New Modified Eremophilanes from the Roots of *Psacalium radulifolium*

María Luisa Garduño-Ramírez,[†,§] Alicia Trejo,[†] Víctor Navarro,[‡] Robert Bye,[⊥] Edelmira Linares,[⊥] and Guillermo Delgado*[,†]

Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D. F., Centro de Investigaciones Químicas de la Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Chamilpa 62210, Cuernavaca, Morelos, México, Unidad de Investigación Biomédica, IMSS, Argentina No. 1, Xochitepec, Morelos, México, and Jardín Botánico del Instituto de Biología de la Universidad Nacional Autónoma de México, Apdo. Postal 70-614, México, D. F.

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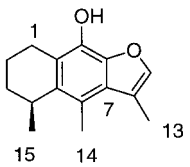
The investigation of the chemical constituents from the roots of *Psacalium radulifolium*, a member of the matarique complex of medicinal plants, which includes several members of the Asteraceae, resulted in the isolation of four new modified eremophilanes: radulifolin A (**4**), *epi*-radulifolin A (**5**), radulifolin B (**6**), and radulifolin C (**7**), together with the known natural substances cacalol, cacalone, *epi*-cacalone, *O*-methyl-1,2-dehydrocacalol, adenostin A, decompostin, and neoadenostylone. Antimicrobial evaluation of the extracts and the isolated compounds indicated that cacalol was the major active compound.

Matarique is a medicinal plant complex of Mexico which includes perennial herbs with thin, fascicled roots extending from a pubescent root crown, the concoction of which is drunk, alone or in combination with other herbs, for treating diabetes, kidney pain, and rheumatism; it can also be applied as a wash or cataplasm to treat wounds and skin ulcers.[1,2] The matarique complex includes *Acourtia thurberi*, *Psacalium decompositum*, *P. palmeri*, *P. peltatum*, *P. sinuatum*, and *P. radulifolium*, a common substitute in the region of San Luis Potosí for the preferred *P. decompositum*, which grows in the pine-oak woodlands of the mountains from western Mexico (Sinaloa and Durango) across central Mexico (Guanajuato and San Luis Potosí) to eastern Mexico (Nuevo Leon and Tamaulipas). The genus *Psacalium* (Asteraceae, Senecioneae, Tussilagininae) includes 40 Mexican species with a few extending into the southwestern United States and Guatemala.[3,4] Antimicrobial assays were conducted given that the decoction and the crushed roots are used to heal skin sores, abrasions, and wounds.

Previous studies on *P. decompositum* have led to the isolation and structural studies of several eremophilanes and rearranged eremophilanes.[5] The structures of cacalol (**1**), cacalone (**2**), and related compounds[6,7] were the subject of several structural revisions,[8] and the final structures were confirmed by chemical synthesis[9−12] and crystallographic analysis.[13] A root decoction of *P. decompositum*, *P. peltatum*, and *A. thurberi* has been shown to lower the glucose levels in mice.[14] The modified eremophilane cacalol (**1**) possesses antimicrobial activity, inhibits the germination of *Amaranthus hypocondriacus* (Amaranthaceae) and *Echinocloa crusgalli* (Poaceae), and is also active against some phytopathogenic fungi.[15] Recently, in vivo bioassay-directed fractionation of an extract of the roots of *P. decompositum* led to the isolation of modified eremophilanolides, which exhibited antihyperglycemic activity in mice.[16] Cacalol (**1**) was also found as an active constituent, but cacalone (**2**) and *epi*-cacalone (**3**) were inactive. As a continuation of our systematic survey on the species included in the complexes of Mexican medicinal plants,[17] we now report the isolation of four new modified eremophilanes—radulifolin A (**4**), *epi*-radulifolin A (**5**), radulifolin B (**6**), and radulifolin C (**7**)—and seven related compounds: **1**−**3**, *O*-methyl-1,2-dehydrocacalol,[18] adenostin A,[19] decompostin,[20] and neoadenostylone,[21] from the roots of *P. radulifolium*.



