Bioactive Abietane and *seco*-Abietane Diterpenoids from *Salvia prionitis*

Xin Chen, Jian Ding, Yong-Mao Ye, and Jin-Sheng Zhang*

Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 294 Tai-Yuan Road, Shanghai 200031, People's Republic of China

Received November 9, 2001

From the roots of *Salvia prionitis* a new tricyclic diterpene, saprirearine (**1**), a new anhydride-type compound, saprionide (**2**), a new 7,8-*seco*-abietane diterpene derivative, 7,8-*seco*-*para*-ferruginone (**3**), and two new 4,5-*seco*-5,10-*friedo*-abietane diterpenoids, 4-hydroxysaprorthoquinone (**4**) and 3-keto-4-hydroxysaprorthoquinone (**5**), were isolated. Their structures were established by spectroscopic methods and chemical transformation. Compound **3** showed antimicrobial activities against two Gram-positive organisms, *Staphylococcus aureus* and *Micrococcus luteus*, with MIC values of 20.0 and 15.0 μM, respectively. Compound **4** showed significant inhibition against topoisomerase I with an IC_{50} value of 0.8 μM. Compound **5** exhibited cytotoxic activities against HL-60 human leukemia and the SGC-7901 and MKN-28 stomach cancer cell lines, with IC_{50} values of 4.6, 0.2, and 0.3 μM, respectively.

As a part of a search for new antitumor agents from Chinese medicinal plants, the roots of *Salvia prionitis* Hance (Labiatae), collected in Jiangxi Province, were investigated. *Salvia prionitis* is used in Chinese folk medicine as an antiphlogistic, antibacterial, and antitubercular drug.[1] More than 40 compounds have been isolated from this plant so far.[2−9] In our continuing study on its bioactive components, two new abietane (**1** and **2**) and three new *seco*-abietane diterpenoids (**3**−**5**) were obtained. We report herein the isolation, structure elucidation, partial synthesis, and bioactivities of these compounds.

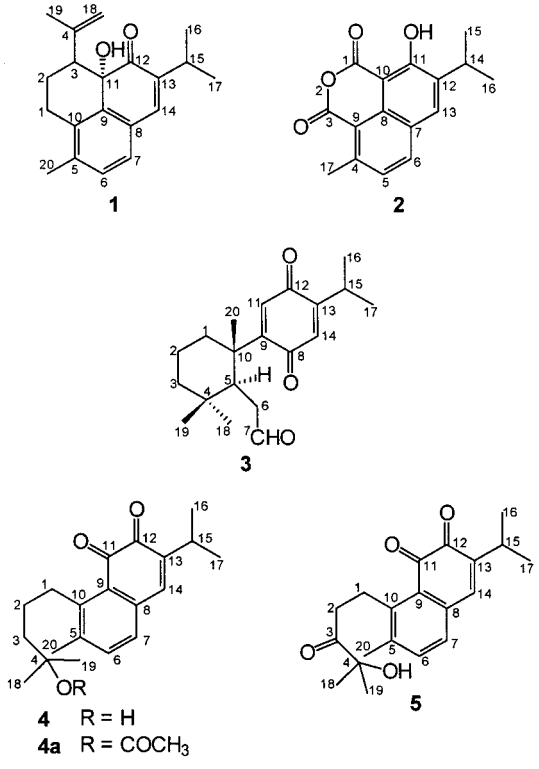


Table 1. 100 Hz ^{13}C NMR Spectral Data[a] and HMBC Correlations for Compounds **1**−**3** ($CDCl_3$)

	1		**2**		**3**	
position	^{13}C	HMBC	^{13}C	HMBC	^{13}C	HMBC
1	25.9	3	167.0		36.0	3, 20
2	26.0				18.7	
3	45.8	18, 19	164.9		40.3	18, 19
4	149.1	2, 3, 18, 19	148.3	6	34.0	2, 18, 19
5	128.6	20	129.4	17	42.1	1, 3, 6, 7, 20
6	130.1	7	134.7	13	42.7	5, 7
7	126.5	14	124.9	5	202.2	5, 6
8	137.3	6,	131.1	6, 13	188.2	11
9	136.3	1, 7, 14, OH	113.9	5, 17	154.1	1, 5, 14
10	137.3	2, 6, 20	99.3	OH	43.5	2, 11
11	74.5	2	164.9	13, 14	135.0	
12	201.7	14, 15	138.9	13, 14	187.7	14, 15
13	141.1	15, 16, 17	133.7	6	152.8	11, 16, 17
14	135.5	7	27.1	15, 16	133.0	15
15	26.8	14, 16, 17	22.2	14, 16	26.2	14, 16, 17
16	21.5	15, 17	22.2	14, 15	21.1	15
17	21.5	16, 17	23.5	5	21.4	15
18	111.7	3, 19			23.0	5, 19
19	24.5	18			33.5	5, 18
20	19.7	6			20.6	5

[a] Assignments based on BB, DEPT, and HMQC spectra; data are in ppm.

