Virgaurols A–D: Novel Asymmetric Eremophilane Dimers from the Roots of *Ligularia virgaurea*

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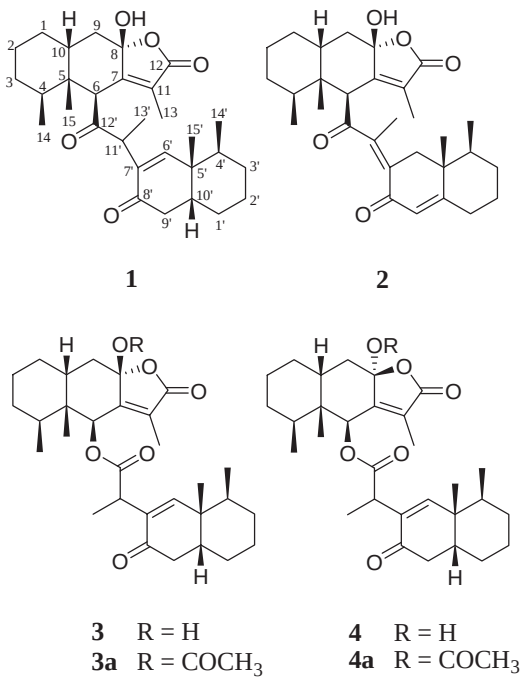
Virgaurols A–D (**1**–**4**), four novel asymmetric eremophilane sesquiterpene dimers were isolated from the roots of *Ligularia virgaurea*. Especially, virgaurols A (**1**) and B (**2**) possessed novel carbon skeleton in which the two sesquiterpene units are uniquely connected by a single C–C bond directly. The structures of these dimers were determined on the basis of comprehensive spectral analysis. Compounds **1** and **3** were found to exhibit weak cytotoxicity against human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cells.

The *Ligularia* species, which have been taxonomically placed in the Compositae, are important medicinal plants, and are receiving phytochemical attention due to the biological and chemical diversities. More than 27 species have long been used as Chinese folk remedies due to their antibiotic, antiphlogistic and antitumor activities.[1] In the *Ligularia* species, sesquiterpenes are found to be the principal secondary metabolites. However, dimeric ones are infrequent, and only several have been isolated over the last twenty years.[2] *Ligularia virgaurea* (Maxim.) Mattf. is widely distributed in northwestern China and is used as a traditional folk medicine for the treatment of stomachache and nausea.[3] In previous studies, some benzofuranosesquiterpenes and eremophilanes have been reported.[2e,2f,4] In our ongoing research for novel bioactive components from *Ligularia* species and the influence of different ecological environments on the chemical constituents of plants, we chose *L. virgaurea* collected in Gannan Tibetan Autonomous Region (S.A. 2200–3800 m), Gansu Province, People's Republic of China.

In this report, we described the isolation and structure elucidation of four novel eremophilane sesquiterpene dimers, named virgaurols A (**1**), B (**2**), C (**3**), and D (**4**) (Figure 1) which are an unusual class of structures containing two asymmetric units, from the roots of *L. virgaurea*. Compounds **1** and **3** exhibited weak cytotoxicity against human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cells.



1 **2**

3 R = H **4** R = H
3a R = $COCH_3$ **4a** R = $COCH_3$

Figure 1. Structures of compounds **1**–**4** isolated from *Ligularia virgaurea*.

Results and Discussion

Compound **1** was obtained as a colorless gum. The HR-ESI-MS (m/z 505.2929 $[M + Na]^+$, calcd for $C_{30}H_{42}O_5Na$: 505.2924) established the molecular formula of **1** as being $C_{30}H_{42}O_5$. Furthermore, the molecular ion peak was observed at m/z 482 $[M]^+$, and significant ion fragments at m/z 249 $[C_{15}H_{21}O_3]^+$, 231 $[C_{15}H_{21}O_3 - H_2O]^+$, and 233 $[C_{15}H_{21}O_2]^+$ in its EIMS, indicating the occurrence of two C_{15} units in **1**. The IR spectrum showed absorption bands characteristic of hydroxy (3423 cm^{-1}), carbonyl (1760 and 1727 cm^{-1}), and double bond (1661 cm^{-1}) groups. The ^1H NMR spectrum of compound **1** (Table 1) showed the presence of three tertiary methyls at δ_H 1.89 (3H, s, H_3-13), 1.06 (3H, s, H_3-15), and 1.12 (3H, s, H_3-15′), three secondary methyls at δ_H 0.85 (3H, d, $J = 6.4$ Hz, H_3-14), 1.28 (3H, d, $J = 7.2$ Hz, H_3-13′), and 0.89 (3H, d, $J = 7.2$ Hz, H_3-14′), an olefinic proton at δ_H 6.66 (1H, s, H-6′), and a hydroxy proton at δ_H 5.58 (1H, s, OH-8), as well as other complicated signals in the upfield region belonging to other methylenes and methines. The ^{13}C NMR spectral data (Table 2) were in good agreement with the above analysis, and exhibited 30 carbon signals ($6 \times CH_3, 8 \times CH_2, 7 \times CH, 9 \times C$), of which the signals in the upfield region appeared in duplicate or twice. The ^1H and ^{13}C NMR spectral data, in combination with the molecular