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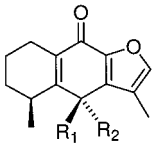
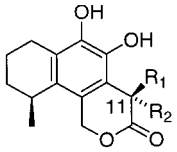
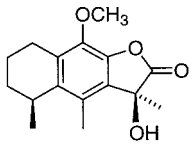


	R_1	R_2
2	CH_3	OH
3	OH	CH_3



	R_1	R_2
4	CH_3	OH
5	OH	CH_3



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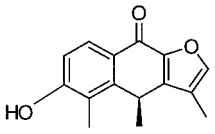

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Results and Discussion

The *n*-hexane and methanolic extracts of the roots of *P. radulifolium* were tested against *S. aureus*, *E. coli*, *P. aeruginosa*, *Proteus micranthus*, and *C. albicans* (see Table 1). The *n*-hexane extract displayed antimicrobial activity against *S. aureus* and *C. albicans*. From this extract were isolated cacalol (**1**),[18] *O*-methyl-1,2-dehydrocacalol ether (*syn*: cacalohastin),[18] adenostin A,[19] decompostin,[20] neoadenostylone,[21] the 1:1 mixture of cacalone (**2**) and *epi*-cacalone (**3**),[31,32] and four new substances whose structures were determined as follows.

Radulifolin A (**4**) and *epi*-radulifolin A (**5**) were isolated as a solid that appeared as one spot on TLC and by HRMS

* To whom correspondence should be addressed. Tel: (52)-5622-4446. Fax: (52)-5616-2217. E-mail: delgado@servidor.unam.mx.
† Instituto de Química de la UNAM.
§ Centro de Investigaciones Químicas de la UAEMor.
‡ Unidad de Investigación Biomédica del IMSS.
⊥ Jardín Botánico de la UNAM.

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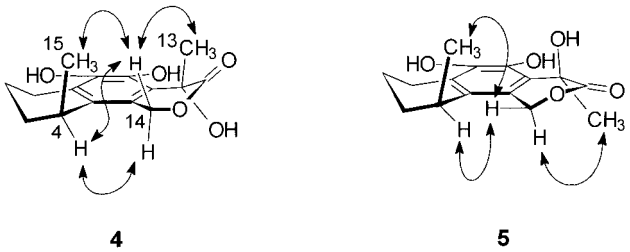
Table 1. Antimicrobial Evaluation and Toxicity toward *Artemia salina* of Extracts of *P. radulifolium* and Isolated Compounds

	MIC (mg/mL)					LD_{50} (ppm)
compound	*S. a.*[a]	*E. c.*[a]	*P. a.*[a]	*P. m.*[a]	*C. a.*[a]	*A. s.*[a]
hexanic extract	0.062	>1.0	>1.0	>1.0	0.062	39.1
methanolic extract	1.0	>1.0	>1.0	>1.0	1.0	34.2
1	0.012	0.025	>0.4	0.012	0.012	12.0
2/3[b]	0.25	>1	>1	>1	>1	269.0
4/5[c]	0.20	>0.40	>0.40	nt[d]	>0.40	230.0
6	0.10	>0.40	>0.40	nt[d]	>0.40	52.5
7	nt	nt	nt	nt	nt	nt
O-methyl-1,2-dehydrocacalol	0.40	>0.40	>0.40	>0.40	>0.40	>1000
adenostin A	0.05	>0.40	>0.40	nt[d]	>0.40	230.0
decompostin	>0.40	>0.40	>0.40	>0.40	>0.40	>1000
neoadenostylone	>0.40	>0.40	>0.40	nt[d]	>0.40	21.7

[a] Microoganisms tested: *S. a.*: *Spahylococcus aureus*; *E. c.*: *Escherichia coli*; *P. a.*: *Pseudomonas aeruginosa*; *P. m.*: *Proteus micranthus*; *C.a.*: *Candida albicans*; *A.s.*: *Artemia salina*. [b] 1:1 mixture of natural epimers. [c] 1.2:1 mixture of natural epimers. [d] Not tested.

