Norditerpenoids and Diterpenoids from *Salvia multicaulis* with Antituberculous Activity

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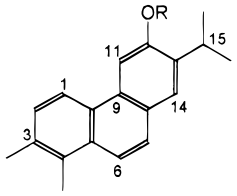
From the roots of *Salvia multicaulis*, four new aromatic norditerpenoids, multicaulin (**1**), 12-demethylmulticauline (**2**), multiorthoquinone (**3**), and 12-demethylmultiorthoquinone (**4**), two new abietane diterpenoids, 12-methyl-5-dehydrohorminone (**5**) and 12-methyl-5-dehydroacetylhorminone (**6**), as well as a new pimarane diterpenoid, salvipimarone (**7**), were isolated. Also obtained in this investigation were the known compounds α-amyrin, hinokione, horminone, lupeol, manool, 1-oxoferruginol, 18-oxoferruginol, pisiferal, and sempervirol. The structures of compounds **1**−**7** were established by 1D and 2D NMR techniques and by chemical methods. The antituberculous activity of **1**−**7** was tested against *Mycobacterium tuberculosis* strain H37Rv, and all compounds were found to be significantly active, with **2** and **4**−**6** being the most potent substances. Six of these novel compounds were evaluated against a number of additional bacterial cultures.

Salvia species have been used in folk medicine all around the world. Several plants of this genus have been associated with antibacterial, antituberculous, and antiphlogistic activities.[1] They are also used in the treatment of hemorrhage, menstrual disorders, and miscarriage[2] as well as in heart disease and hepatitis.[3] There are about 90 *Salvia* species in Turkey, and 44 of them are endemic plants.[4] Such species have many uses in folk medicine, including the treatment of stomach ailments and the common cold, and they also possess antiseptic, antibacterial, carminative, diuretic, hemostatic, and spasmolytic activities.[5] Turkish *Salvia* species are characterized by the presence of abietane-type diterpenoids, as shown in various published studies.[6,7]

In a continuation of our investigation on Turkish *Salvia* species for their chemical composition[8−10] and biological activities,[11−13] we have studied *Salvia multicaulis*, a plant endemic to the Middle East. The plant material was collected from the Mediterranean region of Turkey. From an acetone-soluble extract of the roots of *S. multicaulis* Vahl. (Labiatae), seven new diterpenoids (**1**−**7**) have been isolated, in addition to the known compounds, 1-oxoferruginol,[14] horminone,[15] manool,[16] sempervirol,[17] pisiferal,[18] 18-oxoferruginol,[19] hinokione,[20] lupeol,[21] and α-amyrin.[22] Among the new diterpenoids, four (**1**−**4**) were aromatic norabietanes, two (**5**, **6**) abietanes, and one (**7**) a pimarane. These new secondary metabolites are of interest due to their biological activities and their resemblance to a group of highly active diterpenoids, the tanshinones.[23] All of the new diterpenoids showed same in vitro antituberculous activity, particularly, compounds **2** and **4**−**6**.



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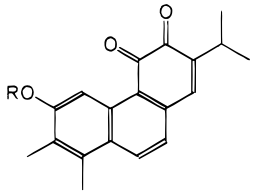
2 R=H



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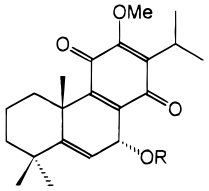

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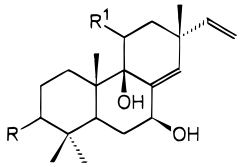
6 R=Ac



7 R=R[1]=O
7a R=β-OH, R[1]=α-OH

Results and Discussion

Compounds **1** and **2** were orange, and compounds **3** and **4** were dark orange to red, indicating extended conjugation in each of these four molecules.

