatty acids 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid (**2**) and 9,12,13-trihydroxyoctadeca-10(*E*)-dienoic acid (**3**), together with 3,4,5-trimethoxyphenyl-β-D-glucopyranoside (**4**),[11,12] 9-hydroxylinaloyl glucoside (**5**),[13] taxiphyllin (**6**),[14,15] *trans*-*N*-feruloyltyramine (**7**),[16−18] and *S*-(−)-*trans*-*N*-feruloyloctopamine (**8**).[16,17,19,20] The antimicrobial activity of **1**−**8** was evaluated against *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, whereas compounds **1** and **4**−**6** were further tested in the *Artemia salina* bioassay.

Following the extraction of the aerial parts of *S. tetrandra* with MeOH and successive partitioning of the dried extract in H_2O/hexane, H_2O/EtOAc, and H_2O/n-BuOH, significant activity (ED_{50} 2.33 μg/mL) of the BuOH fraction was observed in the brine shrimp assay.[21] The active fraction was then subjected to a sequence of normal- and reversed-phase column chromatography, to yield compounds **1**−**6**, whereas the EtOAc fraction afforded **7** and **8**.

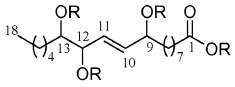
Compound **1** was isolated as a white solid with the molecular formula $C_{19}H_{30}O_9$, calculated from the $[M + Na]^+$ peak at *m*/*z* 425.1776 in the HRESIMS. Its IR spectrum showed absorption bands due to hydroxyl (3452 cm^{-1}) and α,β-unsaturated ketone (1677 cm^{-1}) groups. The NMR spectra (Table 1) showed four methyl groups at δ_H/δ_C 1.07/19.0, 1.31/18.5, 1.63/26.0, and 2.26/27.3, two methylenic protons at δ 1.98 and 2.65/δ_C 39.2, two oxymethine protons at δ_H/δ_C 3.52/91.9 and 4.04/65.0, a high-field tetrasubstituted carbon (δ_C 40.6), two oxygenated quaternary carbons at δ_C 66.5 and 69.5, and a *trans* double bond (J = 15.6 Hz) conjugated to a ketone (δ_C 196.8), as indicated by HMBC correlations. In addition, ^1H and ^{13}C NMR spectra displayed the characteristic resonances of a sugar moiety with several proton signals superposed in the 4.00−5.00 ppm region, resolved by acetylation of **1**. The NMR data of **1** and those of its pentaacetate **1a** supported a 5,6-epoxy-β-ionone basic structure with two



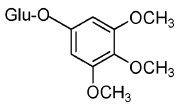
1 R = H
1a R = Ac



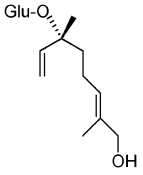
2



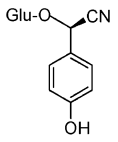
3 R = H
3a R = $SiMe_3$



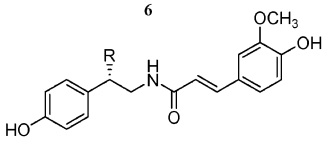
4



5



6



7 R = H
8 R = OH

* Corresponding author. Tel: 351-1-2948354. Fax: 351-1-2948550. E-mail: pma@dq.fct.unl.pt.
[†] Laboratoire de Chimie des Substances Naturelles et de Synthèse Organique, Monastir.
[§] Laboratoire de Microbiologie de l'Environnement, Monastir.
[‡] CQFB/REQUIMTE, Caparica.

10.1021/np060222w CCC: $33.50
Published on Web 08/25/2006

Table 1. NMR Data of Compounds **1** (pyridine) and **1a** ($CDCl_3$)