Results and Discussion

Compound **1**, obtained as pale yellow prisms with mp 45 °C, was assigned a molecular formula of $C_{20}H_{24}O_2$ as determined by HREIMS ($[M]^+$ at *m*/*z* 296.1771). The IR spectrum of **1** showed an α,β-unsaturated carbonyl group at 1678 cm^{-1}, which was supported by a ^{13}C NMR signal δ 201.7, as well as a hydroxyl group at 3442 cm^{-1}, corresponding to an oxygenated ^{13}C NMR signal at δ 74.5 (Table 1). The ^1H NMR spectrum (Table 2) showed the presence of an aromatic isopropyl group at δ 1.13 (6H, d, J = 6.6 Hz) and 2.96 (1H, septet, J = 6.6 Hz), two methyl groups attached to a benzene ring at δ 2.03 and 2.23, an exomethylene singlet at δ 4.90, and a hydroxyl signal at δ 2.88 (1H, s, D_2O exchangeable). Aromatic protons were exhibited at δ 6.90 (1H, s), and two doublets were observed in an AB pattern at δ 6.99 (1H, d, J = 7.7 Hz) and 7.11 (1H, d, J = 7.7 Hz). The HMBC correlations for the H-3 methine proton at δ 2.46 with the carbonyl C-12 at δ 201.7, and with the exomethylene carbon C-18 at δ 111.7, clearly indicated a rearranged abietane skeleton.[10]

* To whom correspondence should be addressed. Tel: 0086-21-64311833. Fax: 0086-21-64370269. E-mail: jszhang@mail.shcnc.ac.cn.

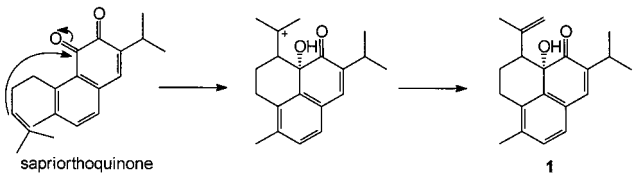
10.1021/np010561j CCC: $22.00
Published on Web 07/04/2002

Table 2. 400 Hz ^{1}H NMR Data for Compounds **1**−**3** ($CDCl_3$)[a]

position	1	2	3
1	2.28 (m), 2.94 (m)		1.31 (m), 2.39 (m)
2	1.82 (m), 2.65 (m)		2.39 (m)
3	2.46 (m)		1.51 (m)
5		7.45 (d), $J_{5,6}$ = 8.2 Hz	2.96 (m)
6	6.99 (d), $J_{6,7}$ = 7.7 Hz	8.01 (d), $J_{5,6}$ = 8.2 Hz	2.38 (m), 2.10 (m)
7	7.11 (d), $J_{6,7}$ = 7.7 Hz		9.50 (s)
11			6.54 (s)
13		7.98 (s)	
14	6.90 (s)	3.51 (m)	6.42 (s)
15	2.96 (m)	1.36 (d), $J_{14,15}$ = 6.6 Hz	2.95 (m)
16	1.13 (d), $J_{15,16}$ = 6.6 Hz	1.36 (d), $J_{14,15}$ = 6.6 Hz	1.12 (d), $J_{15,16}$ = 7.0 Hz
17	1.13 (d), $J_{15,16}$ = 6.6 Hz	2.95 (s)	1.12 (d), $J_{15,16}$ = 7.0 Hz
18	4.90 (s)		0.94 (s)
19	2.23 (s)		0.87 (s)
20	2.03 (s)		1.17 (s)
OH	2.88 (s)	12.2 (s)	

[a] Data are in ppm, with multiplicity of signals given in parentheses: s, singlet; d, doublet; m, multiplet.

