Semisynthesis and Biological Evaluation of Abietane-Type Diterpenes. Revision of the Structure of Rosmaquinone

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Received January 30, 2009

The new aromatic diterpenes 7*β*-*O*-benzylrosmanol (**3**), 7*β*-*O*-benzyl-11,12-di-*O*-methylrosmanol (**4**), and 7α-thiophenylcarnosic acid (**5**) have been obtained by partial synthesis from carnosol (**1**), an abundant natural diterpene present in *Salvia* species. The structures of these compounds were established from their physical and spectroscopic data. The known diterpenes sagequinone methide A (**6**), 7*β*-*O*-methylrosmanol (**7**), 7-*O*-methylrosmanol (**8**), and rosmaquinone B (**9**) were obtained from rosmanol (**2**). The spectroscopic data of these semisynthetic diterpenes were identical to data reported in the literature. In addition, the new semisynthetic isorosmaquinone (**10**) was obtained from isorosmanol (**12**). The proton resonances of rosmaquinone (**11**) are reassigned based on 2D NMR spectroscopy. These compounds, as well as eight known analogues, were evaluated for cytotoxic and antimicrobial activities.

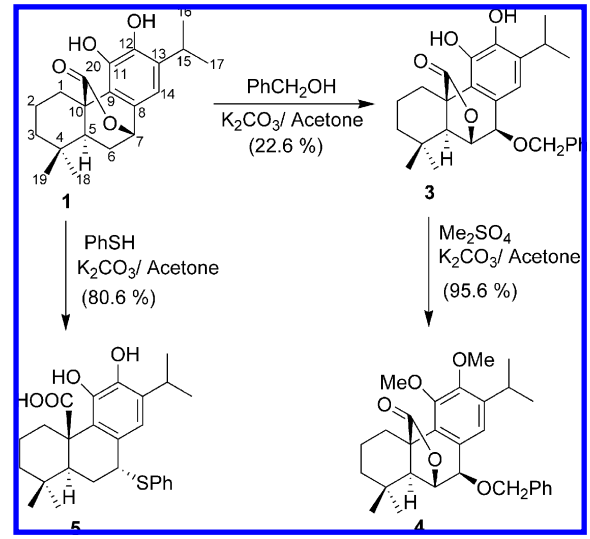
The genus *Salvia* (Lamiaceae) consists of approximately 500 species found worldwide. *Salvia* species are used as traditional medicines for the treatment of a variety of diseases. Studies of their chemical constituents have revealed the presence of a large variety of diterpenoids with antibacterial,[1−4] antioxidant,[5] antidiabetic,[6,7] antitumor,[8,9] leishmanicidal,[10] and antiplasmodial[10] properties. Most of these diterpenes are often present in very low quantities. We have reported an efficient partial synthesis of some of these compounds from the abundant diterpene carnosol (**1**).[11] Moreover, we have obtained new abietane diterpene derivatives with a seco C-ring[12] and new quinone derivatives.[13]

In the course of our work on the partial synthesis of biologically interesting diterpenes, we have prepared four new derivatives of rosmanol (**2**) and now describe a semisynthetic approach to the known diterpene sagequinone methide A (**6**). This is the first semisynthesis of this natural product. Furthermore, these compounds and eight known analogues[12,13] were assayed for their cytotoxic and antimicrobial activities.

Results and Discussion

Following the procedure previously reported,[11] a solution of carnosol (**1**) in acetone was treated with benzyl alcohol and potassium carbonate to give, after purification, compound **3** (Scheme 1). The low-resolution mass spectrum of **3** showed a molecular ion $[M]^+$ at *m/z* 436 ($C_{27}H_{32}O_5$ by HREIMS). The IR spectrum exhibited characteristic absorption bands for phenol (3500 cm^{-1}) and lactone (1770 cm^{-1}) groups. In the ^1H NMR spectrum, signals for a proton heptuplet (*δ* 3.05) together with two methyl groups as doublets at *δ* 1.12 and 1.16 indicated the presence of an aromatic isopropyl group. Two methyl groups at *δ* 0.91 and 1.00 were also observed. The H-5 proton appeared as a singlet at *δ* 2.33, and H-6 as a doublet at 4.66 (J = 3.0 Hz) coupled with another proton doublet at *δ* 4.47 (J = 3.0 Hz) assigned to H-7. This indicated that the lactone closure was at C-6, and the bridgehead proton and H-6 form a 90° angle. In the low-field region of the spectrum, two broad singlets at *δ* 5.83 and 6.30 (interchangeable with D_2O) were assigned to hydroxy groups, and one aromatic proton singlet at *δ* 6.63 was assigned to H-14. An AB system as two one-proton doublets (*δ* 4.77 and 4.89; J = 11.8 Hz) together with five aromatic protons as a multiplet (*δ* 7.26−7.43) were assigned to the benzyl group. Correlation between H-7 and H-5α in the ROESY experiment confirmed that the substituent at C-7 is *β*-oriented. The above data were in accordance with the structure of 7*β*-*O*-benzylrosmanol for **3**.

Scheme 1



Methylation of **3** with dimethyl sulfate in acetone gave the dimethyl ether **4** (Scheme 1), which showed a molecular ion $[M]^+$ at *m/z* 464 ($C_{29}H_{36}O_5$ by HREIMS), while the ^1H NMR spectrum displayed two additional methoxy groups at *δ* 3.75 and 3.77. Treatment of carnosol (**1**) with thiophenol and potassium carbonate in acetone (Scheme 1) yielded **5**. The low-resolution mass spectrum of **5** showed a molecular ion $[M - 1]^+$ at *m/z* 439 corresponding to the molecular formula $C_{26}H_{31}O_4S$ by HREIMS. The IR spectrum showed absorption bands for phenol (3400 cm^{-1}) and carboxylic acid (1682 cm^{-1}) groups, while the lactone group absorption was absent. In the ^1H NMR spectrum, a six-proton doublet at *δ* 1.21 (J = 7.0 Hz) was assigned to the C-16 and C-17 methyls, and two singlets at *δ* 0.88 and 0.83 were assigned to the C-18 and C-19 methyl groups, respectively. In the low-field region, a one-proton singlet at *δ* 6.91 was assigned to the aromatic H-14 and a doublet at *δ* 4.71 (J = 3.1 Hz) to H-7; signals at *δ* 7.28 (3H, m) and 7.48 (2H, d, J = 7.5 Hz) were assigned to the aromatics protons of the thiophenyl group. The ^{13}C NMR spectrum showed signals for 26