	1			**1a**	
position	δ_H (*J* in Hz)	δ_C	HMBC (H−C)	δ_H (*J* in Hz)	δ_C
1		40.6			40.2
2	3.52 d (9.7)	91.9	C-1, C-3, C-11, C-12, C-1′	3.31 d (8.8)	86.0
3	4.04 m	65.0		5.04 m	67.1
4_{ax}	1.98 dd (14.7, 10.2	39.2	C-3	1.85 dd (14.8, 8.7)	35.8
4_{eq}	2.65 dd (14.7, 5.2)		C-2, C-3, C-6	2.50 dd (16.0, 5.2)	
5		66.5			65.4
6		69.6			68.9
7	7.27 d (15.6)	142.5	C-6, C-9	6.96 d (15.6)	140.8
8	6.51 d (15.6)	132.8	C-6, C-9	6.33 d (15.6)	132.6
9		196.8			197.1
10	2.26 s	27.3	C-9	2.27 s	28.9
11	1.31 s	18.5	C-1, C-2, C-6, C-12	0.93 s	18.9
12	1.63 s	26.0	C-1, C-2, C-6, C-11	1.25 s	26.1
13	1.07 s	19.0	C-4, C-5, C-6	1.14 s	19.6
1′	4.92 d (7.8)	106.5	C-2, C-2′	4.53 d (8.0)	102.8
2′	4.04 m	75.0		4.97 t (8.0, 9.4)	71.9
3′	4.17 m	78.3[a]	C-2′, C-4′	5.17 t (9.4, 9.4)	73.1
4′	4.17 m	71.0	C-3′, C-5′	5.07 t (9.4, 9.4)	68.1
5′	4.04 m	78.2[a]		3.66 o (9.4, 4.4, 2.4)	71.7
6_a′	4.29 m	62.1		4.07 dd (12.4, 4.4)	61.9
6_b′	4.52 d (11.0)			4.33 dd (12.4, 2.4)	
OCOC*H*$_3$				1.98, 2.00, 2.06	
OCOCH$_3$					20.5, 20.6, 21.0,
OCOCH$_3$					168.8, 169.3, 169.9, 170.3, 170.6

[a] Assignments in the same column may be exchanged.

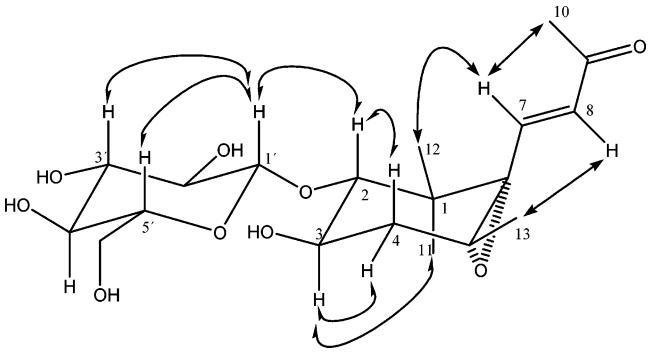


Figure 1. NOE associations of compound **1**.

additional *O*-functions at C-2 and C-3.[22−24] The glycosidic site in **1** was established from the HMBC and NOESY cross-peaks of the anomeric proton H-1′ (δ 4.92) with C-2 (δ_C 91.9) and H-2 (δ 3.52), respectively. The relative configuration of **1** was determined by a combination of coupling constant analysis and NOESY (Figure 1). The axial orientation of the vicinal protons H-2 and H-3 was assigned from the corresponding coupling constant (J = 9.7 Hz) and the NOE interactions H-2/H-4ax, H-3/H-4eq, and H-3/11-CH_3. The equatorial methyl group at C-1 and 13-CH_3 showed NOESY cross-peaks with the olefinic protons H-7 and H-8, respectively. The sugar unit was identified as β-D-glucose, by comparison of the NMR data of **1** and **1a** with those reported for other ionone glucosides[22−25] and the observed NOE interactions between the three *cis*-axial protons H-1′, H-3′, and H-5′. The above data closely resembled those reported for icariside B_3 (2β-hydroxy-5α,6α-epoxy-β-ionone-3β-*O*-β-D-glucopyranoside),[22] except that the resonances of C-2 and C-3 were significantly shifted in **1**, by +16.9 and −10.6 ppm, respectively. These differences suggest that the oxirane γ-effects on C-2 and C-3 are strongly related to the substitution pattern and half-chair conformation of the epoxy-β-ionone ring.[26] Therefore, the structure of **1** was established as 3β-hydroxy-5α,6α-epoxy-β-ionone-2α-*O*-β-D-glucopyranoside.