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Table 1. The ^{1}H NMR Spectral Data of Compounds **1**–**4a**[a)]

No.	**1**	**2**	**3**	**3a**	**4a**
1	1.71 m, 1.38 m	1.80 m, 1.34 m	1.72 m, 1.25 m	1.76 m, 1.38 m	1.73 m, 1.48 m
2	1.46 m	1.62 m	1.47 m	1.60 m	1.56 m
3	1.42 m	1.49 m	1.43 m	1.56 m	1.33 m
4	1.45 m	1.44 m	1.44 m	1.48 m	1.83 m
6	4.30 s	4.17 s	5.49 s	5.91 s	6.19 q (1.4)
9	2.07 dd (12.8, 4.8), 2.03 dd (12.8, 8.0)	2.16 dd (13.6, 4.4), 2.06 brd (13.6)	2.32 brd (13.6), 2.06 brd (13.6)	2.28 brd (14.0), 2.08 brd (14.0)	2.74 brd (12.8), 1.89 brd (12.8)
10	2.56 m	2.68 m	2.18 m	2.18 m	1.92 m
13	1.89 s	1.72 s	1.93 s	1.96 s	1.94 d (1.4)
14	0.85 d (6.4)	0.76 d (6.4)	0.81 d (6.0)	0.85 d (6.0)	1.00 d (7.2)
15	1.06 s	1.15 s	1.07 s	1.06 s	0.85 s
1′	1.76 m, 1.34 m	2.38 m, 1.56 m	1.77 m, 1.25 m	1.82 m, 1.38 m	1.76 m, 1.35 m
2′	1.43 m	1.91 m	1.43 m	1.56 m	1.52 m
3′	1.41 m	1.46 m	1.42 m	1.53 m	1.43 m
4′	1.73 m	1.58 m	1.74 m	1.79 m	1.80 m
6′	6.66 s	2.79 d (14.8), 2.29 d (14.8)	6.68 s	6.72 s	6.74 s
9′	2.64 dd (17.6, 12.0), 2.27 dd (17.6, 4.8)	5.78 s	2.63 dd (17.2, 12.4), 2.24 dd (17.2, 4.0)	2.67 dd (16.8, 12.4), 2.24 dd (16.8, 4.0)	2.69 dd (17.6, 12.4), 2.29 dd (17.6, 4.4)
10′	2.01 m		2.13 m	2.15 m	2.06 m
11′	3.80 q (7.2)		3.33 q (6.8)	3.52 q (6.8)	3.76 q (7.2)
13′	1.28 d (7.2)	1.99 s	1.31 d (6.8)	1.31 d (6.8)	1.39 d (7.2)
14′	0.89 d (7.2)	0.99 d (6.4)	0.92 d (6.8)	0.92 d (6.8)	0.93 d (7.2)
15′	1.12 s	1.07 s	1.17 s	1.11 s	1.14 s
OH	5.58 s	6.36 s	5.64 s		
$COCH_3$				2.01 s	2.06 s

a) ^{1}H NMR data are measured at 400 MHz in $CDCl_3$, and values are reported in parts per million relative to TMS. Proton coupling constants (J) in Hz are given in parentheses.