and gave the molecular formula $C_{15}H_{18}O_5$. The IR spectrum revealed the presence of a hydroxyl group (3377 cm^{-1}), carbonyl (1736 cm^{-1}), and multiple carbon−carbon bonds (1624, 1595, 1470, 1444 cm^{-1}). ^1H and ^{13}C NMR spectra taken at different temperatures (from −5 to 70 °C) showed constant duplicate resonances for protons and carbons, establishing that this sample was a nearly 1.2:1 mixture of closely related compounds. ^{13}C NMR (see Experimental Section) and DEPT experiments exhibited four methyl carbons (two corresponding to methyl carbinols, according to HMBC experiments), eight methylenes (two corresponding to oxymethylenes), two methines (bonded to methyls, in agreement with HMBC experiments), two tertiary carbons bonded to oxygen (which belonged to the above-mentioned methyl carbinols), two lactonic carbonyls, and twelve benzenoid carbons, a pattern that suggests a mixture of cacalol analogues due to the similarities of the chemical shifts of the carbons for the A- and B-rings. The additional carbonyl groups, the extra oxymethylenes, and the methyl carbinol fragments were indicative for the presence of an α-hydroxy-α-methyl-δ-lactone closed at C-14, in agreement with biogenetic considerations and the molecular formula. Therefore, the mixture consists of modified eremophilanolides epimeric at C-11 (**4** and **5**). The observed HMBC correlations between H-13 (δ 1.66; 1.69) and C-12 (δ 175.2; 175.3) and between H-14 (δ 1.07; 1.21) and C-12 confirmed the proposed structures. Resonances for most hydrogens, in particular, H-14 and H-13, for both isomers had small, but clear differences ($\Delta\delta$ = 0.01−0.03). The major difference was observed for H-15 (δ 1.04 for the major isomer (**4**) and δ 1.20 for the minor (**5**) ($\Delta\delta$ = 0.16) and can be explained by the shielding effect of the carbonyl group to the methyl at C-4 in the 11*S*-isomer (**4**, radulifolin A), considering that the hydroxyl is α-oriented and *anti* with the carbonyl (C-12 pseudo-*endo*, see Figure 1), due to the dipole−dipole repulsion that exists between the carbonyl and the C−OH σ-bond in nonpolar solvents. The tertiary alcohol and the carbonyl maintained their *anti*-relationship in the 11*R*-isomer (**5**, *epi*-radulifolin A), due to the conformational change of the δ-lactone, and therefore, the orientation of the C-12 carbonyl is pseudo-*exo* and no longer shields the methyl at C-4 (see Figure 1). In agreement with these assignments, the chemical shifts of H-4 (δ 2.94) and H-14α (δ 5.47) in the 11*S*-isomer (**4**) are shifted downfield with respect to same hydrogens in the 11*R*-isomer (**5**, δ 2.79, 5.20, respectively), due to the deshielding effect of the α-hydroxyl group. The assigned configurations for **4** (11*S*) and **5** (11*R*) were confirmed by NOESY experiments, and relevant correlations are shown in Figure 1.



4 **5**

Figure 1. Selected NOESY interactions for **4** and **5**.

Radulifolin B (**6**) represented a new minor constituent from this species. The IR spectrum of **6** revealed the presence of a hydroxyl (3424 cm^{-1}), β,γ-unsaturated-γ-lactone (1808 cm^{-1}), and carbon−carbon unsaturations (1619, 1466, 1376 cm^{-1}), and the molecular formula $C_{16}H_{20}O_4$ was determined by HRMS. The structure of **6** was determined through the close similarities of the ^{13}C and ^1H NMR data with those reported recently for *epi*-hydroxycacalolide, isolated as an antihyperglycemic constituent from *P. decompositum*,[16] and with those of cacalolide[23] and was determined as *O*-methyl-*epi*-hydroxycacalolide (**6**). The stereochemistry at C-11 was determined by analysis of its CD curve, which showed a positive Cotton effect at 291 nm, which may be attributed to the combination of the n→p* transition of the carbonyl group and the B-band of the aromatic ring. Application of the octant rule[24] indicated a β-orientation of the hydroxyl group. In addition, the dextrorotatory characteristic of **6** was in agreement with the reported optical rotation of its demethyl derivative, whose stereochemistry was determined by ROESY experiments.[16]