Compound **2** showed a molecular weight of 328 amu, as indicated by the pseudomolecular ions at *m/z* 327 $[M - H]^-$, 351 $[M + Na]^+$, and 363 $[M + Cl]^-$ exhibited in the ESIMS. Its IR spectrum displayed OH and C=O absorptions at 3392 and 1700 cm^{-1}, respectively. The profile of the ^{13}C NMR spectrum was characteristic of an unsaturated long-chain fatty acid: a methyl group at δ_C 14.5, several methylene carbons from δ 21.6 to 38.3, four sp^2 carbons at δ_C 125.5, 130.4, 134.3, and 136.6, and a carboxyl carbon at δ_C 178.4. In addition, three low-field oxygenated carbons at δ_C 73.0, 75.8, and 75.9, bearing methine protons at δ 4.08, 3.97, and 3.48, respectively, confirmed the presence of three hydroxyl groups. Analysis of the COSY, HMQC, and HMBC spectra provided evidence for the fragment $CH_3-CH_2-CH=CH-CH_2-CH(OH)-CH(OH)-CH=CH-CH(OH)-CH_2-$. Considering the molecular weight of **2** and the number of CH_2 groups displayed in ^{13}C NMR and DEPT spectra, this fragment should be separated from the terminal carboxyl group by a $-(CH_2)_7-$ chain. The *E* configuration of the C-10−C-11 double bond was assigned on the basis of the coupling constants of the olefinic protons H-10 and H-11, which resonated as a resolved doublet of doublets at δ 5.75 and 5.69, respectively ($J_{10,11}$ = 15.9 Hz, $J_{9,10} = J_{11,12}$ = 5.5 Hz). The coupling constant $J_{15,16}$ = 11.2 Hz suggested a *Z* configuration for the C-15−C-16 double bond, which was corroborated by the high-field chemical shift δ_C 21.6 of the allylic C-17.[27] The structure of **2** was thus determined as 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid, but the lack of available sample precluded the determination of the configuration at C-9, C-12, and C-13. Although this fatty acid has been reported as a substance with prostaglandin-like activity[28−30] and self-defense action against rice disease,[30] so far, only the 9*S*,12*R*,13*S* isomer (malyngic acid) was fully characterized.[30] Since compound **2** and malyngic acid have similar ^1H and ^{13}C NMR spectra, but specific rotations of opposite sign (−8.6 and +7.5, respectively), the above data support their diastereoisomeric relationship.

The molecular formula of compound **3** was determined as $C_{18}H_{34}O_5$ by positive and negative HRESIMS. As for compound **2**, the IR spectrum exhibited absorption bands for OH (3351 cm^{-1}) and C=O (1699 cm^{-1}) groups, whereas its NMR data indicated a monounsaturated long-chain fatty acid structure, with one *E*-double bond (J = 15.9 Hz), part of a $-CH_2-CH(OH)-CH(OH)-CH=CH-CH(OH)-CH_2-$ fragment. The position of the olefin at C-10−C-11 was confirmed by GC-EIMS of the tetramethylsilyl derivative **3a** (Figure 2).[30,33] The above spectroscopic data and the value of the specific rotation $[\alpha]_D^{25}$ −10.0 were in agreement with the reported data for pinellic acid ((9*S*,12*S*,13*S*)-trihydroxy-10*E*-octadecenoic acid, $[\alpha]_D^{28}$ −8.1).[34] Nevertheless, we cannot exclude the

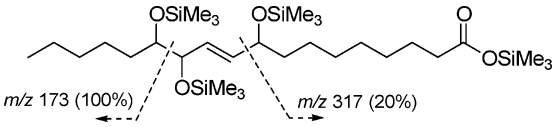


Figure 2. Mass fragmentation of compound **3a**.

alternative structures of the stereoisomers 9*R*,12*S*,13*R* and 9*R*,12*R*,-13*S*, whose specific rotations are −7.1 and −5.3, respectively.[35] Pinellic acid, previously isolated from the tuber of *Pinellia ternata*, is an effective oral adjuvant for nasal influenza vaccine,[34] whose total synthesis, as well as those of all diastereoisomers, has been reported.[35,36]

Following standard protocols,[37,38] compounds **1**−**8** were evaluated for their antimicrobial activities against *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The lowest MIC values were observed for compounds **6** and **8**, which inhibited the growth of *S. aureus* at concentrations of 200 μg/mL, with MBC determined as 500 and 600 μg/mL, respectively (Supporting Information, Table S1). In an *Artemia salina* lethality bioassay, compounds **1** and **4**−**6** showed ED_{50} values of 10.9, 28.9, 21.8, and 0.96 μM, respectively.