Scheme 1



Thus, careful analysis of its HMBC and HMQC spectrum allowed the structural assignment of **1** as 11α-hydroxy-3-isopropenyl-13-isopropyl-5-methyl-1,2,3,11-tetrahydrophenalen-12-one, which has been named saprirearine. Compound **1** possibly may be formed from the rearrangement of sapriorthoquinone[11] as shown in Scheme 1. The same type of rearrangement for aethipinone was proposed by Ulubelen et al. to explain the existence of candidissiol in the roots of *Salvia candidissima*.[10]

Compound **2** was obtained as a pale yellow amorphous powder. Its molecular formula was determined as $C_{16}H_{14}O_4$ by HREIMS. The IR spectrum of **2** exhibited bands attributable to hydroxyl (3435 cm^{-1}), conjugated anhydride (1778, 1711 cm^{-1}), and aromatic groups (1599 cm^{-1}). The ^{1}H NMR data (Table 2) indicated an AB system with *meta*-correlated protons at δ 7.45/8.01 (J = 8.2 Hz) and an aromatic proton singlet at δ 7.98. Additional coupled protons at δ 3.51 (1H) and 1.36 (6H) confirmed an AB_6 system (J = 6.6 Hz) and were in agreement with an isopropyl moiety. Finally, signals were observed for a methyl group at δ 2.95 and a hydroxyl signal at δ 12.20 (due to chelation between a carbonyl and the hydroxyl) that disappeared with D_2O. The presence of this chelated proton led to the conclusion that the anhydride moiety was attached to the *ortho* position to the hydroxy group in the aromatic ring. HMBC correlations (Table 1) for the isopropyl methine proton H-14 at δ 3.51 and the aromatic methine C-13 at δ 133.7, and the aromatic proton H-5 at δ 7.45 with the methyl group C-17 at δ 23.5, indicated that the isopropyl and the methyl groups were located at C-12 and C-4, respectively. Compound **2** (11-hydroxy-12-isopropyl-4-methylbenzo[*de*]isochromene-1,3-dione), which has been named saprionide, is a rare type of new abietane derivative with an anhydride moiety. Chang et al. reported the isolation of similar anhydride-type abietane compounds from *Salvia miltiorrhiza*.[12] Furthermore, Kusumi et al. reported the preparation of an anhydride-type abietane compound with a skeleton similar to **2** by photooxidation of a diterpenequinone.[13] Accordingly, compound **2** might be produced by the same type of bio-oxidation mechanism in the plant.

Compound **3** was isolated as yellow needles. Its molecular formula was deduced as $C_{20}H_{28}O_3$ by HREIMS. The IR spectrum showed absorptions for aldehyde (1716 cm^{-1}), *para*-quinone (1643 and 1627 cm^{-1}), and aromatic (1591 cm^{-1}) functionalities. The ^{1}H NMR spectrum (Table 2) showed two aromatic proton singlets at δ 6.42 and 6.54, an isopropyl moiety with a doublet for two methyls at δ 1.12 (J = 7.0 Hz) and a septet for a proton at δ 2.95, three quaternary methyl groups at δ 0.87, 0.94, and 1.17, and an aldehyde proton at δ 9.50. Its ^{13}C NMR and DEPT spectra (Table 1) exhibited five methyls, four methylenes, and four methine groups, and two saturated carbons together with the signals for a aldehyde carbon and two quinone carbonyl carbons. The HMBC correlations for the C-12 carbonyl at δ 187.7 with the H-15 proton in the isopropyl unit at δ 2.95 and with the H-14 aromatic proton at δ 6.42, and the C-8 carbonyl at δ 188.2 with the H-11 aromatic proton at δ 6.54, suggested that the isopropyl unit should be attached to C-13 and that compound **3** must possess a 7,8-*seco*-abietane skeleton. Interpretation of the 2D NMR spectra (HMQC, HMBC) supported this assignment unambiguously. The stereochemistry at the C-5 position was determined from the ROESY spectrum. Since the methyl group at C-10 could be assigned with a β-configuration for biogenetic reasons and no correlation between this methyl group and the proton at C-5 was observed, H-5 was determined to have an α-configuration. The stereochemistry of **3** was confirmed by chemical transformation. A reference sample, ferruginol,[14] was treated with Fremy's salt and KH_2PO_4 at room temperature for 6 h to give compound **3** in a yield of 83%. The spectral data of the synthetic compound and compound **3** isolated from the plant were identical. Compound **3** is the first compound possessing a 7,8-*seco*-abietane skeleton isolated from a plant in the genus *Salvia* and has been assigned as 7,8-*seco*-*para*-ferruginone. On the basis of the fact that it was synthesized from ferruginol and from a proposal by Gonzalez et al. for the biogenesis of highly oxidized abietatriene diterpenes,[15] this compound may be produced from the radical oxidation of ferruginol in the plant with the participation of singlet-state oxygen appearing to play an important role (Scheme 2). Compound **3** is the first 7,8-*seco*-abietane diterpenoid to have so far been isolated from a plant in the genus *Salvia*.