HREIMS of radulifolin C (**7**) established the molecular formula $C_{15}H_{14}O_3$, and the degree of unsaturation (nine) could be accounted by an aromatic tricyclic sesquiterpene of a modified furanoeremophilane skeleton according to its UV spectrum, which showed a conjugated ketone and aromatic bands at λ_{max} 204, 250, 285, and 329 nm. Three methyls, two *ortho*-benzenoid proton signals, and the characteristic furane proton were observed in its ^1H NMR spectrum, and the assignments were confirmed by HMQC experiments. The HMBC spectrum showed correlations between H-1 (δ 8.18), H-14 (δ 1.43), and H-15 (δ 2.40) with C-5 (δ 147.14), and the hydrogen of the aliphatic methine (H-6, δ 4.25) showed correlations with C-7 (δ 141.49), which, in turn, also correlated with H-13 (δ 1.43), establishing the carbonyl at C-9 and the phenol at C-3 for the structure **7**. The methyl at C-6 is pseudo-axial, to avoid steric interactions with the methyls C-13 and C-15, and therefore, the linear tricyclic system is twisted in the direction opposite the methyl at C-6, as a consequence of the molecular strain. The B-ring is a relatively rigid pseudo-boat, and the CD curve of **7** showed a strong positive ellipticity in the region of 240−300 nm.[25] The application of the octant rule showed that the furanic oxygen is located in a negative octant, that the hydroxyl at C-3 is almost coplanar with the horizontal plane, and that C-1, C-2, and C-13 contribute significantly to the positive octants for the 6*S*-stereoisomer, establishing this configuration for the natural product, in agreement with biogenetic considerations.

The extracts and most of the isolated compounds were tested for their antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Candida albicans* (Table 1). The results showed antimicrobial activity in the *n*-hexane

extract and a broad activity for **1**, as previously noted.[15] The mixture of cacalone (**2**) and *epi*-cacalone (**3**), the mixture of radulifolin A (**4**) and *epi*-radulifolin A (**5**), radulifolin B (**6**), *O*-methyl-1,2-dehydrocacalol, and adenostin A displayed selective but moderate activities against *S. aureus*. In addition, the toxicities toward brine shrimp were tested, **1** being the most active substance. These results are in agreement with the general uses in traditional medicine of some preparations of the title plant as an antiseptic agent.

Experimental Section

General Experimental Procedures. Melting points were obtained in a Fisher-Johns apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus-500 instrument, and the chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. Infrared spectra were recorded with Nicolet Magna IR TM 750 and Perkin-Elmer 283B instruments. MS data were recorded with a JEOL JMS-AX 505 HA mass spectrometer. EIMS were obtained at 70 eV ionization energy. Vacuum chromatography was performed on Merck Kieselgel 60 (0.040−0.863 mm). The eluent is specified in each experiment. TLC analyses were performed on ALUGRAM SIL G/UV$_{254}$ silica gel plates. TLC visualization was accomplished with either a UV lamp or a charring solution (12 g of ceric ammonium sulfate dihydrate, 22.2 mL of concentrated H_2SO_4, and 350 g of ice).

Plant Material. The roots of *Psacalium radulifolium* (HBK.) H. Rob. & Brettell were obtained in San Luis Potosí, and the corroborative specimen was collected in Sierra Madre Oriental of San Luis Potosí in July 1995 (voucher specimen Bye & Linares 20028 and corroborative specimen Bye & Linares 20149 are deposited in the Ethnobotanical Collection and National Herbarium, respectively, of the Instituto de Biología de la Universidad Nacional Autónoma de México).

Extraction and Isolation. Air-dried roots of *P. radulifolium* (974 g) were powdered and extracted consecutively three times (each for 48 h) with *n*-hexane, CH_2Cl_2−EtOH (3:2), and MeOH at room temperature. The solvents were removed under reduced pressure to give the corresponding residues (50.4, 59.8, and 36.0 g, respectively). The *n*-hexane extract, which displayed the major antimicrobial activity, was subjected to column chromatography under reduced pressure with a gradient of *n*-hexanes−EtOAc to afford seven main fractions (A−G). Fraction A (2.5 g) was rechromatographed using *n*-hexane as constant eluent to obtain *O*-methyl-1,2-dehydrocacalol (50 mg). Mp: 79−82 °C [lit. 79.5−81 °C].[18] From the rechromatography of fractions B (34 g) and C (11 g), eluted with *n*-hexanes−EtOAc gradient, and subsequent rechromatographies of the residues, were isolated cacalol (**1**, 4.4 g), mp 90−92 °C [lit. 92−94 °C],[18] neoadenostylone (15 mg), mp 98−100 °C,[21] decompostin (1.9 g), mp 184−185 °C [lit. 183−184 °C],[20] and adenostin A (60 mg), mp 185−188 °C [lit. 186−188 °C].[19] Column rechromatography of fraction D (2 g) using *n*-hexanes−EtOAc as gradient elution system yielded radulifolin B (**6**, 8 mg) and the 1.2:1 mixture of radulifolin A (**4**) and *epi*-radulifolin A (**5**) (45 mg). Fractions E (1.5 g), F (1.4 g), and G (0.7 g) were shown to contain one major spot and were pooled. The residue was then separated by column chromatography over silica gel using *n*-hexanes−EtOAc gradient, and the residue obtained from some fractions was subjected to preparative TLC with *n*-hexanes−EtOAc (65:35) as developing solvent (two developments), affording 168 mg of the 1:1 mixture of **2** and **3**, mp 115−118 [lit. 120−121 °C].[5] From the polar fractions of the column rechromatography of the E−G subfractions, a residue was obtained (157 mg), which was purified by TLC (*n*-hexanes−EtOAc, 88:12) to obtain radulifolin C (**7**, 8.4 mg).