Experimental Section

General Experimental Procedures. Mp's were determined on a Reichert microscope. Optical rotations were recorded on a Perkin-Elmer 241-MC polarimeter. UV and FTIR spectra were recorded on a Milton Roy Spectronic 1201 spectrophotometer and Perkin-Elmer 157G infrared spectrophotometer, respectively. ^{1}H (400 MHz) and ^{13}C (100.61 MHz) NMR spectra were recorded on a Bruker ARX-400 spectrometer. EIMS (70 eV), ESIMS, and HRESIMS were recorded on a Micromass GCTOF spectrometer, Agilent MSD1100 single quadropole spectrometer, and Agilent ESI-TOF instrument, respectively. GC-MS was performed on a Hewlett-Packard MSD 5972 operating at 70 eV, under the following conditions: column, fused-silica HP5 MS (30 m × 0.25 mm, 0.25 μm); He as carrier gas; flow rate 1.2 mL/min; split ratio 1:50; oven temperature program, 50 °C for 1 min, 50−280 °C at 5 °C/min, and 20 min at 280 °C; injector temperature 250 °C; detector temperature 280 °C. CC was performed over Si gel (35−70 mesh, MN), whereas Si gel (230−400 mesh, MN) and RP-18 Si gel (40−63 μm, Merck) were used for flash chromatography. Michel-Miller columns filled with LiChroprep RP-18 Si gel (40−63 μm, Merck) were used in low-pressure liquid chromatography (LPLC). The mobile phase was delivered by a Fluid Metering QSY pump at a maximum flow rate of 15 mL/min. TLC was performed using precoated Si gel 60 F_{254} (MN) and RP-18 Si gel F_{254} (Merck) plates.

Plant Material. *Salsola tetrandra* was collected at Monastir (Tunisia) in March 2002 and identified by Dr. F. Harzallah-Skhiri, Ecole Supérieure d'Horticulture et d'Elevage de Chott Mériem, Université de Sousse, Tunisia, where a voucher (ST-100) is deposited.

Extraction and Isolation. Air-dried and powdered aerial parts of *S. tetrandra* (1.8 kg) were extracted in a Soxhlet apparatus with MeOH. After evaporation of the solvent under reduced pressure, the extract (380 g) was suspended in H_2O (2 L) and partitioned successively with hexane, EtOAc, and n-BuOH. The BuOH fraction (16 g) was applied to a Si gel column and eluted with a gradient of CH_2Cl_2/MeOH (100: 0; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 20:80; 0:100) to give nine fractions (A_1−A_9), two of which (A_5 and A_6) were active in the brine shrimp assay. Successive RP-18 flash CC and LPLC of fraction A_5 (1.8 g) with H_2O/MeOH gradient elution afforded compounds **2** (9.4 mg), **3** (10.6 mg), and **5** (26 mg). Flash CC of fraction A_6 (3.22 g) with CH_2Cl_2/MeOH (85:15) elution yielded 340 mg of **6** and a mixture of **1** and **4**, which was separated by RP-18 flash CC with H_2O/MeOH (80:20) elution to obtain 16 mg of **1** and 10.8 mg of **4**. The EtOAc fraction (27 g) was chromatographed over Si gel with successive petroleum ether/EtOAc (80:20; 60:40; 40:60; 20:80; 0:100) and EtOAc/MeOH (90:10; 80:20; 70:30; 50:50; 0:100) elution, to give 12 subfractions, B_1−B_{12}. Si gel CC of B_8 (1.36 g) with $CHCl_3$/MeOH elution (9.5:0.5 and 9:1) afforded **7** (8 mg) and **8** (5 mg).