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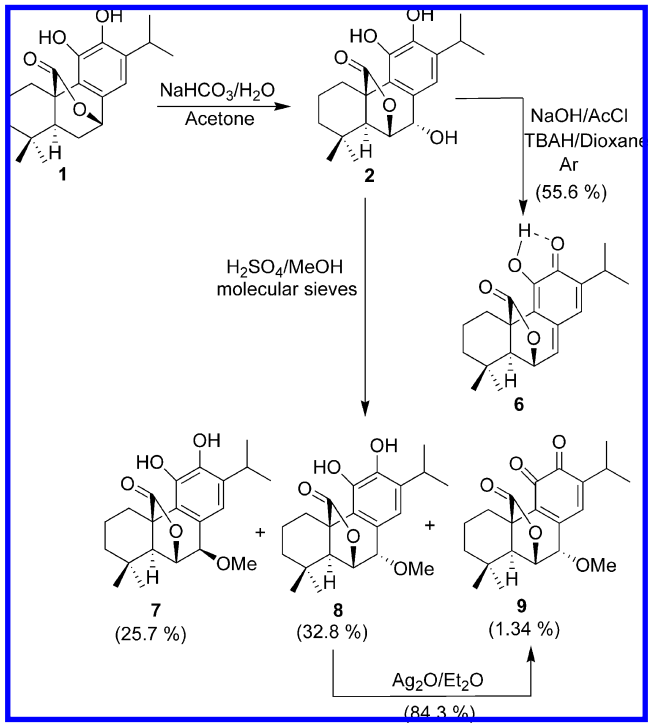
10.1021/np900047p CCC: $40.75
Published on Web 07/15/2009

Scheme 2



carbon atoms, including a signal at δ 181.9 (s) assigned to the carboxylic acid group (C-20) and signals at δ 141.8 (s, C-12) and 142.7 (s, C-11) assignable to two oxygen-bearing aromatic carbons. The above data are in accordance with the structure of 7α-thiophenylcarnosic acid for **5**.

We were also interested in obtaining 7β-*O*-methylrosmanol (**7**) and isorosmaquinone (**10**) from **2** and **12**, respectively, in order to test their biological activity. Rosmanol (**2**) was obtained from carnosol (**1**) as previously reported.[11] In our initial attempts, we carried out the protection of phenolic groups, followed by transformation of the C-7 hydroxy group into a good leaving group, followed by substitution with a methoxy group by an S_N2 reaction. Treatment of rosmanol (**2**) with acetyl chloride, solid sodium hydroxide, and tetrabutylammonium hydrogen sulfate in dioxane (Scheme 2)[14] yielded **6**, which showed physical data identical to natural sagequinone methide A.[15]

Treatment of rosmanol (**2**) with sulfuric acid in methanol at room temperature yielded a mixture of three products, which were separated by preparative TLC and identified as 7β-*O*-methylrosmanol (**7**), 7-*O*-methylrosmanol (**8**), and quinone (**9**) (Scheme 2).

Compound **9** showed a molecular ion $[M]^+$ at *m*/*z* 358 ($C_{21}H_{26}O_5$ by HREIMS). The IR spectrum displayed characteristic bands for lactone (1786 cm^{-1}) and *o*-quinone (1667 cm^{-1}) groups. The presence of the *o*-quinone group was confirmed by UV absorption (340 and 270 nm).[16] In the ^1H NMR spectrum, signals for a six-proton doublet for two methyls (δ 1.12, $J = 7.0$ Hz) and two three-proton singlets at δ 0.89 and 1.01 for two geminal methyl groups were observed. An AB spin system of two one-proton doublets at δ 3.88 and 4.64 was assigned to H-7 and H-6, respectively, and a one-proton singlet at δ 6.62 to the vinylic proton H-14. A three-proton singlet assigned to a methoxy group appears at δ 3.68. The ^{13}C NMR spectrum showed signals for 20 carbon atoms, including a signal at δ 175.6 (s) assigned to the lactone carbonyl and signals at δ 59.6 (q), 72.5 (d), 76.9 (d), 179.6 (s), and 180.0 (s) assignable to five oxygen-bearing carbon atoms.

The structure of **9** was confirmed by Ag_2O oxidation of 7-*O*-methylrosmanol (**8**) (Scheme 2), which yielded a product identical to **9**, therefore establishing **9** as rosmaquinone B.[17]

In previous work[18] the assignment of protons H-6 and H-7 in rosmaquinone (**11**) was made on the basis of $^1H-^1H$ COSY, HMBC, and HMQC experiments of rosmanol (**2**), which confirmed that H-7 always appeared downfield from H-6. However, in the case of rosmaquinone B (**9**) this is reversed, prompting us to revise the proton assignment of rosmaquinone (**11**), which was prepared by oxidation of rosmanol (**2**) with silver oxide (Scheme 3).[18] HMBC correlations (H-6/C-20, C-8) established the lactone ring closure at C-6 for **11**. A $^1H-^1H$ COSY correlation between H-6 [δ 4.52 (d, $J = 3.3$ Hz)] and H-7 [δ 4.40 (d, $J = 3.3$ Hz)] together with the ^{13}C NMR, HSQC, and HMBC (Table 1) experiments allowed the full NMR assignment of rosmaquinone (**11**), which unambiguously established H-6 as being downfield compared to H-7.