Multicauline (**1**) had the molecular formula $C_{20}H_{22}O$ as deduced from its HRCIMS (*m*/*z* 279.1678, $[M + 1]^+$), indicating 10 degrees of unsaturation as double bond equivalents, of which three were accounted for the presence of a tricyclic skeleton and the remaining seven degrees of unsaturation indicated the presence of seven double bonds. The lack of a carbonyl group was apparent from the IR and ^{13}C NMR spectra of multicaulin (**1**). The structure was further investigated from an examination of its ^1H-, ^{13}C-, and HETCOR NMR spectra (Table 1). The ^1H NMR spectrum revealed the presence of two sets of *ortho*-coupled protons δ 8.39 and 7.70 (each

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**4**[a]

	1		**2**		**3**		**4**	
position	δH	δC	δH	δC	δH	δC	δH	δC
1	8.39 (9)	120.9 d	8.30 (9)	121.0 d	7.17 s	152.8 d	7.12 s	153.0 d
2	7.70 (9)	128.0 d	7.60 (9)	128.2 d		133.9 s		133.2 s
3		140.0 s		140.0 s		140.6 s		141.0 s
4		135.6 s		135.5 s		133.4 s		133.5 s
5		122.7 s		123.0 s		127.6 s		127.0 s
6	7.88 d (8.7)	125.5 d	7.85 (8.8)	125.6 d	7.50 d (8)	125.5 d	7.42 d (8)	122.6 d
7	7.41 d (8.7)	127.9 d	7.40 (8.8)	127.8 d	7.10 d (8)	132.4 d	7.10 d (8)	133.0 d
8		126.1 s		126.3 s		128.5 s		128.9 s
9		147.8 s		147.8 s		126.0 s		127.0 s
10		133.4 s		133.4 s		152.4 s		150.1 s
11	7.95 s	110.6 d	7.91 s	109.9 d		183.3 s[b]		183.2 s[c]
12		151.4 s		148.9 s		181.4 s[b]		183.0 s[c]
13		130.3 s		130.0 s		135.3 s		134.9 s
14	7.67 s	125.5 d	7.57 s	124.9 d	7.05 s	126.6 d	7.03 s	126.5 d
15	3.46 sept (7)	25.5 d	3.39 sept (7)	26.0 d	3.30 sept (7)	26.3 d	3.21 sept (7)	25.8 d
16	1.32 d (7)	22.7 q	1.38 d (7)	22.8 q	1.22 d (7)	23.0 q	1.26 d (7)	22.9 q
17	1.32 d (7)	22.7 q	1.38 d (7)	22.8 q	1.22 d (7)	23.2 q	1.26 d (7)	23.0 q
18	2.65 s	20.9 q	2.64 s	21.0 q	2.33 s	20.8 q	2.32 s	21.0 q
19	2.52 s	18.3 q	2.52 s	18.4 q	2.25 s	17.9 q	2.25 s	17.6 q
OMe	4.06 s	55.4 q			3.88 s	56.0 q		

[a] *J* values are given in parentheses (Hz). [b,c] Assignments marked with the same letter are interchangeable.

Table 2. COLOC Correlations of Compounds **1** and **3**

	1	**3**
proton(s)	correlated carbon	correlated carbon
H−1	C−3, C−5, C−9, C−10	C−3, C−5, C−10, C−11
H−2	C−4, C−10, C−11	
H−6	C−4, C−7, C−8, C−10	C−4, C−7, C−10
H−7	C−5, C−6, C−14	C−5, C−14
H−11	C−1, C−10, C−13	
H−14	C−7, C−9, C−15	C−7, C−12, C−15
H−15	C−11, C−12, C−14	C−12, C−12, C−14

1H, d, J = 9 Hz) for H-1 and H-2 and at δ 7.88 and 7.41 (each 1H, d, J = 8.7 Hz) for H-6 and H-7, as well as two aromatic proton singlet signals at δ 7.95 and 7.67 for H-11 and H-14, indicating a fully aromatized tricyclic ring structure for **1** (Table 1). The ^{13}C NMR (APT) spectrum of **1** displayed signals of four methyl quartets for five methyl groups (one being a methoxyl and two being part of an isopropyl group) and seven methine doublets, six of them at lowfield. The appearance of eight downfield carbon singlets verified the aromatic nature of the compound. The presence of ^{1}H NMR signals for an isopropyl group at δ 1.32 (6H, d, J = 7 Hz, Me-16 and Me-17) and δ 3.46 (1H, septet, J = 7 Hz, H-15) and for two aromatic methyl singlets at δ 2.52 and 2.63 (each 3H, s) indicated an abietane-type diterpenoid.[24,25] Biogenetic considerations also supported the abietane structure for multicauline (**1**); however, the lack of a third methyl signal showed that **1** is a norabietane diterpenoid.[26] The unambiguous assignment of the entire structure was possible by a COLOC experiment (Table 2). The spectral data therefore permitted the assignment of multicauline (**1**) as 12-methoxyabieta-1,3,5(10),6,8,11,13-heptaene.