3β-Hydroxy-5α,6α-epoxy-β-ionone-2α-*O*-β-D-glucopyranoside (1): white solid; $[\alpha]_D^{25}$ −145 (*c* 0.14, MeOH); IR ν_{max} 3452, 2933, 1678 cm^{-1}; HREIMS *m*/*z* 425.1776 [M + Na]$^+$ (calcd for $C_{19}H_{30}O_9Na$, 425.1788); NMR data, see Table 1. Compound **1** was acetylated with Ac_2O/pyridine (overnight, rt) to yield the corresponding pentaacetate **1a**: HREIMS *m*/*z* 635.2302 [M + Na]$^+$ (calcd for $C_{29}H_{40}NaO_{14}$ 635.2316); NMR data, see Table 1.

9,12,13-Trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid (2): gummy, white solid; $[\alpha]_D^{25}$ −8.6 (*c* 0.86, MeOH); ESIMS *m*/*z* 363 [M + Cl]$^-$; 351 [M + Na]$^+$, 327 [M − H]$^+$; IR ν_{max} 3392, 2930, 2856, 1700, 973 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$/MeOD) δ 0.96 (3H, *t*, *J* = 7.5, H-18), 1.34 (11H, bs, H-4, H-5, H-6, H-7, H-8a), 1.52 (1H, m, H-8b), 1.61 (2H, m, H-3), 2.07 (2H, t, *J* = 6.6, H-17), 2.17 (1H, m, H-14a), 2.28 (2H, m, H-2), 2.33 (1H, m, H-14b), 3.48 (1H, m, H-13), 3.97 (1H, t, *J* = 5.5 Hz, H-12), 4.08 (1H, m, H-9), 5.44 (1H, o, *J* = 11.2, 5.2 Hz, H-16), 5.49 (1H, o, *J* = 11.2, 5.2 Hz, H-15), 5.69 (1H, dd, *J* = 15.9, 5.5 Hz, H-11), 5.75 (1H, dd, *J* = 15.9, 5.5 Hz, H-10); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD) δ 14.5 (C-18), 21.6 (C-17), 26.3 (C-3), 26.4 (C-7), 30.2 (C-4, C-5, or C-6), 30.4 (C-4, C-5, or C-6), 30.5 (C-4, C-5, or C-6), 31.6 (C-14), 36.6 (C-2), 38.3 (C-8), 72.3 (C-9), 75.3 (C-12, C-13), 125.5 (C-15), 130.9 (C-10), 134.3 (C-16), 136.3 (C-11), 178.1 (C-1).

9,12,13-Trihydroxy-10(*E*)-octadecenoic acid (3): gummy, white solid; $[\alpha]_D^{25}$ −10.0 (*c* 0.36, MeOH); HRESIMS *m*/*z* 353.2297 [M + Na]$^+$ (calc for $C_{18}H_{34}NaO_5$, 353.2304); *m*/*z* 329.2374 [M − H]$^+$ (calc for $C_{18}H_{33}O_5$, 329.2328); IR_{max} 3351, 2931, 2850, 1699, 974 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$/MeOD) δ 0.91 (3H, t, *J* = 7.5, H-18), 1.34 (16H, bs, H-4, H-5, H-6, H-7, H-8b, H-14a, H-15, H-16, H-17), 1.52 (1H, m, H-8b), 1.61 (3H, m, H-3, H-14b), 2.29 (2H, m, H-2), (3.42 (1H, m, H-13), 3.91 (1H, t, *J* = 5.5 Hz, H-12), 4.06 (1H, m, H-9), 5.67 (1H, dd, *J* = 15.9, 5.5 Hz, H-11), 5.73 (1H, dd, *J* = 15.9, 5.5 Hz, H-10); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD) δ 14.37 (C-18), 23.5 (C-17), 25.9 (C-3 or C-15), 26.2 C-3 or C-15, 26.3 (C-7), 29.9 (C-4), 30.1 (C-5 or C-6), 30.3 (C-5 or C-6), 32.8 (C-16), 33.2 (C-14), 35.1 (C-2), 38.8 (C-8), 72.7 (C-9), 75.5 (C-13), 76.2 (C-12), 130.4 (C-10), 136.4 (C-11), 177.9 (C-1). Compound **3** was silylated with *N*,*O*-bis-(trimethylsilyl) acetamide, according to a described procedure,[39] to yield **3a**, which was analyzed by GC-EIMS.