Scheme 3

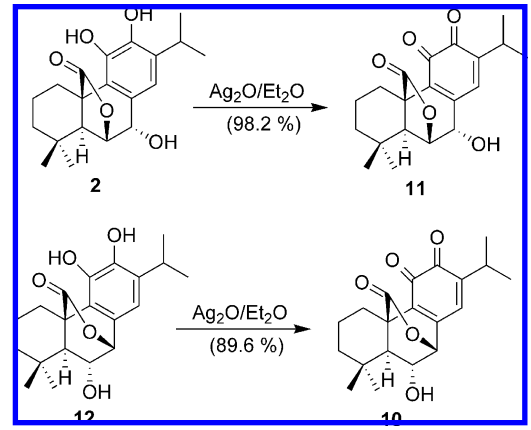


Table 1. HMBC Data of Compound **11** in $CDCl_3$

proton	three-bond correlation	two-bond correlation
H-1α	C-9, C-20	C-2, C-10
H-1β	C-3	C-10
H-2	C-4, C-10	C-1
H-5	C-4, C-9, C-18, C-20	C-10
H-6	C-4, C-8, C-10, C-20	
H-14	C-12, C-15	
H-15	C-12, C-16, C-17	C-13
H-16 and -17	C-13, C-15	
H-18 and -19	C-3, C-4	

Oxidation of isorosmanol (**12**) with freshly prepared Ag_2O in diethyl ether yielded **10** (Scheme 3). The mass spectrum of **10** showed a molecular ion $[M]^+$ at *m*/*z* 344 ($C_{20}H_{24}O_5$ by HREIMS). The IR spectrum displayed characteristic absorption bands for lactone (1760 cm^{-1}) and *o*-quinone (1668 cm^{-1}) groups. In the ^1H NMR spectrum, signals for the six-proton methyl doublet (6H, δ 1.11, $J = 7.0$ Hz), two geminal methyls singlets (δ 0.86 and 1.05), and an aromatic proton at δ 6.73 (H-14) were observed. The δ-lactone required closure at C-7, which is in accordance with the lower chemical shift of H-7 (δ 5.12, $J = 4.5$ Hz) compared to H-6 (δ 4.48, $J = 4.5$ Hz). The ^{13}C NMR spectrum showed signals for 20 carbons, including a signal at δ 172.4 (s) assigned to the lactone carbonyl and signals at δ 69.2 (d), 77.9 (d), 176.3 (s), and 179.3 (s) corresponding to four oxygen-bearing carbons, confirming the structure of isorosmaquinone as **10**.

The antimicrobial activity of compounds **1**−**4**, **6**−**9**, and **11**−**20** (Figure 1) was weak (Table 2), showing no activity against Gram-negative bacteria and *Candida albicans* (MICs > 40 μg/mL), except for **1** (MIC 20 μg/mL).

Compounds **1**−**20** can be categorized into three groups based on their chemical features. Compounds **1**, **10**, and **12**−**15** are C-7 γ-lactones, among which isorosmanol (**12**) and carnosol (**1**) showed marginal activity.[3] Accordingly, we speculate that two free hydroxy groups in the C-ring (**1** versus **13**) increase the antibiotic activity, but an additional hydroxy group at C-6 (**1** versus **12**) decreases it. Compounds **2**−**4**, **6**−**9**, **11**, and **16** are C-6 γ-lactones. In this case, the activity on *Staphylococcus aureus* depends on the presence of

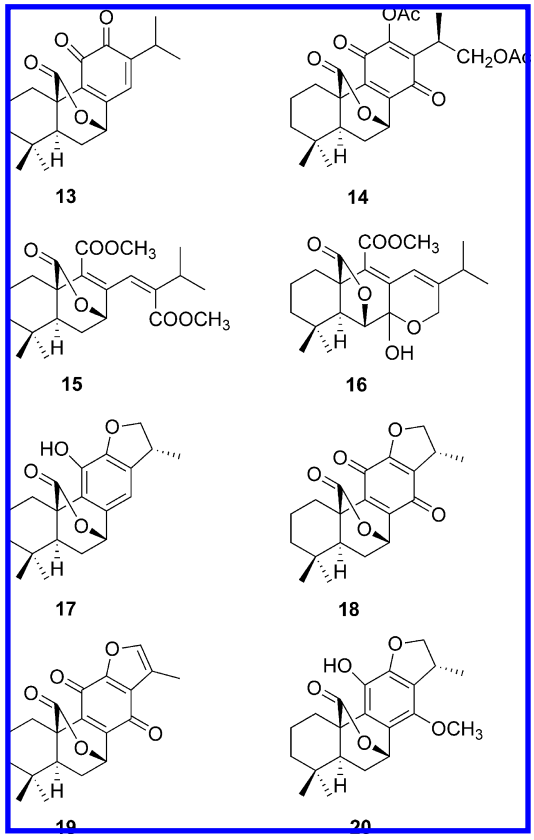


Figure 1

Table 2. Antimicrobial Activity of **1**−**4**, **6**−**9**, and **11**−**20** (MIC in μg/mL)

compound[a,b]	*S. aureus*	*S. epidermidis*	*B. subtilis*	*B. cereus*	*C. albicans*
1	30−25	10−5	6−4	10−5	20
2	25−20	20−10	20−10	40−20	>40
3	>40	>40	40−20	20−10	>40
6	40−20	40	20−10	20−10	>40
7	>40	40−20	40−20	40−20	>40
8	>40	40−20	>40	40−20	>40
9	40	>40	40−20	40−20	>40
11	20	40	8	20−10	>40
12	40	40−20	35−30	40−20	>40
cefotaxime[c]	2.5−1.25	2.5	8	10	nt[d]

[a] Diterpenes **4** and **13**−**20** were inactive against all microorganisms (MIC > 40 μg/mL). [b] All compounds were inactive against *M. smegmatis*, *E. faecalis*, and Gram-negative bacteria (MIC > 40 μg/mL). [c] Cefotaxime was used as positive control. [d] nt: not tested.

a hydroxy group at C-7 (**2** and **11**), since a 7β-benzyl or a methoxy group in this position leads to a loss of the activity (**3** and **8**). Moreover, the MIC values of **2** and **11** against *Bacilus subtilis* indicate that a carbonyl group in the C-ring increases the antibiotic activity. These results reinforce the structure−activity relationships as previously reported.[3] Within the third type, **17**−**20** are characterized by a C-7 γ-lactone group and a 12,16-epoxy group with no activity against all microorganisms assayed, indicating that the epoxy group leads to a loss of activity.