The second new compound (**2**) was the 12-demethyl derivative of **1**. The HREIMS (*m*/*z* 264.1522) corresponded to the molecular formula $C_{19}H_{20}O$, indicating 10 degrees of unsaturation, similar to compound **1**. The ^{1}H NMR spectrum of **2** exhibited signals quite similar to those of **1**, with the methoxyl group being absent (Table 1). The ^{13}C NMR (APT) spectrum correlated with the aromatic norditerpene structure giving signals for four methyl quartets, seven methine doublets, and eight carbon singlets. All of these 15 signals, with one exception (C-15), appeared downfield. The HETCOR experiment showed a correlation between the carbons and protons in the molecule of **2** (Table 1). Analysis of that spectral data enabled the structure 12-hydroxyabieta-1,3,5(10),6,8,11,13-heptaene to be assigned to **2**.

The other two novel norabietane derivatives (**3** and **4**) had orthoquinoid structures with aromatic A and B rings and are among the first examples of this type of norditerpene.[27] The HREIMS of multiorthoquinone (**3**) showed a molecular formula of $C_{20}H_{20}O_3$ (*m*/*z* 308.1422), indicating 11 degrees of unsaturation, of which two were accounted for by the presence of two quinoid carbonyl groups and three by a tricyclic skeleton, and the remaining six degrees of unsaturation indicated the presence of six double bonds. The IR and ^{13}C NMR spectra showed the presence of quinoid carbonyl groups at 1680 and 1654 cm^{-1} and at δ 183.1 and 181.4, respectively.[28] The ^{1}H NMR spectrum exhibited two *ortho* protons present in ring B and two aromatic proton singlets in the A and C rings of the molecule. The chemical shifts of H-6 (δ_H 7.50, d, J = 8 Hz) and H-7 (δ_H 7.10, d, J = 8 Hz) were characteristic for aromatic-ring protons conjugated with an orthoquinoid ring system, as observed in saprorthoquinone.[28] The other signals showed the presence of one methoxyl at δ 3.88 (3H, s) and two aromatic methyl groups at δ 2.33 and 2.25 (each 3H, s) as well as the presence of an isopropyl group at δ 1.22 (6H, d, J = 7 Hz, Me-16 and Me-17) and δ 3.30 (1H, septet, J = 7 Hz, H-15) (Table 1). The ^{13}C NMR (APT) spectrum of **3** indicated five methyl quartets, with one being a methoxyl group, and five methine doublets, of which four appeared at lowfield, together with 10 carbon singlets. Two of the carbon singlets, observed at δ 183.3 and 181.4, verified the quinoid structure of ring C.[28] The HETCOR experiment revealed the carbon−proton correlations for compound **3** (Table 1). The COLOC experiment enabled the unambiguous assignment of the structure of multiorthoquinone (**3**) as 2-methoxy-11,12-dioxoabieta-1,3,5(10),6,8,13-hexaene.

Along with compound **3**, its 2-demethyl derivative (**4**) was also isolated. The molecular formula of **4**, $C_{19}H_{18}O_3$, was calculated from its HREIMS (*m*/*z* 294.1260), indi-

Table 3. ^{1}H NMR Data of Compounds **5** and **6** and ^{13}C NMR Data of **5**−**7**[a]

	5		**6**		**7**
position	$^\delta$H	$^\delta$C	$^\delta$H	$^\delta$C	$^\delta$C
1α	1.50 br dd(12.8, 6.0)	37.4 t	1.48 br dd (12.5, 6.0)	37.5 t	39.1 t
1*β*	2.71 td(14.2,13.1)		2.69 td (14.0, 13.0)		
2α	1.62 m	18.2 t	1.59 m	18.2 t	34.3 t
2*β*	2.56 tt(14, 5.5)		2.55 tt (14.0, 5.5)		
3α	1.38 td(13.5,6.0)	40.8 t	1.37 td (14.0, 6.0)	40.7 t	214.0 s
3*β*	2.18 m		2.16 m		
4		33.4 s		33.4 s	42.1 s
5		127.7 s		127.6 s	46.2 s
6	6.91 brs	106.7 d	6.92 brs	108.0 d	29.3 t
7	4.70 brs	77.8 d	5.35 brs	76.9 d	73.4 d
8		124.7 s		124.8 s	144.1 s
9		142.6 s		142.6 s	78.0 s
10		41.2 s		41.2 s	39.1 s
11		183.2 s		183.2 s	210.1 s
12		154.5 s		154.4 s	42.1 t
13		129.4 s		129.5 s	37.3 s
14		181.4 s		182.0 s	134.0 d
15	3.30 sept (6.8)	26.4 d	3.29 sept (6.8)	25.9 d	148.3 d
16	1.21 d (6.8)	21.4 q	1.21 d (6.8)	21.5 q	110.6 t
17	1.18 d (6.8)	21.6 q	1.18 d (6.8)	21.5 q	25.6 q
18	1.05 s	21.6 q	1.05 s	22.0 q	33.4 q
19	1.05 s	33.4 q	1.05 s	33.0 q	21.9 q
20	1.09 s	20.1 q	1.08 s	20.2 q	14.2 q
OMe	3.82 s	55.9 q	3.81 s	55.9 q	
C═O				172.6 s	
CH_3			2.03 s	22.3 q	