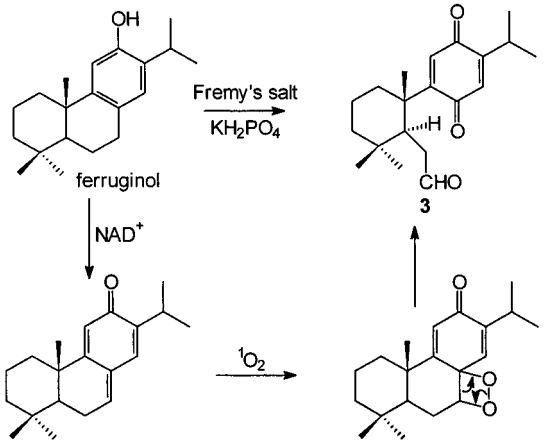
4-Hydroxysaprorthoquinone (**4**) was isolated as red syrup. Its molecular formula was deduced as $C_{20}H_{26}O_3$ by HREIMS ($[M]^+$ at m/z 314.1825). Its IR spectrum showed peaks at 3390 cm^{-1} (hydroxyl) and 1662, 1640, and 1630 cm^{-1} (*ortho*-quinoid). This compound exhibited NMR data

Table 3. NMR Data for Compounds **4**, **4a**, and **5**[a]

	4		4a		5	
position	^{1}H (*J*)	^{13}C	^{1}H (*J*)	1H (*J*)	^{13}C	HMBC
1	2.99 (m)	30.6	2.98 (m)	3.25 (m)	24.9	2, 3, 5, 10
2	1.62 (m)	23.8	1.50 (m)	2.78 (m)	23.2	1, 3,
3	1.62 (m)	44.2	1.95 (m)		213.6	
4		71.1			76.4	
5		148.6			146.2	
6	7.03 (d, 7.5)	140.3	7.03 (d, 7.6)	7.07 (d, 7.6)	134.0	8, 10, 20
7	7.34 (d, 7.5)	136.7	7.34 (d, 7.6)	7.37 (d, 7.6)	137.0	5, 9, 14
8		128.2			128.6	
9		134.9			135.0	
10		140.0			140.0	
11		182.3			182.4	
12		181.4			181.2	
13		144.6			144.9	
14	7.07 (s)	128.1	7.06 (s)	7.07 (s)	128.6	7, 9, 12, 13, 15
15	2.99 (m)	26.9	2.98 (m)	2.99 (m)	26.9	12, 14, 16(17)
16	1.14 (d, 7.0)	21.5	1.14 (d, 7.0)	1.14 (d, 7.0)	21.4	13, 15
17	1.14 (d, 7.0)	21.5	1.14 (d, 7.0)	1.14 (d, 7.0)	21.4	13, 15
18	1.21 (s)	29.2	1.42 (s)	1.40 (s)	26.5	3, 19
19	1.21 (s)	29.2	1.42 (s)	1.40 (s)	26.5	3, 18
20	2.36 (s)	19.8	2.34 (s)	2.35 (s)	19.7	6, 10
OAc			1.93 (s)			

[a] ^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz; run in $CDCl_3$; data in ppm (*J* in Hz). Multiplicity of signals is given in parentheses: s, singlet; d, doublet; m, multiplet.