The cytotoxicity of the diterpenes was studied *in vitro* against A-549, HeLa, Hep-2, and MCF-7 human tumor and Vero cells lines, using 6-mercaptopurine as a positive control (Table 3). Diterpenes **5** and **6** were the most active against HeLa and Hep-2 cell lines after 48 h of continuous exposure; however, the cytotoxicity of **5** decreased and that of **6** increased when the compounds were incorporated in the log or exponential growth phase. Similarly, the activity increased considerably in the case of **6**, **9**, **11**, and **18** against different cancer cells, which indicates that the mechanism of these compounds could be related to a biosynthetic process as for 6-mercaptopurine.[19] It is important to emphasize that **6**, **9**−**11**, and **18** show selective cytotoxicity, as demonstrated by their IC_{50} values against the nontumoral mammalian Vero cells (IC_{50} > 60 μM).

The structure−activity relationship also revealed that the closing position of the lactone ring does not affect cytotoxic activity, but the *o*-quinone group (**10** versus **12**) and the C-6 hydroxy group (**10** versus **13**) increase the activity. On the contrary, the benzyl group leads to a loss of activity (**3** versus **6**), and the hydroxy group is preferable to an *o*-quinone in the C-ring (**2** versus **11**). With respect to diterpenes with an epoxy group, they show only moderate activity when possessing a *p*-quinone C-ring and with no double bond at C-15 and C-16 (**18** versus **19**).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter in $CHCl_3$ at 20 °C, and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. UV spectra were obtained in absolute EtOH on a JASCO V-560 instrument. IR spectra were obtained on a Bruker IFS 28/55 (FTIR) spectrometer. The NMR spectra were recorded on Bruker Avance 300 MHz and Bruker Avance 400 MHz spectrometers in $CDCl_3$, unless otherwise noted. Chemical shifts are given in ppm with TMS as the internal standard. Low-resolution mass spectra were run on a VG Micromass ZAB-2F and high-resolution mass spectra on a VG Micromass ZAB-2F at 70 eV. Merck silica gel (0.063−0.2) was used for column chromatography. Analytical thin-layer chromatography (TLC) and preparative TLC were carried out on precoated Schleicher and Schüll plates.

Plant Material. A voucher specimen of *Salvia canariensis* L. (No. 25252) was deposited in the herbarium of the Department of Botany, Faculty of Biology, Universidad de La Laguna. Carnosol (**1**) and isorosmanol (**12**) were isolated from a dried extract of stems and leaves of *S. canariensis* L.[11]

7β-*O*-Benzylrosmanol (3). Carnosol (**1**) (109.8 mg, 0.32 mmol), benzyl alcohol (0.2 mL, 1.93 mmol, 6.1 equiv), and solid K_2CO_3 (267.0 mg, 1.93 mmol, 6.09 equiv) in acetone (10 mL) were stirred at room temperature under Ar for 24 h, followed by evaporation of the solvent under reduced pressure. The reaction mixture was acidified with 5% HCl, extracted with EtOAc (3 × 20 mL), washed with brine, and dried over anhydrous Na_2SO_4. The residue was purified by silica gel column chromatography eluting with *n*-hexane/acetone (9:1), yielding **3** (32.8 mg, 22.6%): $[\alpha]^{20}_D$ +7.4 (*c* 0.09, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 289 (3.68) nm; IR (film) ν_{max} 3500, 2958, 2927, 2870, 1770, 1455, 1395, 1372, 1355, 1327, 1303, 1269, 1217, 1175, 1070, 1016, 964, 909, 757 cm^{-1}; ^{1}H NMR (300 MHz) δ 0.91 (3H, s, Me-19), 1.00 (3H, s, Me-18), 1.12 (3H, d, J = 7.0 Hz, Me-16), 1.16 (3H, d, J = 7.0 Hz, Me-17), 1.20 (1H, overlap, H-3α), 1.44 (1H, bd, J = 13.3 Hz, H-3β), 1.62 (2H, m, H-2), 1.96 (1H, td, J_1 = 5.4 Hz, J_2 = 13.5 Hz, H-1α), 2.33 (1H, s, H-5), 3.05 (1H, hept, J = 7.0 Hz, H-15), 3.17 (1H, bd, J = 13.7 Hz, H-1β), 4.47 (1H, d, J = 3.0 Hz, H-7), 4.66 (1H, d, J = 3.0 Hz, H-6), 4.77 (1H, d, J = 11.8 Hz, −C$\underline{H}_2$-Ph), 4.89 (1H, d, J = 11.8 Hz, −C$\underline{H}_2$-Ph), 5.83 (1H, bs, O$\underline{H}$-11), 6.30 (1H, bs, O$\underline{H}$-12), 6.63 (1H, s, H-14), 7.26−7.43 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′); ^{13}C NMR (75 MHz) δ 19.0 (t, C-2), 21.9 (q, C-19), 22.0 (q, C-16), 22.1 (q, C-17), 27.1 (d, C-15), 27.1 (t, C-1), 31.4 (q, C-18), 31.5 (s, C-4), 38.0 (t, C-3), 47.1 (s, C-10), 51.0 (d, C-5), 72.8 (t, −C$\underline{H}_2$-Ph), 75.0 (d, C-7), 75.4 (d, C-6), 120.8 (d, C-14), 124.5 (s, C-9), 127.0 (d, C-4′), 127.4 (s, C-8), 128.3 (d, C-3′ and C-5′), 128.8 (d, C-2′ and C-6′), 134.8 (s, C-13), 137.8 (s, C-1′), 141.7 (s, C-12), 142.7 (s, C-11), 179.2 (s, C-20); EIMS *m*/*z* 436 [M]$^+$ (14), 300 (12), 284 (24), 268 (15), 239 (13), 215 (44), 91 (100), 79 (22); HREIMS *m*/*z* 436.2275 [M]$^+$ (calcd for $C_{27}H_{32}O_5$, 436.2250).