[a] *J* values are given in parentheses (Hz).

cating 11 degrees of unsaturation similar to that of **3**. The ^{1}H NMR spectrum showed signals similar to those of **3**, with the lack of a methoxyl group. The ^{13}C NMR (APT) spectrum of **4** exhibited two carbonyl carbons at δ 183.2 and 183.0 indicating its quinoid ring C. There were four methyl quartets, five methine doublets, and eight carbon singlets indicating a norditerpene structure, with spectral data similar to that of **3** (Table 1). Thus, the structure of **4** was established as 2-hydroxy-11,12-dioxoabieta-1,3,5(10),6,8,13-hexaene.

In addition to the norabietanes (**1**−**4**), two quinoid abietanes (**5** and **6**) and a pimarane diterpenoid (**7**) were obtained.

Compound **5** had a molecular formula of $C_{21}H_{28}O_4$, as deduced from its HREIMS (*m*/*z* 344.1992), indicating eight degrees of unsaturation, of which two were assigned to two quinoid carbonyl groups, and three degrees of unsaturation to a tricyclic skeleton, and the remaining three degrees of unsaturation were accounted for by three double bonds in the molecule. The IR and ^{13}C NMR spectra indicated the presence of quinoid carbonyl groups at 1689 cm^{-1} and δ 183.2, 181.4, respectively. The ^{1}H and ^{13}C NMR spectra showed clearly that the structure of **5** resembles that of horminone with the presence of a double bond that could only be at Δ.[5] The observed signals at δ 4.70 (1H, brs, H-7), 3.30 (1H, septet, J = 6.8 Hz, H-15), 1.21 (3H, d, J = 6.8 Hz), 1.18 (3H, d, J = 6.8 Hz) (Me-16 and Me-17), 1.09 (3H, s, Me-20), 1.05 (6H, s, Me-18 and Me-19) in the ^{1}H NMR of compound **5** are all similar to analogous data for horminone.[15] Compound **5** differed from horminone with the additional ^{1}H NMR spectrum signals at δ 3.82 (3H, s, OMe) and 6.91 (1H, brs, H-6). Examination of the structure proposed for **5** using a Dreiding model indicated an angle nearly 90° between H-6 and H-7, thus supporting the presence of singlets for H-6 and H-7. The ^{13}C NMR spectrum displayed the presence of six methyl quartets as five signals, with one being a methoxyl, along with three methylene triplets, three methine doublets, and nine carbon singlets (Table 3). Analyzing the spectral data suggested the structure of **5** as 7-hydroxy-12-methoxy-11,14-dioxoabieta-5,8,12-triene.

Compound **6** was the acetyl derivative of **5**. The HREIMS showed the molecular formula to be $C_{23}H_{30}O_5$ (*m*/*z* 386.2122). The ^{1}H NMR spectrum was quite similar to that of **5**, only the H-7 signal was shifted to δ 5.35 (1H, brs, H-7) from δ 4.70 together with the appearance of an acetyl signal at δ 2.03 (3H, s). The other signals were similar for both compounds. The ^{13}C NMR (APT) spectrum of **6** was the same as that of **5** with an additional carbonyl signal at δ 172.6 and an acetyl methyl signal at δ 22.3 verifying the presence of the acetyl group (Table 3). The structure of **6** was therefore assigned as 7-acetyl-12-methoxy-11,14-dioxoabieta-5,8,12-triene.