Antimicrobial Assays. The antimicrobial activities of **1**−**8** were evaluated against Gram-positive *Staphylococcus aureus* ATCC 29213, *S. epidermidis* NCIMB 8853, and *Micrococcus luteus* NCIMB 8166 and Gram-negative *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 using microdilution methods on liquid medium, as previously described.[37,38] All organisms were stored at −70 °C in glycerol Mueller-Hinton broth. Fresh subcultures were used for each experiment, and the final bacterial concentration in the tubes was adjusted to (1−5) × 10^5 cfu/mL. The minimal inhibitory concentration (MIC) was defined as the lowest concentrations that prevent visible growth, and the minimal bactericidal concentration (MBC) was determined as a concentration where ≥99.9% of the initial inoculum is killed. To confirm the results of MBC, 100 μL of each dilution suspensions was subcultured on TSA agar plates, which were incubated at 30 °C for 18−24 h.

Acknowledgment. We thank GRICES (Portugal) for a travel grant (M.H.O.).

Supporting Information Available: Data of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of compounds **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Woldu, Y; Abegaz, B. *Phytochemistry* **1990**, *29*, 2013−2015.
(2) Hichri, F.; Ben Jannet, H.; Abreu, P. M.; Mighri, Z. *J. Soc. Alg. Chim.* **2003**, *13*, 187−196.
(3) Braham, H.; Mighri, Z.; Ben Jannet, H.; Matthew, S.; Abreu, P. M. *J. Nat. Prod.* **2005**, *68*, 517−522.
(4) Oueslati, M. H.; Ben Jannet, H.; Abreu, P.; Mighri, Z. *J. Soc. Alger. Chim.* **2004**, *14*, 181−187.
(5) Oueslati, M. H.; Ben Jannet, H.; Abreu, P.; Mighri, Z. *J. Soc. Alger. Chim.* **2004**, *14*, 245−258.
(6) Oueslati, M. H.; Ben Jannet, H.; Mighri, Z.; Abreu, P. M. *Lipids* **2005**, *40*, 1075−1079.
(7) Alapetite, G. P. *Flore de la Tunisie*; Publications Scientifiques Tunisiennes, Imprimerie Officielle de la République Tunisienne: Tunis, Tunisie, 1981.
(8) Le Floc'h, E. *Contribution à une étude ethnobotanique de la flore Tunisienne*; Publications Scientifiques Tunisiennes, Imprimerie Officielle de la République Tunisienne: Tunis, Tunisie, 1983.