7β-*O*-Benzyl-11,12-di-*O*-methylrosmanol (4). Anhydrous K_2CO_3 (44.5 mg, 0.3 mmol, 13.1 equiv) was suspended in acetone (5 mL), and 7-*O*-benzylrosmanol (**3**) (10.7 mg, 0.0245 mmol) added. The solution was treated with Me_2SO_4 (0.1 mL, 1.05 mmol, 42.9 equiv). After 12 h, the reaction mixture was acidified with 5% HCl, the acetone was evaporated under reduced pressure, and the product was extracted with EtOAc (3 × 15 mL), washed with brine, and dried over anhydrous Na_2SO_4. The crude reaction product was chromatographed over silica gel using CH_2Cl_2 as eluent to yield **4** (10.9 mg, 95.6%): $[\alpha]^{20}_D$ +4.2 (*c* 0.04, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 280 (3.58); IR (film) ν_{max} 2955, 2925, 2854, 1778, 1456, 1411, 1395, 1375, 1353, 1328, 1306, 1226, 1173, 1109, 1071, 1049, 1034, 957, 944 cm^{-1}; ^{1}H NMR (300 MHz) δ

Table 3. Cytotoxicity (IC_{50} μM) of **1**−**20** against Human Cancer and Vero Cell Lines

compound[a]	HeLa[b]	HeLa[c]	Hep-2[b]	Hep-2[c]	A-549[b]	A-549[c]	MCF-7[b]	MCF-7[c]	Vero[b]	Vero[c]
1	43.9	>60	nt[e]	nt	>60	47	40.9	44.5	>60	51.5
2	20.2	51.4	nt	nt	43.4	27.7	17.1	15.3	19.9	36.4
5	4.8	11.1	15.2	18.9	nt	nt	nt	nt	6.1	21.1
6	14.0	9.5	32.0	19.2	>60	26.5	46.0	26.8	>60	>60
7	40.8	>60	nt	nt	35.6	50.8	23.3	41.1	36.7	33.9
8	42.2	>60	nt	nt	42.5	>60	37.5	55.6	42.2	47.2
9	36.0	33.8	42.5	>60	>60	25.4	43.3	22.1	>60	>60
10	25.0	30.8	39.5	39.2	nt	nt	nt	nt	>60	>60
11	>60	>60	nt	nt	>60	28.8	39.5	>60	>60	>60
12	>60	>60	nt	nt	>60	46.0	54.3	36.1	>60	>60
13	>60	40.2	>60	>60	nt	nt	nt	nt	nt	nt
14	33.8	40.5	>45	>45	nt	nt	>45	>45	>45	>45
16	>60	>60	>60	>60	>60	39.4	>60	42.6	>60	>60
17	56.1	37.8	>60	>60	>60	51.5	>60	>60	>60	>60
18	50.3	17.5	>58.4	39.8	>58.4	40.4	33.0	18.4	>58.4	>58.4
19	>58.8	>58.8	nt	nt	48.8	>58.8	>58.8	>58.8	>58.8	>58.8
6-mercaptopurine[d]	2.9	4.1	431	317.8	47	49.3	1.4	5.8	67.5	118.6

[a] Compounds **3**, **4**, **15**, and **20** were inactive against all cell lines used. [b] Compounds were added in lag phase of growth. [c] Compounds were added in log or exponential phase of growth. [d] 6-Mercaptopurine was used as positive control. [e] nt: not tested.

0.94 (3H, s, Me-19), 1.01 (3H, s, Me-18), 1.11 (3H, d, J = 7.0 Hz, Me-16), 1.16 (3H, d, J = 7.0 Hz, Me-17), 1.48 (4H, m, H-2 and H-3), 1.91 (1H, td, J_1 = 5.5 Hz, J_2 = 12.2 Hz, H-1α), 2.35 (1H, s, H-5), 3.16 (1H, bd, J = 12.2 Hz, H-1β), 3.23 (1H, m, H-15), 3.75 (3H, s, Ar-OC$\underline{H}_3$), 3.77 (3H, s, Ar-OC$\underline{H}_3$), 4.48 (1H, d, J = 2.8 Hz, H-7), 4.66 (1H, d, J = 2.8 Hz, H-6), 4.78 (1H, d, J = 11.7 Hz, -C$\underline{H}_2$-Ph), 4.90 (1H, d, J = 11.7 Hz, -C$\underline{H}_2$-Ph), 6.80 (1H, s, H-14), 7.35−7.46 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′); EIMS m/z 464 $[M]^+$ (64), 358 (63), 314 (42), 299 (23), 287 (30), 271 (83), 243 (27), 201 (29), 111 (30), 91 (100), 69 (73); HREIMS m/z 464.2511 $[M]^+$ (calcd for $C_{29}H_{36}O_5$, 464.2563).

7α-Thiophenylcarnosic Acid (5). Carnosol (**1**) (22.6 mg, 0.07 mmol) in acetone (5 mL) was treated with thiophenol (0.05 mL, 0.49 mmol, 7.2 equiv) and solid K_2CO_3 (104.0 mg, 0.75 mmol, 11.0 equiv), and the mixture was stirred at room temperature under Ar for 6 h, after which the acetone was evaporated under reduced pressure. The reaction mixture was acidified with 5% HCl, extracted with EtOAc (3 × 15 mL), washed with brine, and dried over anhydrous Na_2SO_4. The residue was purified by silica gel column chromatography eluting with CH_2Cl_2, yielding **5** (24.1 mg, 80.6%): $[\alpha]^{20}_D$ −3.1 (c 0.04, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 287 (3.82), 232 (4.46) nm; IR (film) ν_{max} 3400, 2961, 2870, 1682, 1583, 1439, 1424, 1391, 1367, 1326, 1292, 1217, 1165, 1129, 1086, 1025, 987, 951, 756, 692, 667 cm^{-1}; ^1H NMR (300 MHz) δ 0.83 (3H, s, Me-19), 0.88 (3H, s, Me-18), 1.21 (6H, d, J = 7.0 Hz, Me-16 and Me-17), 1.47 (5H, m, H-2, H-3 and H-6α), 1.94 (1H, d, J = 12.5 Hz, H-5), 2.36 (1H, d, J = 12.4 Hz, H-6β), 2.72 (1H, td, J_1 = 3.5 Hz, J_2 = 13.5 Hz, H-1α), 3.17 (1H, hept, J = 7.0 Hz, H-15), 3.30 (1H, bd, J = 13.7 Hz, H-1β), 4.71 (1H, d, J = 3.1 Hz, H-7), 5.90 (2H, bs, Ar-O$\underline{H}$), 6.91 (1H, s, H-14), 7.28 (3H, m, H-3′, H-4′, H-5′), 7.48 (2H, d, J=7.5 Hz, H-2′ and H-6′); ^{13}C NMR (75 MHz) δ 20.3 (t, C-2), 22.0 (q, C-19), 22.3 (q, C-16), 22.5 (q, C-17), 23.9 (t, C-6), 27.3 (d, C-15), 32.1 (q, C-18), 34.0 (s, C-4), 34.5 (t, C-1), 41.5 (t, C-3), 47.0 (d, C-5), 48.6 (s, C-10), 50.0 (d, C-7), 122.1 (d, C-14), 122.9 (s, C-8), 126.9 (s, C-9), 127.0 (d, C-4′), 128.9 (d, C-3′ and C-5′), 131.8 (d, C-2′ and C-6′), 133.9 (s, C-1′), 136.3 (s, C-13), 141.8 (s, C-12), 142.7 (s, C-11), 181.9 (s, C-20); EIMS m/z 439 $[M - 1]^+$ (5), 331 (100), 285 (33), 215 (20), 154 (19), 136 (18), 69 (18), 55 (16); HREIMS m/z 439.1968 $[M - 1]^+$ (calcd for $C_{26}H_{31}O_4S$, 439.1943).