The final novel compound (**7**) had a molecular formula $C_{20}H_{28}O_4$ (*m*/*z* 332.1991) as deduced from its HREIMS, indicating seven degrees of unsaturation, two of which were accounted for two carbonyl groups, two others by two double bonds of the molecule, and the remaining three unsaturations indicated a tricyclic skeleton. The ^{1}H NMR spectrum showed a vinyl side-chain from signals at δ 5.79 (1H, dd, J = 11 and 16.5 Hz), 4.96 (1H, dd, J = 2 and 16.5 Hz) and 4.92 (1H, dd, J = 2 and 11 Hz), which together with four methyl singlet signals at δ 0.78, 0.85, 0.90 and 1.04 (each 3H, s) indicated a pimarane-type compound.[29] The chemical shift of a broad singlet at δ 5.50 indicated an olefinic proton that could be placed at $\Delta^{8(14)}$. A narrow triplet at δ 4.20 (1H, t, J = 1.5 Hz, H-7α) was indicative of the presence of a *β*-hydroxyl group at C-7. Similar compounds with a C-7*β* hydroxyl group and a double bond at $\Delta^{8(14)}$ were previously isolated from *Arelia cordata* and *A. racemosa* and showed comparable chemical shifts and splitting patterns for H-14 and H-7α.[30,31] The IR spectrum of **7** indicated the presence of carbonyl group(s) at 1737 and 1720 cm^{-1} which could be correlated with the ^{13}C NMR

Table 4. HMBC Data of Compound **7**

proton(s)	correlated carbon(s)
H−7	C−5, C−6, C−9, C−14
H−15	C−11, C−12
H−16	C−13
H−17	C−11, C−13, C−14, C−15, C−16
H−18	C−2, C−3, C−5, C−6
H−19	C−3, C−5, C−6
H−20	C−1, C−2, C−9

Table 5. Activity of Compounds **1**−**7** against *Mycobacterium tuberculosis* Strain H37Rv

compd	MIC value[a]	compd	MIC value[a]
1	5.6	**5**	1.2
2	0.46	**6**	0.89
3	2.0	**7**	7.3
4	1.2		

[a] Minimal inhibiting concentrations are given in μg/mL.

(DEPT) signals at δ 214.0 and 210.1. The ^{13}C NMR signals for the vinyl side chain were observed at δ 148.3 (d) and 110.6 (t), and the signals at δ 134.0 (d) and 144.1 (s) were assigned to C-14 and C-8, respectively. A doublet signal at δ 73.4 showed the presence of a secondary hydroxyl group at C-7, and the singlet signal at δ 78.0 revealed the presence of a tertiary hydroxyl group. The locations of the latter hydroxyl group as well as the two oxo groups were assigned to C-9, C-3, and C-11, respectively, on the basis of SINEPT and HMBC data (Table 4). To verify the placement of the two oxo groups in **7**, $NaBH_4$ reduction was carried out. In the IR spectrum of the reduced product (**7a**), the signals for the carbonyl groups of **7** disappeared while the hydroxyl band became stronger. The ^{1}H NMR spectrum showed two additional signals at δ 3.96 (H-3α) and 3.68 (H-11β). In a NOESY experiment on **7**, the interactions between H-7α and H-14 were observed as well as between Me-20, Me-17, and C-9 OH, indicating the β orientation of the latter groups. These data confirmed that compound **7** is a pimarane diterpenoid.[29] Therefore, based on the interpretation of its spectral and chemical data, compound **7** was elucidated as 7β,9β-dihydroxypimara-8(14)-ene-3,11-dione, to which we have accorded the trivial name salvipimarone.

All the test compounds (**1**−**7**) showed antituberculous activity according to the broth microdilution method (Table 5).[32,33] When our MIC results were compared to standard tuberculostatic agents as indicated in the literature,[34] such data are as follows: streptomycin (2−10 μg/mL), kanamycin (5−10 μg/mL), rifampicin (0.5 μg/mL), PAS (*p*-aminosalicylic acid) (5−10 μg/mL), INH (isonicotinic acid hydrazide) (0.2−5 μg/mL), ETA (ethionamide) (5−10 μg/mL), and EMB (ethambutol) (5−10 μg/mL). Thus, the test compounds **1**−**7** gave comparable values to these standards as antituberculous agents. However, data were not obtained in this study for any of these standard compounds.