Radulifolin A and *epi*-radulifolin A (4 and 5): white amorphous powder, mp 117−119 °C; R_f 0.440 (hex−AcOEt, 75:25); $[\alpha]_D$ +18.45 (*c* 0.206, MeOH); UV λ_{max} (log ϵ) 208 (4.48), 287 (3.53) nm; IR ($CHCl_3$) 3337, 3026, 2933, 2878, 1736, 1663, 1624, 1595, 1470, 1444, 1406 cm^{-1}; ^{1}H NMR for **4** (Cl_2-$CDCDCl_2$, 500 MHz) δ 5.47 (1H, d, J = 14.5, H-14α), 5.19 (1H, d, J = 14.5, H-14β), 2.94 (1H, ddq, J = 7,4,3, H-4), 2.84 (1H, m, H-1a), 2.53 (1H, m, H-1b), 1.74 (2H, m, H-2a,b), 1.71 (2H, m, H-3a,b), 1.67 (3H, s, H-13), 1.04 (3H, d, J = 7, H-15); ^{1}H NMR for **5** ($Cl_2CDCDCl_2$, 500 MHz) δ 5.50 (1H, d, J = 14.5, H-14β), 5.20 (1H, d, J = 14.5, H-14α), 2.79 (1H, ddq, J = 7,4,3, H-4), 2.87 (1H, m, H-1a), 2.49 (1H, m, H-1b), 1.77 (2H, m, H-2a,b), 1.69 (2H, m, H-3a,b), 1.64 (3H, s, H-13), 1.20 (3H, d, J = 7, H-15); ^{13}C NMR for **4** ($CDCl_3$, 125 MHz) δ 175.33 (s, C-12), 143.15 (s, C-9), 138.70 (C-8), 130.37 (s, C-5), 123.81 (s, C-10), 117.93 (s, C-7), 116.77 (s, C-6), 72.46 (s, C-11), 67.18 (t, C-14), 30.02 (t, C-3), 28.48 (d, C-4), 24.92 (q, C-13), 23.31 (t, C-1), 22.24 (q, C-15), 16.55 (t, C-2); ^{13}C NMR for **5** ($CDCl_3$, 125 MHz) δ 175.24 (s, C-12), 143.07 (s, C-9), 138.71 (C-8), 129.86 (s, C-5), 123.47 (s, C-10), 118.09 (s, C-7), 117.37 (s, C-6), 72.22 (s, C-11), 67.73 (t, C-14), 28.97 (t, C-3), 27.81 (d, C-4), 24.94 (q, C-13), 22.23 (t, C-1), 21.60 (q, C-15), 16.05 (t, C-2); EIMS *m*/*z* 278 [M$^+$] 835), 260 (100), 245 (80), 227 (11), 217 (11), 187 (15), 176 (20), 161 (5), 128 (6), 115 (8), 91 (7), 77 (5), 55 (6), 43 (22), 41 (6); HREIMS *m*/*z* [M$^+$] 278.1163 (calcd for $C_{15}H_{18}O_5$ 278.1154).