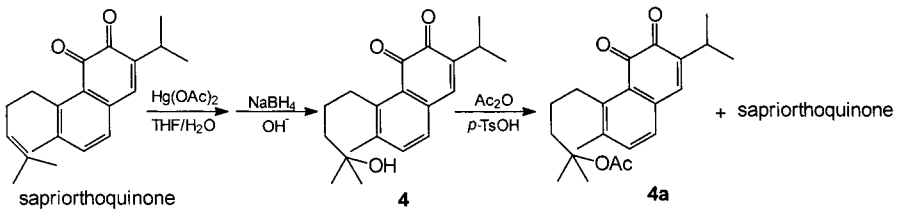
Scheme 2



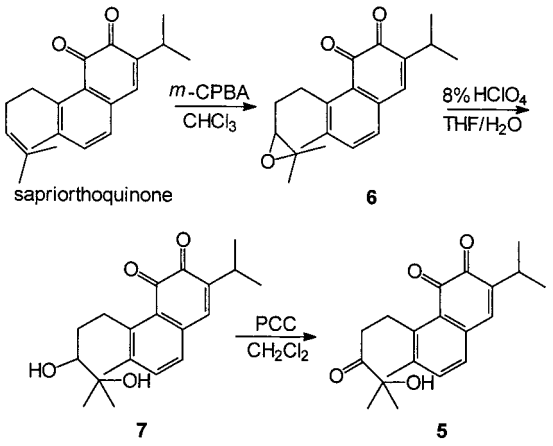
(Table 3) very similar to that of saprorthoquinone.[11] The main differences observed were the missing signals due to an olefinic methine at δ 5.28/123.8 and additional signals due to a methylene at δ 1.62/44.2, which indicated that there was an oxygenated carbon instead of two olefinic carbons in the side chain of this new compound. Its ^{13}C NMR spectrum showed two carbonyl signals, eight aromatic signals, and an oxygenated quaternary carbon. Careful analysis of its NMR spectrum led to the deduction of its structure as 4-hydroxysaprothoquinone (**4**). To support this assignment, compound **4** was synthesized from saprorthoquinone[11] (Scheme 3). All the spectral data of the synthetic compound were identical with those of the isolated compound. Compound **4** was treated with acetic anhydride with *p*-TsOH used as a catalyst to give its acetyl derivative **4a** (Table 3) in 44% yield, along with the dehydration product saprorthoquinone (Scheme 3). This result further proved the structure of compound **4**, which is a 4,5-*seco*-5,10-*friedo*-abietane diterpenoid.

3-Keto-4-hydroxysaprorthoquinone (**5**), a red syrup, was assigned a molecular formula of $C_{20}H_{24}O_4$, as determined by HREIMS. The IR spectrum exhibited frequencies for a hydroxyl at 3487 cm^{-1}, a carbonyl group at 1708 cm^{-1}, and an *ortho*-qunonoid group at 1688, 1658, and 1633 cm^{-1}. The ^{1}H NMR data (Table 3) were also very similar to those of saprirorthoquinone,[11] except for the loss of an olefinic proton, which suggested that both compounds possess the same skeleton but with different side chains. The chemical shifts of the two methyl groups at δ 1.40 as well as the ^{13}C NMR signal at δ 76.4 indicated the presence of a hydroxyl group at C-4. The HMBC spectrum showed correlations for CH_3-4 at δ 1.40 with the carbonyl group at δ 213.6, suggesting that the carbonyl group was placed at C-3. Thus, compound **5** was characterized as 3-keto-4-hydroxy-4,5-*seco*-5,10-*friedo*-abieta-3,5(10),6,8,13-pentaene-11,12-dione and has been given the trivial name 3-keto-4-hydroxysaprorthoquinone. Earlier, Ulubelen et al. reported the compound sclareapinone, isolated from *Salvia sclarea*, with the same structure as **5** based on interpretation of its 1D ^{1}H and ^{13}C NMR spectra.[16] However, on comparing the spectral data of both compounds, we found some differences. The isolate obtained in the present study was assigned with the structure **5** not only from the HMBC spectrum but also by chemical transformation. Compound **5** was synthesized from the known compound saprirorthoquinone[11] in three steps (Scheme 4) to confirm its structure. According to the structure of the starting material, two hydroxyl groups in the intermediate **6** could be formed only

Scheme 3



Scheme 4



at C-3 and C-4, respectively. Furthermore, only the hydroxyl group at C-3 could be oxidized to a carbonyl group. Like compound **4**, **5** is also a 4,5-*seco*-5,10-*friedo*-abietane diterpenoid.

The bioactivities of compounds **1**−**5** have been tested. Compound **3** displayed antimicrobial activity against the Gram-positive organisms, *Staphylococcus aureus* and *Micrococcus luteus*, when tested according to established protocols[17] (MIC values of 20.0 and 15.0 μM, respectively). Compound **4** showed a significant inhibitory effect (IC_{50} 0.8 μM) on topoisomerase I according to a method developed by Isabella et al.[18] Compound **5** was evaluated for its cytotoxic activities against HL-60 human leukemia cells and the SGC-7901 and MKN-28 stomach cancer cell lines by a microculture tetrazolium colorimetric assay (MTT).[19] This substance exhibited potent activities against the three cancer cell lines with IC_{50} values of 4.6, 0.2, and 0.3 μM, respectively. Compounds **1** and **2** were not found to be active in any of these three assays.