Sagequinone Methide A (6). Anhydrous NaOH (46.2 mg, 1.2 mmol, 2.8 equiv) was suspended in dioxane (10 mL) and treated with solid rosmanol (**2**) (144.2 mg, 0.42 mmol), tetrabutylammonium hydrogen sulfate (4.2 mg, 0.01 mmol, 0.03 equiv), and AcCl (0.14 mL, 2.0 mmol, 4.7 equiv), and the mixture was stirred at room temperature under Ar for 7 days. The mixture was filtered and the solvent evaporated. The crude product was chromatographed over silica gel using CH_2Cl_2/Me_2CO (99:1) as eluent to give **6** (76.0 mg, 55.6%): $[\alpha]^{20}_D$ −25.2 (c 0.10, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 287 (3.34) nm; IR (film) ν_{max} 3410, 2920, 1760, 1610 cm^{-1}; ^1H NMR (300 MHz) δ 0.93 (3H, s, Me-19), 1.03 (3H, s, Me-18), 1.13 (6H, d, J = 7.0 Hz, Me-16 and Me-17), 1.20 (1H, dd, J_1 = 3.4 Hz, J_2 = 13.5 Hz, H-3α), 1.44 (1H, bd, J = 13.1 Hz, H-3β), 1.57 (2H, m, H-2), 1.96 (1H, td, J_1 = 5.6 Hz, J_2 = 14.0 Hz, H-1α), 2.30 (1H, s, H-5), 3.05 (1H, hept, J = 8.1 Hz, H-15), 3.17 (1H, bd, J = 14.6 Hz, H-1β), 4.87 (1H, d, J = 5.9 Hz, H-6), 6.84 (1H, s, H-14), 6.96 (1H, d, J = 5.9 Hz, H-7), 7.63 (1H, bs, Ar-O$\underline{H}$); ^{13}C NMR (75 MHz) δ 18.8 (t, C-2), 21.4 (q, C-16 and C-17), 22.3 (q, C-19), 26.9 (d, C-15), 27.4 (t, C-1), 32.3 (q, C-18), 32.4 (s, C-4), 38.4 (t, C-3), 48.9 (s, C-10), 60.9 (d, C-5), 72.9 (d, C-6), 115.8 (s, C-8), 133.8 (s, C-9), 134.8 (d, C-14), 139.9 (d, C-7), 143.8 (s, C-13), 145.8 (s, C-11), 176.2 (s, C-20), 180.4 (s, C-12); EIMS m/z 328 $[M]^+$ (12), 300 (13), 284 (44), 269 (13), 228 (11), 215 (100), 149 (11), 69 (11), 57 (20); HREIMS m/z 328.1698 $[M]^+$ (calcd for $C_{20}H_{24}O_4$, 328.1674).

Treatment of Rosmanol (2) with H_2SO_4/MeOH. To a round bottoned flask with molecular sieves under Ar was added a solution of rosmanol (**2**) (96.8 mg, 0.28 mmol) in MeOH (10 mL) and concentrated H_2SO_4 (0.03 mL). After 24 h, the reaction mixture was filtered over Celite, the solvent was evaporated under reduced pressure, and the product was extracted with EtOAc (3 × 15 mL), washed with H_2O and brine, and dried over anhydrous Na_2SO_4. The crude reaction product was chromatographed by preparative TLC using CH_2Cl_2/Me_2CO (96:4) as eluent to yield 7β-*O*-methylrosmanol (**7**) (25.9 mg, 25.7%), 7-*O*-methylrosmanol (**8**) (33.1 mg, 32.8%), and rosmaquinone B (**9**) (1.3 mg, 1.3%).

7β-*O*-Methylrosmanol (7): $[\alpha]^{20}_D$ +4.5 (c 0.02, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 289 (3.33), 220 (4.08) nm; IR (film) ν_{max} 3500, 1750 cm^{-1}; ^1H NMR (300 MHz) δ 0.96 (3H, s, Me-19), 1.00 (3H, s, Me-18), 1.10, 1.17 (3H each one, d, J = 7.0 Hz, Me-16 and Me-17), 1.96 (1H, s, H-5), 3.01 (1H, hept, J = 6.8 Hz, H-15), 3.21 (1H, bd, J = 14.2 Hz, H-1β), 3.58 (3H, s, −OC$\underline{H}_3$), 4.42 (1H, d, J = 2.0 Hz, H-6), 4.93 (1H, d, J = 2.0 Hz, H-7), 5.69 (1H, bs, Ar-O$\underline{H}$), 5.95 (1H, bs, Ar-O$\underline{H}$), 6.83 (1H, s, H-14); EIMS m/z 361 $[M + 1]^+$ (7), 347 (21), 300 (21), 271 (60), 255 (74), 246 (66), 231 (50), 201 (46), 177 (40), 137 (59), 128 (44), 105 (37), 69 (100); HREIMS m/z 360.1951 $[M]^+$ (calcd for $C_{21}H_{28}O_5$, 360.1937).