All of the new compounds except **5** were also tested against standard bacterial strains,[35] and compound **2** was found to be active against β-hemolytic *Streptococcus* while **1** and **3** were particularly active against *S. aureus* and β-hemolytic *Streptococcus*. Compound **1** was also active against *E. coli* and *P. mirabilis* and **3** against *E. faecalis* and *P. aeruginosa*. Compound **7** exhibited inhibitory effects against *P. mirabilis* and *E. faecalis* (Table 6).

Experimental Section

General Experimental Procedures. IR spectra were recorded on Perkin-Elmer 983 spectrophotometer in $CHCl_3$. UV spectra were measured on a Varian Techtron 635 instrument using MeOH as solvent. DEPT, SINEPT, and HMBC experiments were carried out in a Bruker AMX 500 MHz NMR spectrometer, and all other spectra were recorded on a Bruker AC 200 L instrument in $CDCl_3$. HRMS were measured on a VG ZabSpec mass spectrometer. Also used were Kieselgel 60F 254 precoated Al sheets for TLC (0.2 mm, Merck), silica gel (70−200 mesh) for column chromatography, silica gel 5554 (Merck) for vacuum−liquid chromatography (VLC), and Sephadex LH-20 (Fluka) for gel permation chromatography.

Plant Material. The roots of *S. multicaulis* Vahl. were collected from southern Turkey (Adana-Armutoluk district) at an altitude of 1850 m in July 1995 and identified by Prof. Dr. N. Özhatay (Istanbul). A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 68325).

Extraction and Isolation. The powdered roots of *S. multicaulis* (940 g) were extracted with acetone in a Soxhlet apparatus. The extract was evaporated in vacuo to give 23 g of a residue. The residue was fractionated by column chromatography on a silica gel column, eluted with petroleum ether, followed by a gradient of EtOAc up to 100% and then with EtOH. After TLC analysis, the combined fractions were purified by vacuum−liquid chromatography (VLC), eluting with petroleum ether and ethyl acetate. When necessary, Sephadex LH-20 columns and/or preparative TLC plates were used for final purification. The following compounds were obtained: **1** (16 mg), **2** (12 mg), **3** (20 mg), 1-oxoferruginol (8 mg), hinokione (15 mg), sempervirol (15 mg), manool (10 mg), **6** (10 mg), **5** (8 mg), **4** (12 mg), horminone (15 mg), **7** (12 mg), lupeol (20 mg), and α-amyrin (25 mg).

Multicaulin (1): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 450 (sh), 355 (2.5), 340 (2.6), 310 (3.2), 260 (3.5), 222 (3.4) nm; IR ($CHCl_3$) ν_{max} 3050, 1605, 1590, 1520, 1492, 1246, 1171, 1098, 949, 889, 842, 802, 766 cm^{-1}; ^{1}H and ^{13}C NMR (200 MHz, $CDCl_3$), see Table 1; CIMS *m/z* 279 [M + 1]$^+$ (100), 263 [M − 16]$^+$ (25), 248 [M − 31]$^+$ (7), 237 [M − 42]$^+$ (18); HRCIMS *m/z* 279.1678, calcd for $C_{20}H_{23}O$ 279.1743.

12-Demethylmulticaulin (2): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 452 (sh), 360 (2.6), 340 (2.6), 308 (3.3), 260 (3.5), 220 (3.4) nm; IR ($CHCl_3$) ν_{max} 3460, 3050, 1600, 1590, 1522, 1495, 1250, 1070, 1150, 1090, 950, 880, 840, 760 cm^{-1}; ^{1}H and ^{13}C NMR (200 MHz, in $CDCl_3$), see Table 1; EIMS *m/z* 264 [M]$^+$ (100), 249 [M − 15]$^+$ (94), 234 (30), 216 (35), 202 (20), 189 (24), 132 (13), 117 (22), 71 (9), 57 (13); HREIMS *m/z* 264.1522, calcd for $C_{19}H_{20}O$ 264.1514.

Multiorthoquinone (3): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 430 (3.4), 350 (2.5), 310 (3.1), 290 (3.0), 266 (3.9), 228 (3.8) nm; IR ($CHCl_3$) ν_{max} 3040, 1680, 1654, 1615, 1559, 1505, 1477, 1393, 1163, 1099, 1052, 965, 888, 849, 814, 771 cm^{-1}; ^{1}H and ^{13}C NMR (200 MHz, in $CDCl_3$), see Table 1; EIMS *m/z* 308 [M]$^+$ (5), 280 [M − 28]$^+$ (82), 265 [M − 28−15]$^+$ (100), 250 (12), 235 (15), 220 (12), 203 (16), 165 (8), 125 (14); HREIMS *m/z* 308.1422 [M]$^+$, calcd for $C_{20}H_{20}O_3$ 308.1412.