Radulifolin B (6): yellow oil, R_f 0.714 (hex−AcOEt, 75:25); $[\alpha]_D$ 10.6 (*c* 0.235, MeOH); UV λ_{max} (log ϵ) 206 (3.86), 286 (3.05) nm; CD (*c* 0.003525, MeOH) $[\theta]_{214}$ 5708, $[\theta]_{235}$ 0, $[\theta]_{245}$ −1777, $[\theta]_{280}$ 0, $[\theta]_{291}$ +227; IR ($CHCl_3$) 3424, 2970, 2870, 1808, 1619, 1466, 1376, 1325 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.94 (3H, s, OCH_3), 3.06 (1H, ddq, J = 7,5,4, H-4), 2.89 (1H, ddd, J = 15, 4, 4, H-1b), 2.48 (1H, ddd, J = 15, 10, 4, H-1a), 2.38 (3H, s, H-14), 1.79 (2H, m, H-2a,b), 1.78 (3H, s, H-13), 1.74 (1H, dddd, J = 14, 5, 5, 4, H-3a), 1.69 (1H, dddd, J = 14, 10, 5, 4, H-3b), 1.15 (3H, d, J = 7, H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.74 (s, C-12), 140.54 (s, C-8), 139.47 (C-9), 138.66 (s, C-5), 131.39 (s, C-10), 127.94 (s, C-6), 125.62 (s, C-7), 73.42 (s, C-11), 60.17 (q, OCH_3), 29.62 (t, C-3), 28.76 (d, C-4), 24.62 (q, C-13), 23.79 (t, C-1), 20.80 (q, C-15), 16.49 (t, C-2), 12.86 (q, C-14); EIMS *m*/*z* 276 [M$^+$] (35), 272 (12), 248 (66), 233 (100), 229 (21) 203 (7), 189 (6), 176 (6), 159 (8), 157 (7), 129 (8), 115 (9), 91 (8), 84 (14), 43 (35); HREIMS *m*/*z* [M$^+$] 276.1369 (calcd for $C_{16}H_{20}O_4$ 276.1362).

Radulifolin C (7): yellow amorphous powder, mp 163−165 °C; R_f 0.32 (hex−AcOEt, 7:3); $[\alpha]_D$ −24.21 (*c* 0.190, MeOH); UV λ_{max} (log ϵ) 204 (3.75), 250 (3.63), 285 (3.42), 329 (3.75) nm; CD (*c* 0.0019, MeOH) $[\theta]_{200}$ −2037, $[\theta]_{217}$ 0, $[\theta]_{222}$ +223, $[\theta]_{228}$ 0, $[\theta]_{241}$ − 569, $[\theta]_{263}$ 0, $[\theta]_{302}$ + 724, $[\theta]_{319}$ 0, $[\theta]_{333}$ −164; IR ($CHCl_3$) 3586, 3270, 1655, 1578, 1541, 1466, 1427, 1363, 1275, 1169, 1125, 1005, 992, 932, 900, 863, 836 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.18 (1H, d, J = 8.5, H-1), 7.50 (1H, q, J = 1, H-12), 6.92 (1H, d, J = 8.5, H-2), 4.25 (1H, q, J = 7, H-6), 2.40 (3H, s, H-15), 2.19 (3H, d, J = 1, H-13), 1.43 (3H, d, J = 7, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.10 (d, C-9), 157.75 (s, C-3), 147.14 (s, C-5), 146.00 (s, C-8), 144.98 (d, C-12), 141.49 (s, C-7), 127.25 (d, C-1), 126.39 (s, C-10), 121.57 (s, C-4), 120.13 (s, C-11), 114.14 (d, C-2), 31.35 (d, C-6), 22.04 (q, C-14), 11.71 (q, C-15), 8.05 (q, C-13); EIMS *m*/*z* 242 [M$^+$] 242 (48), 227 (100), 214 (4), 199 (6), 171 (5), 141 (5), 128 (5), 115 (4), 107 (3), 57 (4); HREIMS *m*/*z* [M$^+$] 242.0937 (calcd for $C_{15}H_{14}O_3$ 242.0943).

Biological Activities. Antimicrobial Activity. The bacteria *S. aureus* (ATCC6358), *E. coli* (ATCC8937), *P. aeruginosa* (ATCC27853), and *P. mirabilis* (ATCC12453) were maintained in Trypticase soya agar, and *C. albicans* (ATCC10231) was maintained on Sabourand's dextrose agar. The method used was based on the standard described procedures for the study of natural products. Each natural product was suspended in 10% DMSO and added at various concentrations to the melted agar in Petri dishes. The plates were incubated for 24 h at 37 °C. Gentamicin and nystatin were used as reference standards, and the experiments were performed in duplicate.[26] The bioassays of toxicities toward brine shrimp were performed as described in the literature.[27]

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References and Notes

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