composition, strongly showed compound **1** to be a dimeric sesquiterpene derivative. The structure of **1** was finally determined by the extensive study of 2D NMR experiments (especially ^{1}H–^{1}H COSY and gHMBC). From the ^{1}H–^{1}H COSY spectrum of **1**, it was possible to establish the proton sequences between H-1/H-2; H-3/H-4; H-4/H_3-14; and H-9/H-10. From the gHMBC spectrum (Figure 2), the long-range correlations between the following protons and carbons were observed: H_3-13/C-7, C-11, C-12; H_3-15/C-4, C-5, C-6, C-10; H_3-14/C-3, C-4, C-5; H-6/C-4, C-5, C-7, C-8, C-10, C-11, C-15; H_2-9/C-5, C-7, C-8, C-10; and hydroxy proton/C-8. Based on these data, the connectivity from C-1 to C-15 could be established. In association with characteristic carbon chemical shifts at δ_C 171.1 (ester carbonyl, C-12), 103.9 (acetal, C-8), 154.1 and 126.8 (olefinic, C-7 and C-11), the partial structure of unit I was determined as 8-hydroxyeremophil-7(11)-en-8,12-olide skeleton (Figure 3). In addition, the presence of the other unit II in **1** was revealed by analysis of ^{1}H–^{1}H COSY and gHMBC spectra. The proton sequences (H-1′/H-2′; H-3′/H-4′; H-4′/H_3-14′; H-9′/H-10′; and H-11′/H_3-13′) in the ^{1}H–^{1}H COSY, and the H/C correlations in the gHMBC spectrum (H_3-13′/C-7′, C-11′, C-12′; H_3-15′/C-4′, C-5′, C-6′, C-10′; H_3-14′/C-3′, C-4′, C-5′; H-6′/C-4′, C-5′, C-7′, C-8′, C-10′, C-11′, C-15′; H_2-9′/C-1′, C-5′, C-8′, C-10′; and H-11′/C-6′, C-7′, C-8′, C-12′, C-13′), together with typical carbon chemical shifts at δ_C 200.9 and 208.0 (ketone carbonyl, C-8′ and C-12′), and 160.1 and 135.1 (olefinic, C-6′ and C-7′), established the second unit II as eremophil-6′-en-8′,12′-dione (Figure 3). Finally, the connected position of the two units was characterized from the key gHMBC correlation between H-6/C-12′. Therefore, the units I and II were uniquely connected from C-6 to C-12′ by a single C–C bond directly.

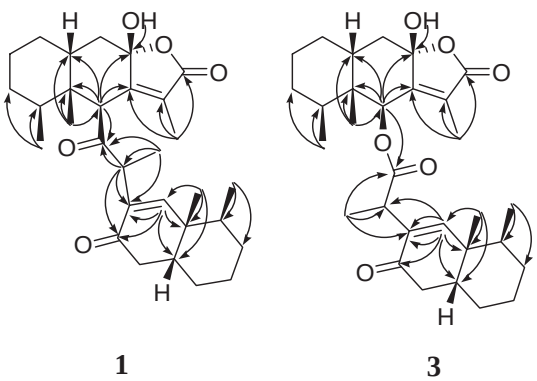
Stereochemically, in the biogenetic consideration of eremophilane derivatives isolated from Compositae species, the methyls at C-4 (or C-4′) and C-5 (or C-5′) were both assigned as β-orientation.[5] In differential NOE experiments, the enhancement between H_3-15 (or H_3-15′) and H_3-14 (or H_3-14′) suggested β-oriented H_3-14 (or H_3-14′); the enhancement between H_3-15 (or H_3-15′) and H-10 (or H-10′) suggested a cis fused A/B (A′/B′) ring system. In the ^{1}H NMR spectrum, the chemical shift of H_3-15 at δ_H 1.06 (3H, s) was downfield compared with H_3-14 at δ_H 0.85 (3H, d, $J = 6.4$ Hz), indicating that OH-8 was β-oriented, which agrees with the empirical rules reported by Naya et al.[6] In addition, the absence of homoallylic coupling between H-6 and H_3-13 indicates that H-6 is α-oriented.[7] Thus, we conclude that **1** is a novel asymmetric eremophilane sesquiterpene dimer, and named virgaurol A.

The molecular formula of compound **2** was determined to be $C_{30}H_{40}O_5$ by its HR-ESI-MS (m/z 481.2950 $[M + H]^+$, calcd for $C_{30}H_{41}O_5$: 481.2949), indicating 2 mass units less than compound **1**. Most of the ^{1}H and ^{13}C signals of **2** (Tables 1 and 2) strikingly matched those of **1** and revealed a dimeric sesquiterpene skeleton. Further analysis of its 2D spectra indicated that the unit I was identical with compound **1** and the significant differences were in unit II. The ^{1}H–^{1}H COSY spectrum indicated proton sequences between H-

Table 2. The ^{13}C NMR (DEPT) Spectral Data of Compounds **1**–**4a**[a)]