Experimental Section

General Experimental Procedures. The melting points were determined on a Kofler hot-stage apparatus and are uncorrected. UV spectra were measured on a Beckman DU-600 spectrophotometer. IR spectra were measured on a Nicolet Magna 750 spectrophotometer. ^{1}H, ^{13}C, HMBC, and ROESY NMR spectra were recorded on a Bruker AM-400 (^{1}H) or a Bruker AC-100 (^{13}C) spectrometer with TMS as internal standard and $CDCl_3$ as solvent. Mass spectra were obtained on a MAT 711 mass spectrometer. Silica gel (200−300 mesh) was used for column chromatography and silica gel GF_{254} for preparative TLC.

Plant Material. *Salvia prionitis* was collected at Yujiang, Jiangxi Province, People's Republic of China, in July 1995, and authenticated by Prof. X. L. Huang of our institute. A voucher specimen is maintained in the Herbarium of Shanghai Institute of Materia Medica (SIMMP 95068).

Extraction and Isolation. Dried roots of *S. prionitis* (5 kg) were extracted with EtOAc for 6 days at room temperature. The solvent was removed in vacuo to yield 350 g of a gummy residue. The extract was subjected to repeated chromatography over silica gel eluted with mixture of cyclohexane−EtOAc of increasing polarity (100:1 to 5:1). The fraction that eluted with cyclohexance−EtOAc (30:1) was further chromatographed using the same adsorbent and solvent system as presented above to give 90 mg of compound **1** together with a mixture containing compounds **2** and **3**. This mixture was purified by preparative TLC using cyclohexane−EtOAc (10:1) to afford compound **2** (R_f = 0.4, 10 mg) and compound **3** (R_f = 0.3, 15 mg). The fraction that eluted with cyclohexane−EtOAc (5:1) was further purified by preparative TLC using cyclohexane−EtOAc (4:1) to afford compound **4** (R_f = 0.7, 60 mg) and compound **5** (R_f = 0.3, 15 mg).

Compound 1: pale yellow prisms, mp 45 °C; UV (MeOH) λ_{max} (log ϵ) 243 (4.22), 335 (3.85) nm; IR (KBr) ν_{max} 3442, 2958, 1678, 1633, 1458, 1377, 868 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 296 [M]$^+$ (40), 268 (56), 253 (32), 228 (38), 225 (100), 185 (18); HREIMS *m*/*z* 296.1771 (calcd for $C_{20}H_{24}O_2$, 296.1776).

Compound 2: pale yellow powder; UV ($CHCl_3$) λ_{max} (log ϵ) 333 (3.17), 358 (3.14), 374 (3.43) nm; IR (KBr) ν_{max} 3435, 2953, 1778, 1711, 1648, 1599, 1506, 1159, 1095, 959 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 270 [M]$^+$ (84), 255 (100), 242 (31), 237 (20), 224 (6), 209 (10), 185 (17), 153 (16), 149 (30); HREIMS *m*/*z* 270.0905 (calcd for $C_{16}H_{14}O_4$, 270.0892).

Compound 3: yellow needles; mp 151−152 °C; UV (MeOH) λ_{max} (log ϵ) 260 (4.07) nm; IR (KBr) ν_{max} 2964, 1716, 1643, 1627, 1591, 1236, cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 316 [M]$^+$ (23), 301 (6), 298 (8), 283 (7), 272 (100), 257 (24), 229 (12), 216 (20), 203 (40); HREIMS *m*/*z* 316.2029 (calcd for $C_{20}H_{28}O_3$, 316.2038).

Oxidation of Ferruginol to Compound 3. Fremy's salt (0.7 g) and KH_2PO_4 (2.12 g, 15.6 mmol) were dissolved in water (60 mL). To this solution was added ferruginol[14] (0.26 g, 0.91 mmol) in 40 mL of acetone. The mixture was stirred in the dark under N_2 overnight. On concentration in vacuo, the mixture was extracted with ether three times. The organic phase was washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography on a silica gel column (20 g) eluted with cyclohexane−EtOAc (20:1) to give compound **3** (0.64 g, 83% yield).