Table 6. Antibacterial Activity (MIC)[a] of Compounds **1**−**4**, **6** and **7**

	organisms[c]						
compd[b]	*S. aureus*	*E. coli*	*P. mirabilis*	*K. pneumonia*	*β*-hem. *Strep.*	*E. faecalis*	*P. aeruginosa*
1	0.2	0.7	1.4	NA[d]	0.2	NA	NA
2	NA	NA	NA	15.6	2.0	NA	15.6
3	0.1	NA	NA	NA	0.1	2.0	0.5
4	NA	4.6	NA	NA	NA	NA	NA
6	NA	NA	NA	7.2	NA	NA	NA
7	NA	NA	3.6	NA	NA	3.6	NA

[a] Minimal inhibitory concentrations of the compounds are given in μg/mL. [b] The highest concentration used were as follows: **1**, 11.2 μg/mL; **2**, 12.5 μg/mL; **3**, 8.12 μg/mL; **4**, 18.8 μg/mL; **6**, 28.6 μg/mL; **7**, 29.2 μg/mL. [c] Key to organisms: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Proteus mirabilis* ATCC 14153, *Klebsiella pneumonia* ATCC 4352, *Pseudomonas aeruginosa* ATCC 9027, beta-hemolytic *Streptococcus* (clinical isolate), and *Enterococcus faecalis* (ATCC 29212). [d] NA = not active (determined by the disk-diffusion method as defined in the Experimental Section).

2-Demethylmultiorthoquinone (4): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 435 (3.6), 352 (2.6), 315 (3.0), 290 (3.0), 266 (3.9), 230 (3.9) nm; IR ($CHCl_3$) ν_{max} 3480, 3040, 1680, 1650, 1620, 1560, 1505, 1477, 1393, 1165, 1100, 1050, 970, 888, 849, 770 cm^{-1}; ^1H and ^{13}C NMR (200 MHz, in $CDCl_3$), see Table 1; EIMS *m/z* 294 $[M]^+$ (3), 266 $[M - 28]^+$ (72), 251 $[M - 28 - 15]^+$ (100), 233 (10), 218 (12), 203 (15), 189 (10), 165 (8), 118 (12); HREIMS *m/z* 294.1260 $[M]^+$, calcd for $C_{19}H_{18}O_3$ 294.1255.

12-Methyl-5-dehydrohorminone (5): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 395 (2.6), 280 (3.0), 228 (4.1) nm; IR ($CHCl_3$) ν_{max} 3420, 2930, 2869, 1689, 1502, 1463, 1412, 1347, 1257, 1107, 991, 969, 884, 848, 740 cm^{-1}; ^1H and ^{13}C NMR (200 MHz, in $CDCl_3$), see Table 3; EIMS *m/z* 344 $[M]^+$ (25), 329 $[M - 15]^+$ (10), 298 $[M - 15 - 31]^+$ (100), 270 (58), 255 (47), 171 (21), 128 (10); HREIMS *m/z* 344.1992 $[M]^+$, calcd for $C_{21}H_{28}O_4$ 344.1987.

12-Methyl-5-dehydroacetylhorminone (6): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 395 (2.6), 285 (3.1), 230 (4.1) nm; IR ($CHCl_3$) ν max 2950, 2870, 1720, 1690, 1610, 1500, 1463, 1410, 1350, 1260, 1240, 1110, 970, 885, 850, 740 cm^{-1}; ^1H and ^{13}C NMR (200 MHz, in $CHCl_3$), see Table 3; EIMS *m/z* 386 $[M]^+$ (5), 344 $[M - 42]^+$ (60), 327 $[M - 59]^+$ (95), 296 $[M - 60 - 30]^+$ (40), 270 (50), 213 (40), 171 (18), 128 (5); HREIMS *m/z* 386.2122 $[M]^+$ calcd for $C_{23}H_{30}O_5$, 386.2093.