No.	1	2	3	3a	4a
1	26.7 (t)	25.7 (t)	26.7 (t)	26.9 (t)	28.1 (t)
2	20.3 (t)	19.7 (t)	20.3 (t)	20.4 (t)	20.4 (t)
3	30.4 (t)	30.5 (t)	30.1 (t)	30.2 (t)	28.7 (t)
4	30.2 (d)	30.0 (d)	29.0 (d)	29.2 (d)	32.0 (d)
5	42.4 (s)	43.1 (s)	41.8 (s)	42.1 (s)	44.6 (s)
6	54.1 (d)	54.7 (d)	72.2 (d)	70.7 (d)	71.7 (d)
7	154.1 (s)	154.2 (s)	151.8 (s)	150.0 (s)	154.6 (s)
8	103.9 (s)	104.3 (s)	104.3 (s)	103.7 (s)	104.8 (s)
9	38.8 (t)	39.3 (t)	38.7 (t)	38.2 (t)	36.4 (t)
10	35.4 (d)	36.1 (d)	35.2 (d)	34.8 (d)	36.2 (d)
11	126.8 (s)	126.6 (s)	128.6 (s)	129.3 (s)	126.0 (s)
12	171.1 (s)	171.2 (s)	171.2 (s)	170.5 (s)	170.8 (s)
13	8.9 (q)	9.0 (q)	8.7 (q)	8.8 (q)	9.0 (q)
14	16.3 (q)	16.3 (q)	16.4 (q)	16.4 (q)	15.6 (q)
15	17.3 (q)	17.6 (q)	16.1 (q)	16.1 (q)	19.4 (q)
1′	25.6 (t)	33.1 (t)	25.5 (t)	25.4 (t)	26.9 (t)
2′	19.6 (t)	26.3 (t)	19.9 (t)	19.7 (t)	20.0 (t)
3′	30.0 (t)	30.3 (t)	30.4 (t)	30.4 (t)	30.1 (t)
4′	36.2 (d)	42.6 (d)	35.8 (d)	35.8 (d)	35.9 (d)
5′	39.3[b)] (s)	41.6 (s)	39.0[b)] (s)	38.9 (s)	39.0 (s)
6′	160.1 (d)	39.8 (t)	157.8 (d)	157.5 (d)	157.0 (d)
7′	135.1 (s)	130.7 (s)	135.6 (s)	135.8 (s)	135.7 (s)
8′	200.9 (s)	187.5 (s)	201.1 (s)	198.3 (s)	198.4 (s)
9′	39.2 (t)	124.5 (d)	39.0[b)] (t)	39.4 (t)	39.2 (t)
10′	39.3[b)] (d)	173.7 (s)	39.2 (d)	39.3 (d)	39.5 (d)
11′	45.4 (d)	146.6 (s)	39.1 (d)	39.0 (d)	38.4 (d)
12′	208.0 (s)	204.8 (s)	174.5 (s)	173.3 (s)	173.3 (s)
13′	14.7 (q)	16.8 (q)	14.5 (q)	17.8 (q)	16.6 (q)
14′	15.8 (q)	15.5 (q)	15.9 (q)	16.0 (q)	15.9 (q)
15′	20.6 (q)	18.0 (q)	20.3 (q)	20.3 (q)	20.5 (q)
$COCH_3$				21.8 (q)	22.1 (q)
				168.5 (s)	168.1 (s)

a) ^{13}C NMR data are measured at 100 MHz in $CDCl_3$, and values are reported in parts per million relative to TMS. b) Overlapping signal.



1 **3**

Figure 2. The selected gHMBC correlations (from H to C) of compounds **1** and **3**.

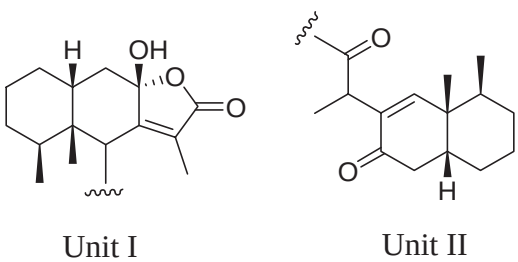


Figure 3. The partial structures of compound **1**.