Compound 4: red syrup, UV (MeOH) λ_{max} (log ϵ) 211 (4.48), 249 (4.47), 342 (3.57), 436 (3.25) nm; IR (film) ν_{max} 3390, 2930, 1662, 1640, 1508, 1457, 1251, 1035 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; EIMS *m*/*z* 314 [M]$^+$ (3), 312 (28), 286 (5), 268 (7), 253 (3), 243 (100), 227 (10); HREIMS *m*/*z* 314.1825 (calcd for $C_{20}H_{26}O_3$, 314.1882).

Acetylation of 4. A portion (20 mg, 0.064 mmol) of compound **4** was dissolved in 2 mL of Ac_2O, and a trace amount of *p*-TsOH was added. The mixture was stirred at room temperature with protection from light for 2 h. After the usual workup, the reaction mixture was purified by preparative TLC (cyclohexane−EtOAc, 10:1; R_f = 0.4) to afford 10 mg of **4a**. Compound **4a**: red syrup, UV (MeOH) λ_{max} (log ϵ) 213 (3.81), 220 (3.83), 247 (3.87), 407 (2.65), 443 (2.60) nm; IR (film) ν_{max} 2860, 1732, 1662, 1433, 1367, 1256, 1175, 1020, 756 cm^{-1}; ^{1}H NMR data, see Table 3; EIMS *m*/*z* 356 [M]$^+$ (4), 298 (12), 296 (36), 268 (36), 227 (100), 141 (14); HREIMS *m*/*z* 356.1993 (calcd for $C_{22}H_{28}O_4$, 356.1988).

Synthesis of 4 from Sapriorthoquinone. $Hg(OAc)_2$ (190 mg, 0.50 mmol) was placed in a 50 mL round-bottomed flask, and 5 mL of water together with 10 mL of THF were added, followed by 150 mg of sapriorthoquinone[11] (0.50 mmol). The mixture was stirred at room temperature for 30 min. Then, 0.5 mL of a 3 M NaOH solution and 0.5 mL of 0.5 M $NaBH_4$ in a 3 M NaOH solution were added, and the reaction was stopped immediately. After the usual workup, the crude product was chromatographed by silica gel column chromatography (cyclohexane−EtOAc, 7:1) to furnish 40 mg of compound **4** (yield 20%).

Compound 5: red syrup; UV (MeOH) λ_{max} (log ϵ) 353 (3.21), 435 (3.41) nm; IR (KBr) ν_{max} 3487, 2966, 2936, 2872, 1709, 1689, 1659, 1633, 1568, 1464, 1254 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; EIMS *m*/*z* 328 [M]$^+$ (2), 316 (8), 286 (2), 270 (100), 242 (50), 228 (90), 224 (64), 200 (47); HREIMS *m*/*z* 328.1654 (calcd for $C_{20}H_{24}O_4$ 328.1675).

Synthesis of Compound 5. To a stirred solution of *m*-chloroperbenzoic acid (43 mg, 0.025 mmol) in $CHCl_3$ (10 mL) held at 0 °C was added a solution of sapriorthoquinone[11] (60 mg, 0.02 mmol) dissolved in 6 mL of $CHCl_3$. The mixture was stirred overnight at room temperature, washed with 10% $NaHCO_3$ solution, and dried over anhydrous $MgSO_4$. The solvent was concentrated in vacuo, and the residue was purified by silica gel column chromatography by elution with cyclohexane−EtOAc (9:1) to afford epoxide **6** (50 mg, 79%

yield). Then, 50 mg (0.16 mmol) of **6** were dissolved in 12 mL of tetrahydrofuran. After the addition of 2 mL of water, followed by 0.4 mL of 8% perchloric acid, the mixture was stirred under N_2 at room temperature for 6 h. Then, 30 mL of brine were added and the solution was extracted several times with ether. The organic phase was washed with dilute $NaHCO_3$ and brine and dried ($MgSO_4$). On evaporation, the residue was purified by preparative TLC (cyclohexane−EtOAc 1:2) to give red crystals of compound **7** (R_f = 0.6, 34 mg, 65% yield). A portion (34 mg, 0.1 mmol) of **7** was further treated with PCC (110 mg, 0.51 mmol) in 10 mL of CH_2Cl_2. The solution was stirred at room temperature for 30 min. Then, the mixture was filtered and the filtrate dried over $MgSO_4$ and evaporated. The residue was purified by preparative TLC (cyclohexane−EtOAc 4:1) to furnish compound **5** (R_f = 0.3, 20 mg, 30% yield from saprirthoquinone).