(9) Karawya, M. S.; Wassel, G.; Rücker, M. G.; Baghdadi, H. H.; Ahmed, Z. F. *Phytochemistry* **1971**, *10*, 3303−3304.
(10) Wassel, G. M.; Baghdadi, H. H.; El Difrawy, S. M. *Fitoterapia* **1979**, *50*, 51−52.
(11) Steinbeck, C.; Schneider, C.; Rotscheidt, K.; Breitmaier, E. *Phytochemistry* **1995**, *40*, 1313−1315.
(12) Achenbach, H.; Benirschke, G. *Phytochemistry* **1997**, *45*, 149−157.
(13) Uchiyama, T.; Miyase, T.; Ueno, A.; Usmanghani. K. *Phytochemistry* **1989**, *28*, 3369−3372.
(14) Towers G. H. N.; Mcinnes A. G.; Neish A. C. *Tetrahedron* **1964**, *20*, 71−77.
(15) Seigler, D. S.; Pauli, G. F.; Fröhlich, R.; Wegelius, E.; Nahrstedt, A.; Glander, K. E.; Ebinger, J. E. *Phytochemistry* **2005**, *66*, 1567−1580.
(16) Yoshihara, T.; Takamatsu, S.; Sakamura, S. *Agric. Biol. Chem.* **1978**, *42*, 623−627.
(17) Yoshihara, T.; Yamagushi, K.; Takamatsu, S.; Sakamura, S. *Agric. Biol. Chem.* **1981**, *45*, 2593−2598.
(18) Hussain, S. F.; Gözler, B.; Shamma, M.; Gözler. T. *Phytochemistry* **1982**, *21*, 2979−2980.
(19) Hussein, N. S.; El-Bassuony, A. A. *Rev. Latinoam. Quím.* **2004**, *32*, 15−20.
(20) King, R. R.; Calhoun, L. A. *Phytochemistry* **2005**, *66*, 2468−2473.
(21) McLaughlin, J. L. In *Methods in Plant Biochemistry*; Hostettman, K., Ed.; Academic: London, 1991; Vol. 6, pp 1−32.
(22) Miyase, T.; Ueno, A.; Takizawa, N.; Kobayashi, H.; Oguchi, H. *Chem. Pharm. Bull.* **1987**, *35*, 3713−3719.
(23) Humpf H.-U.; Schreier, P. *J. Agric. Food Chem.* **1992**, *40*, 1898−1901.
(24) Skouroumounis G. K.; Winterhalter, P. *J. Agric. Food Chem.* **1994**, *42*, 1068−1072.
(25) Güldner, A.; Winterhalter, P. *J. Agric. Food Chem.* **1991**, *39*, 2142−2146.
(26) Carda, M.; Sans, J. F.; Marco, J. A. *J. Org. Chem.* **1992**, *57*, 804−811.
(27) Rossi, R.; Carpita, A.; Quirici, M. G.; Veracini, C. A. *Tetrahedron* **1982**, *38*, 639−644.
(28) Panosyan, A. G.; Avetisyan, G. M.; Mnatsakanyan, V. A.; Asatryan, T. A.; Vartanyan, S. A.; Boroyan, R. G.; Batrakov, S. G. *Bioorg. Khim.* **1979**, *5*, 242−253.
(29) Panosyan, A. G.; Barikyan, M. L.; Lebedeva, M. N.; Amroyan, E. A.; Gabrielyan, E. S. *Khim. Prir. Soedin.* **1980**, *6*, 825−826.
(30) Panosyan, A. G.; Avetisyan, G. M.; Mnatsakanyan, V. A.; Batrakov, S. G.; Vartanyan, S. A.; Gabrielyan, E. S.; Amroyan, E. A. *Planta Med.* **1983**, *47*, 17−25.
(31) Kato, T.; Yamaguchi, Y.; Abe, N.; Uyehara, T.; Namai T.; Kodama, M.; Shiobara, Y. *Tetrahedron Lett.* **1985**, *26*, 2357−2360.
(32) Cardellina, J. H.; Moore, R. E. *Tetrahedron* **1980**, *36*, 993−996.
(33) Üstünes, L.; Claeys, M.; Laekeman, G.; Herman, A. G.; Vlietinck, A. J.; Özer, A. *Prostaglandins* **1985**, *29*, 847−862.
(34) Nagai, T.; Kiyohara, H.; Munakata, K.; Shirahata, T.; Sunazuka, T.; Harigaya, Y.; Yamada, H. *Int. Immunopharm.* **2002**, *2*, 1183−1193.
(35) Shirahata, T.; Sunazuka, T.; Yoshida, K.; Yamamoto, D.; Harigaya, Y.; Nagai, T.; Kiyohara, H. Yamada, H.; Kuwajima, I.; Omura, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 937−941.
(36) Sunazuka, T.; Shirahata, T.; Yoshida, K.; Yamamoto, D.; Harigaya, Y.; Nagai, T.; Kiyohara, H.; Yamada, H.; Kuwajima, I.; Omura, S. *Tetrahedron Lett.* **2002**, *43*, 1256−1268.
(37) May J.; Chan, C. H.; King, A.; Williams, L.; French, G. L. *J. Antimicrob. Chemother.* **2000**, *45*, 639−643.
(38) Canillac, N.; Mourey, A. *Food Microbiol.* **2001**, *18*, 261−268.
(39) Eglinton, G. D.; Hunneman, H.; McCormick, A. *Org. Mass Spectrom.* **1968**, *1*, 593−611.

NP060222W