1′/H-2′; H-3′/H-4′; and H-4′/H_3-14′. The gHMBC spectrum indicated long-range correlations between H_3-13′/C-7′, C-11′, C-12′; H_3-15′/C-4′, C-5′, C-6′, C-10′; H_3-14′/C-3′, C-4′, C-5′; H-9′/C-1′, C-5′, C-7′; and H_2-6′/C-4′, C-5′, C-7′, C-8′, C-10′, C-11′, C-15′; Based on these correlations, together with representative carbon chemical shifts at δ_C 187.5 and 204.8 (ketone carbonyl, C-8′ and C-12′), 130.7 and 146.6 (olefinic, C-7′ and C-11′), and 124.5 and 173.7 (olefinic, C-9′ and C-10′), the connectivity from C-1′ to C-15′ could be established, as eremophil-7′(11′),9′-dien-8′,12′-dione. As compound **1**, the connected position of the two units was from C-6 to C-12′ by a single C–C bond, which was confirmed by the key gHMBC correlation between H-6 and C-12′. In the differential NOE experiments, the enhancement between H_3-13′ and H-6′ suggested the double bond at C-7′ and C-11′ was Z. We named compound **2** as virgaurol B.

The HR-ESI-MS of compound **3** (m/z 516.3310 $[M + NH_4]^+$, calcd for $C_{30}H_{46}O_6N$: 516.3320) gave a molecular formula of $C_{30}H_{42}O_6$, having an extra oxygen atom com-

pared to **1**. The NMR spectral data (Tables 1 and 2) of compound **3** revealed it was also an eremophilane dimer, and the partial structures of two units were very similar to those of **1**. The same ^{1}H–^{1}H COSY and gHMBC key correlations as those of **1** were observed for compound **3**. However, in compound **3**, analysis of its ^{1}H and ^{13}C NMR spectral data indicated that: the chemical shift of H-6 was downfield 1.19 ppm (from δ_H 4.30 to 5.49); H-11′ was upfield 0.47 ppm (from δ_H 3.80 to 3.33); C-6 was downfield 18.1 ppm (from δ_C 54.1 to 72.2); C-11′ was upfield 6.3 ppm (from δ_C 45.4 to 39.1); and C-12′ was upfield 33.5 ppm (from δ_C 208.0 to 174.5). These observations indicated that the connected center between the two units was different from that of compound **1**: an ester carbonyl (δ_C 174.5, C-12′) in **3** instead of the ketone carbonyl (δ_C 208.0) in **1**. The remaining signals were very similar to those of **1**. Thus, in **3**, the units I and II are connected from C-6 to C-12′ by an oxygen atom. We named compound **3** as virgaurol C.

The 8-acetyl derivative **3a** was obtained by treatment with a mixture of Ac_2O/pyridine (1:1, 0.5 mL each) at room temperature (see experimental).

Compound **4** was obtained in the form of its acetate **4a**. For compound **4a**, its HR-ESI-MS showed an $[M + NH_4]^+$ peak at m/z 558.3431 (calcd for 558.3425), indicating a molecular formula of $C_{32}H_{44}O_7$, the same as **3a**. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) were similar to those of **3a**, except for the relative proton chemical shift of H_3-15 and H_3-14 was reversed: the H_3-15 at δ_H 0.85 (3H, s) was upfield compared with H_3-14 at δ_H 1.00 (3H, d, $J = 7.2$ Hz) in **4a**, indicating the lactone was β-oriented,[6] which was the only difference from **3a**. The presence of homoallylic coupling ($J = 1.4$ Hz) between H-6 and H_3-13 further confirmed the above conclusion.[7] We named **4** as virgaurol D.

The cytotoxicity of compounds **1** and **3** against human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cells, was studied. The results showed that compounds **1** and **3** possessed weak cytotoxicity against HL-60 (IC_{50} 21.9 and 17.9 μg mL^{-1}), SMMC-7721 (IC_{50} 47.6 and 46.1 μg mL^{-1}), and HeLa cells (IC_{50} 50.8 and 55.4 μg mL^{-1}), respectively.

Experimental

General Experimental Procedures. The IR spectra were taken on a Nicolet NEXUS 670 FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. NMR spectra were recorded on a Varian Mercury plus-400 NMR spectrometer with TMS as internal standard. HR-ESI-MS data were measured on a Bruker Daltonics APEX II 47e spectrometer. EIMS data were obtained on an HP5988 GC/MS spectrometer. Silica gel (200–300 mesh) used for column chromatography, silica gel GF_{254} (10–40 μm) plates used for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, P. R. China, and reversed phase pre-coated TLC plates RP-18 F_{254}s (size 20 × 20 cm, Schichtdicke 0.25 mm) were supplied by E. Merck Factory, Germany. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v).