Bioassays. Antimicrobial Assay. The microbial strains were from the American Type Culture Collection, and the antimicrobial susceptibility tests were carried out by a microdilution assay.[17] The microbial cells were suspended in Mueller-Hinton broth to form a final density of 5×10^{-5}−10^{-6} CFU/mL and incubated at 37 °C for 18 h under aerobic conditions with the respective compounds, which were dissolved in DMSO. The blank controls of microbial culture were incubated with a limited amount of DMSO under the same conditions. DMSO was determined not to be toxic under these experimental conditions.

Topoisomerase I Inhibition Assay. The topoisomerase I inhibition assay was performed using the topoisomerase I extracted from Ehrlich carcinoma cells according to a method developed by Isabella et al.[18] The activity was measured by topoisomerase I mediated supercoiled pBR322 relaxation.

Cytotoxicity Assay. Cytotoxic activities were carried out against HL-60 human leukemia cells and two stomach cancer cell lines (SGC-7901 and MKN-28). The three cell lines were exposed to the compounds for 48 h. The cytotoxic activities were measured by a microculture tetrazolium colorimetric assay (MTT).[19]

Acknowledgment. This work was supported by a grant from the National Natural Science Foundation of China (Project No. 39830440). The authors thank the National Center for Drug Screening of China for the antimicrobial assays, and the Division of Antitumor Pharmacology, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, for the topoisomerase I inhibition and cytotoxicity assays.

References and Notes

(1) Luo, X. R., Ed. *Colored Icons of Chinese Medicine*; Guangdong Science and Technology Press: Guangdong, 1994; Vol. 3.
(2) Zhang, J. S.; Huang, Y. *Nat. Prod. Res. Dev.* **1995**, *7*, 1−4.
(3) Huang, X. L.; Wang, X. M.; Huang, Y.; Zhang, J. S.; Lin L. Z. *Acta Bot. Sin.* **1990**, *32*, 490−491.
(4) Lin, L. Z.; Wang, X. M.; Huang, X. L.; Huang, Y. *Acta Pharm. Sin.* **1990**, *25*, 154−156.
(5) Lin, L. Z.; Wang, X. M.; Huang, X. L.; Yang, B. J. *Planta Med.* **1988**, *54*, 443−444.
(6) Lin, L. Z.; Blasko, G.; Cordell, G. A. *Phytochemistry* **1989**, *28*, 177−181.
(7) Lin, L. Z.; Cordell, G. A.; Lin, P. *Phytochemistry* **1995**, *40*, 1469−1471.
(8) Li, M.; Zhang, J. S.; Ye, Y. M.; Fang, J. N. *J. Nat. Prod.* **2000**, *63*, 139−40.
(9) Li, M.; Zhang, J. S.; Chen, M. Q. *J. Nat. Prod.* **2001**, *64*, 971−972.
(10) Ulubelen, A.; Topcu, G.; Tan, N. *Tetrahedron Lett.* **1992**, *33*, 7241−7244.
(11) Yang, B. J.; Huang, X. L.; Huang, Y.; Wang, X. M.; Lin, L. Z.; But, P. H.; Zhuang, G. F. *Acta Bot. Sin.* **1988**, *30*, 524−527.
(12) Chang, H. M.; Choang, T. F.; Chui, K. Y.; Hon, P. M.; Lee, C. M.; Mak, T. C. W.; Wong, H. N. C. *J. Chem. Res. (S)* **1990**, 114−115.
(13) Kusumi, T.; Kishi, T.; Kakisawa, H.; Kinoshita, T. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1716−1718.
(14) Mayer, W. L.; Clemans, G. B.; Manning, R. A. *J. Org. Chem.* **1975**, *46*, 3686−3694.
(15) Gonzalez, A. G.; Andes, L. S.; Aguiar, Z. E.; Luis, J. G. *Phytochemistry* **1992**, *31*, 1297−1305.
(16) Ulubelen, A.; Sonmez, U.; Topcu, G. *Phytochemistry* **1997**, *44*, 1297−1299.
(17) Demetzos, C.; Stahl, B.; Anastassaki, M.; Tzouveleki, L.; Rallis, M. *Planta Med.* **1999**, *65*, 76−80.
(18) Isabella, D. P.; Capranico, G.; Binashi, M.; Tinelli, S.; Zunino, F. *Mol. Pharmacol.* **1990**, *37*, 11−16.
(19) Mossman, T. *J. Immunol. Methods* **1983**, *65*, 55−63.

NP010561J