7-*O*-Methylrosmanol (8): $[\alpha]^{20}_D$ −3.4 (c 0.02, $CHCl_3$); IR (film) ν_{max} 3580, 3500, 2940, 2920, 2840, 1760, 1450, 1365, 1240, 1080, 1035, 955 cm^{-1}; ^1H NMR (300 MHz) δ 0.94 (3H, s, Me-19), 1.02 (3H, s, Me-18), 1.23 (6H, d, J = 7.0 Hz, Me-16 and Me-17), 2.00 (1H, td, J_1 = 5.6 Hz, J_2 = 13.8 Hz, H-1α), 2.25 (1H, s, H-5), 3.07 (1H, hept, J = 7.0 Hz, H-15), 3.17 (1H, bd, J = 14.6 Hz, H-1β), 3.66 (3H, s, −OC$\underline{H}_3$), 4.27 (1H, d, J = 3.2 Hz, H-6), 4.71 (1H, d, J = 3.2 Hz, H-7), 5.48 (1H, bs, Ar-O$\underline{H}$), 6.00 (1H, bs, Ar-O$\underline{H}$), 6.80 (1H, s, H-14); EIMS m/z 360 $[M]^+$ (100), 314 (81), 298 (80), 284 (88), 269 (84), 245 (93), 228 (38), 215 (93); HREIMS m/z 360.1931 $[M]^+$ (calcd for $C_{21}H_{28}O_5$, 360.1937).

Rosmaquinone B (9): $[\alpha]^{20}_D$ −2.8 (c 0.01, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 340 (2.02), 270 (2.51) nm; IR (film) ν_{max} 2964, 1786, 1667, 1460, 1395, 1350, 1260, 1216, 1170, 1091, 995, 960, 756 cm^{-1}; ^1H NMR (300 MHz) δ 0.89 (3H, s, Me-19), 1.01 (3H, s, Me-18), 1.12 (6H, d, J = 7.0 Hz, Me-16 and Me-17), 1.98 (1H, s, H-5), 2.91 (1H, hept, J = 7.0 Hz, H-15), 3.20 (1H, bd, J = 11.1 Hz, H-1β), 3.68 (3H, s, −OC$\underline{H}_3$), 3.88 (1H, d, J = 2.9 Hz, H-7), 4.64 (1H, d, J = 2.9 Hz, H-6), 6.62 (1H, s, H-14); ^{13}C NMR (75 MHz) δ 18.4 (t, C-2), 21.3 (q, C-16), 21.9 (q, C-19), 22.0 (q, C-17), 25.1 (t, C-1), 27.5 (d, C-15), 31.1 (s, C-4), 31.5 (q, C-18), 38.0 (t, C-3), 45.8 (s, C-10), 50.2 (d, C-5), 59.6 (q, O-C$\underline{H}_3$), 72.5 (d, C-6), 76.9 (d, C-7), 133.6 (d, C-14),

138.3 (s, C-9), 145.7 (s, C-8), 150.0 (s, C-13), 175.6 (s, C-20), 179.6 (s, C-11), 180.0 (s, C-12); EIMS *m/z* 358 $[M]^+$ (24), 314 (30), 284 (11), 269 (26), 258 (60), 245 (100), 243 (23), 215 (31), 69 (10); HREIMS *m/z* 358.1741 $[M]^+$ (calcd for $C_{21}H_{26}O_5$, 358.1780).

Oxidation of 7-*O*-Methylrosmanol (8) with Ag_2O. Freshly prepared Ag_2O (80.7 mg, 0.35 mmol, 4.8 equiv) was added to a solution of 7-*O*-methylrosmanol (**8**) (26.2 mg, 0.07 mmol) in Et_2O (10 mL). After being shaken for 2 h, the solution was filtered, the solvent was evaporated under reduced pressure, and the product was chromatographed over silica gel using CH_2Cl_2 as eluent to yield rosmaquinone B (**9**) (22 mg, 84.3%).

Rosmaquinone (11). Freshly prepared Ag_2O (55.9 mg, 0.241 mmol, 2.03 equiv) was added to a solution of rosmanol (**7**) (41.3 mg, 0.12 mmol) in Et_2O (5 mL). After being stirred for 2 h, the solution was filtered, the solvent was evaporated under reduced pressure, and the product was chromatographed over silica gel using CH_2Cl_2 as eluent to yield **11** (37 mg, 90.4%): $[\alpha]^{20}_D$ −5.4 (*c* 0.06, $CHCl_3$); ^{1}H NMR (300 MHz) δ 0.88 (3H, s, Me-19), 1.02 (3H, s, Me-18), 1.06 (3H, d, J = 7.0 Hz, Me-16), 1.08 (3H, d, J = 7.0 Hz, Me-17), 1.22 (1H, m, H-3α), 1.45 (3H, overlap, H-1α, H-2β and H-3β), 1.60 (1H, m, H-2α), 2.05 (1H, s, H-5), 2.93 (1H, hept, J = 7.0 Hz, H-15), 3.21 (1H, bd, J = 10.5 Hz, H-1β), 4.40 (1H, d, J = 3.3 Hz, H-7), 4.52 (1H, d, J = 3.3 Hz, H-6), 6.79 (1H, s, H-14); ^{13}C NMR (75 MHz) δ 18.2 (t, C-2), 21.1 (q, C-16 and C-17), 21.7 (q, C-19), 25.0 (t, C-1), 27.3 (d, C-15), 30.9 (q, C-18), 31.2 (s, C-4), 37.8 (t, C-3), 45.7 (s, C-10), 49.8 (d, C-5), 68.0 (d, C-7), 76.1 (d, C-6), 133.5 (d, C-14), 138.1 (s, C-9), 146.7 (s, C-8), 150.1 (s, C-13), 175.5 (s, C-20), 179.5 (s, C-11), 179.9 (s, C-12); EIMS *m/z* 344 $[M]^+$ (15), 316 (25), 285 (27), 257 (48), 238 (21), 231 (27), 230 (44), 229 (38), 215 (33), 211 (13), 203 (45), 201 (48), 187 (43), 173 (21), 161 (25), 159 (21), 141 (25), 131 (22), 128 (39), 115 (57), 105 (26), 91 (66), 55 (100); HREIMS *m/z* 344.1586 $[M]^+$ (calcd for $C_{20}H_{24}O_5$, 344.1548).