Salvipimarone (7): amorphous powder: UV (MeOH) λ_{max} (log ϵ) 225 (3.9) nm; IR ($CHCl_3$) ν_{max} 3400, 2960, 2940, 2860, 1737, 1720, 1625, 1460, 1385, 1370, 1240, 1220, 1180, 1140, 910, 870 cm^{-1}; ^1H NMR (200 MHz in CDCl3) 5.79 (1H, dd, J = 11, 16.5 Hz, H-15), 4.96 (1H, dd, J = 2, 16.5 Hz, H-16), 4.92 (1H, dd, J = 2, 11 Hz, H-16′), 5.50 (1H, br s, H-14), 4.20 (1H, t, J = 1.5 Hz, H-7α), 1.04, 0.90, 0.85, 0.78 (each 3H, s); ^{13}C NMR (500 MHz, in $CDCl_3$), see Table 3; EIMS *m/z* 332 (M^+, 100), 315 (12), 303 (8), 255 (40), 223 (80), 210 (38), 165 (8), 105 (7), 77 (16); HREIMS *m/z* 332.1991 (M^+) calcd for $C_{20}H_{28}O_4$, 332.1987.

Reduction of 7. Ten mg of **7** was dissolved in 1 mL of MeOH, 50 mg of $NaBH_4$ was added, and the mixture was left at room temperature for 24 h. Acetic acid (25%) was added to the mixture, which was then extracted with $CHCl_3$ and evaporated to dryness. On workup, residue **7a** (6 mg) was obtained.

3*β*,7*β*,9*β*,11α-Tetrahydroxypimara-8(14),15-diene (7a): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 227 (4.1) nm; IR ($CHCl_3$) ν_{max} 3450, 2980, 2960, 2850, 1630, 1460, 1380, 1370, 1220, 1140, 1080, 1060, 910, 860 cm^{-1}; ^1H NMR (200 MHz, in $CDCl_3$) δ 5.80 (1H, dd, J = 11, 16 Hz, H-15), 4.96 (1H, dd, J = 2, 16 Hz, H-16), 4.92 (1H, dd, J = 2 and 11 Hz, H-16′), 5.45 (1H, br s, H-14), 4.20 (1H, t, J = 1.5 Hz, H-7α), 3.96 (1H, dd, J = 4, 10 Hz, H-3α), 3.68 (1H, dd, J = 8, 12 Hz, H-11*β*), 1.04, 0.92, 0.85, 0.79 (12 H, each 3H, s); EIMS *m/z* 336 $[M]^+$ (70), 318 $[M - 18]^+$ (100), 300 $[M - 18 - 18]^+$ (45), 257 (18), 226 (55), 169 (10), 105 (12), 77 (25).

Antimycobacterial Activity Tests. The broth microdilution method was used.[32,33] *M. tuberculosis* standard strain H37Rv was subcultured into Middlebrook 7H9 broth in screw-capped tubes and incubated at 37 °C for 3 weeks. After observation of the growth, the tubes were left at room temperature for 30 min for sedimentation of solid particules. The homogeneous suspension at the upper part of each tube was transferred into sterile screw-capped plastic tubes and the inoculum size was adjusted with Middlebrook 7H9 broth to approximately 10^8 cfu/mL according to the MacFarland No. 0.5 turbidity standard. This suspension was further diluted 10-fold and used for MIC determinations. Microdilution susceptibility testing was performed in clear 96-well polystyrene plates containing 50 μL of liquid medium in each well. The compounds were dissolved in chloroform. Drug solutions (50 μL) were dispensed into the microplates beginning from the first well. Two-fold dilutions were made for compounds **1**−**7**. The last well in each row was used for bacterial controls. The inocula (50 μL) were dispensed into the wells, and the plates were tested in plastic bags and incubated at 37 °C in a humid atmosphere for 21 days. The same test was carried out with chloroform as control. MIC values were determined as the lowest concentration of the compound for which no visible growth was observed.

Antimicrobial Activity Tests. The disk-diffusion method[35,36] was used to determine the inhibition zones of the diterpenoids. Compounds **1**−**4**, **6**, and **7**, with inhibition zones greater than 7 mm, were selected for tube dilution tests[37] to determine the antibacterial activity quantitatively as minimum inhibition concentrations (MIC). The standard bacterial strains used were *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Proteus mirabilis* ATCC 14153, *Klebsiella pneumonia* ATCC 4352, *Pseudomonas aeruginosa* ATCC 9027, *β*-hemolytic *Streptococcus* (clinical isolate), and *Enterococcus faecalis* (ATCC 29212).

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