Isorosmaquinone (10). Freshly prepared Ag_2O (235.6 mg, 1.02 mmol, 6.4 equiv) was added to a solution of isorosmanol (**12**) (56.8 mg, 0.16 mmol) in Et_2O (10 mL). After being shaken for 4.5 h, the solution was filtered, the solvent was evaporated under reduced pressure, and the product was chromatographed over silica gel using CH_2Cl_2 as eluent to yield **10** (51.9 mg, 91.4%): $[\alpha]^{20}_D$ −4.6 (*c* 0.03, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 420 (2.21), 290 (2.62), 212.5 (3.10) nm; IR (film) ν_{max} 3462, 2963, 2876, 1760, 1668, 1462, 1329, 1219, 1110, 757, 612 cm^{-1}; ^{1}H NMR (300 MHz) δ 0.86 (3H, s, Me-19), 1.05 (3H, s, Me-18), 1.11 (6H, d, J = 7.0 Hz, Me-16 and Me-17), 1.26 (1H, bd, J = 13.9 Hz, H-3β), 1.49 (1H, bd, J = 13.9 Hz, H-3α), 1.61 (1H, bd, J = 13.9 Hz, H-2β), 1.79, 1.83 (1H, dt, J_1 = 3.3 Hz, J_2 = 13.8 Hz, H-2α), 2.28 (1H, td, J_1 = 4.8 Hz, J_2 = 13.6 Hz, H-1α), 2.62 (1H, bd, J = 13.6 Hz, H-1β), 2.93 (1H, hept, J = 7.0 Hz, H-15), 4.48 (1H, d, J = 4.5 Hz, H-6), 5.12 (1H, d, J = 4.5 Hz, H-7), 6.73 (1H, s, H-14); ^{13}C NMR (75 MHz) δ 18.1 (t, C-2), 20.5 (q, C-19), 21.2 (q, C-16), 21.6 (q, C-17), 26.9 (t, C-1), 27.8 (d, C-15), 32.5 (q, C-18), 34.2 (s, C-4), 40.3 (t, C-3), 48.4 (s, C-10), 56.1 (d, C-5), 69.2 (d, C-6), 77.9 (d, C-7), 131.3 (d, C-14), 135.6 (s, C-9), 150.3 (s, C-13), 151.9 (s, C-8), 172.4 (s, C-20), 176.3 (s, C-11), 179.3 (s, C-12); EIMS *m/z* 344 $[M]^+$ (17), 300 (57), 287 (50), 270 (49), 255 (100), 231 (47), 215 (27), 165 (10), 115 (15), 69 (15); HREIMS *m/z* 344.1619 $[M]^+$ (calcd for $C_{20}H_{24}O_5$, 344.1624).

Antimicrobial Activity. Antimicrobial activity was determined against Gram-positive (*Bacillus subtilis* ATCC 6051, *B. cereus* ATCC 21772, *Staphylococcus aureus* ATCC 6538, *S. epidermidis* ATCC 14990, *Enterococcus faecalis* ATCC 29212, *Mycobacterium smegmatis* ATCC 19420) and Gram-negative (*Escherichia coli* ATCC 9637, *Proteus mirabilis* CECT 170, *Pseudomonas aeruginosa* AK 958, *Salmonella sp* CECT 4569) bacteria and the yeast *Candida albicans* CECT 1039.

The bacteria cultures were developed in nutrient broth (NB) or brain heart infusion broth (for *E. faecalis*, and *M. smegmatis* containing 0.06% Tween 80), and the yeast was cultured in Sabouraud liquid medium at 37 °C. All media were purchased from Oxoid.

The minimal inhibitory concentration (MIC) was determined for each compound in triplicate, by the broth microdilution method as previously described.[20] The compounds were dissolved in DMSO, and wells with the same proportions of DMSO were used as controls and never exceeded 1% (v/v). The starting microorganism concentration was approximately (1−5) × 10^5 cfu/mL, and growth was monitored by measuring the increase in optical density at 550 nm (OD_{550}) with a microplate reader (Multiskan Plus II). All wells with no visible growth after 24 h, except for *M. smegmatis* (48 h), in a rotatory shaker at 37 °C were subcultured by transferring in duplicate (0.1 mL) to nutrient, brain heart infusion, or Sabouraud agar plates. After overnight incubation, colony counts were performed and the MIC was defined as the lowest concentration of compound at which growth was inhibited after 24 h of incubation.

Cytotoxic Activity. HeLa (human carcinoma of the cervix), A-549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), Hep-2 (human carcinoma of larynx), and Vero (African green monkey kidney) cell lines were each grown as a monolayer in Dulbecco's modified Eagle's medium, DMEM (Sigma), supplemented with 5% fetal calf serum (Gibco) and 1% of penicillin−streptomycin mixture (10.000 UI/mL). The cells were maintained at 37 °C in 5% CO_2 and 98% humidity. Cytotoxicity was assessed using the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] reduction assay.[21] Cell suspensions (0.1 mL of 2 × 10^4 cells/well) in lag phase and log phase of growth were incubated in a microtiter well plate (96-well Iwaki) with the compounds at different concentrations predissolved in DMSO. After 48 h the optical density was measured using a microELISA reader (Multiskan Plus II) at 550 nm after dissolving the MTT formazan with DMSO (150 μL). The percentage viability (IC_{50}) was calculated from the curve. All the experiments were repeated three times.

Acknowledgment. This work was supported by a Grant from the Ministerio de Ciencia y Tecnología (BQU2006-13376/BQU) and FICIC-GI no. 05/2008. L.A. and N.P.M. are grateful to the University of Andes (Mérida, Venezuela) and to the CajaCanarias, respectively, for their fellowships.

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NP900047P