Plant Material. The roots of *Ligularia virgaurea* (Maxim.) Mattf. were collected in Gannan Tibetan Autonomous Region (S.A. 2200–3800 m), Gansu Province, People's Republic of China, in August 2005, and identified by Prof. Guo-Liang Zhang, School of Life Sciences, Lanzhou University. A voucher specimen (No. 20050801) was deposited at the College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation. The powdered air-dried roots (3.8 kg) of *L. virgaurea* were extracted successively with a mixed solvent of petroleum ether/Et_2O/methanol (1:1:1) three times at room temperature. The solvent was evaporated under reduced pressure to give a residue (256 g). This residue was subjected to silica gel column chromatography using petroleum ether (60–90 °C) mixed with gradually increasing amounts of acetone (30:1, 15:1, 8:1, 5:1, 3:1, 1:1, and 0:1). Then seven crude fractions (A–G) were obtained according to the differences in the composition indicated by TLC analysis. Fraction C (50 g) was subjected to a second silica gel column chromatography eluting with $CHCl_3$/EtOAc (80:1, 50:1, 30:1, and 10:1). Fraction C-4 (18.5 g) was chromatographed on a silica gel column eluting with petroleum ether/acetone (10:1, 7:1, 5:1, and 3:1). Subfraction C-4–8 (0.56 g), after being chromatographed with petroleum ether/EtOAc (5:1 and 3:1) and then preparative TLC (silica gel GF_{254}, petroleum ether/EtOAc, 3:1), yielded compound **1** (10 mg, R_f 0.394). Subfraction C-4–9 (3.6 g), after being chromatographed with $CHCl_3$/acetone (50:1, 30:1, and 10:1) and preparative TLC (silica gel GF_{254}, petroleum ether/EtOAc, 9:2), yielded compound **3** (5 mg, R_f 0.188), and mixture of **3** and **4** (8 mg). The crude mixture of **3** and **4**, which cannot be separated after further isolated through CC, was treated by acetylation and then purified by preparative TLC (silica gel GF_{254}, petroleum ether/EtOAc, 9:2) to give **3a** (3 mg, R_f 0.377) and **4a** (4 mg, R_f 0.261). Fraction D (6.65 g) was chromatographed on a silica gel column with petroleum ether/EtOAc (4:1, 3:1, and 2:1). Subfraction D-8 (65 mg), after preparative reversed-phase TLC (RP-18, MeOH/H_2O, 6:1), afforded compound **2** (3 mg, R_f 0.244).

Virgaurol A (1): $C_{30}H_{42}O_5$; colorless gum; $[\alpha]_D^{26} = +133$ (c 0.3, $CHCl_3$); IR (KBr) ν_{max} 3423, 2929, 2865, 1760, 1727, 1661, 1455, 1381, 1117, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2; HR-ESI-MS m/z 505.2929 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_5Na$, 505.2924); EIMS m/z 482 (1, $[M]^+$), 464 (1, $[M - H_2O]^+$), 277 (14, $[C_{15}H_{21}O_3 + CO]^+$), 259 (5, $[C_{15}H_{21}O_3 - H_2O + CO]^+$), 249 (4, $[C_{15}H_{21}O_3]^+$), 233 (60, $[C_{15}H_{21}O_2]^+$), 231 (97, $[C_{15}H_{21}O_3 - H_2O]^+$), 205 (100, $[C_{15}H_{21}O_2 - CO]^+$).

Virgaurol B (2): $C_{30}H_{40}O_5$; colorless gum; $[\alpha]_D^{26} = +13$ (c 0.4, $CHCl_3$); IR (KBr) ν_{max} 3442, 2931, 2868, 1757, 1670, 1456, 1380, 1218, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2; HR-ESI-MS m/z 481.2950 $[M + H]^+$ (calcd for $C_{30}H_{41}O_5$, 481.2949); EIMS m/z 480 (1, $[M]^+$), 462 (1, $[M - H_2O]^+$), 249 (3, $[C_{15}H_{21}O_3]^+$), 231 (100, $[C_{15}H_{21}O_3 - H_2O]^+$ and $[C_{15}H_{19}O_2]^+$).

Virgaurol C (3): $C_{30}H_{42}O_6$; colorless gum; $[\alpha]_D^{26} = +15$ (c 0.3, $CHCl_3$); IR (KBr) ν_{max} 3447, 2928, 2867, 1762, 1734, 1668, 1459, 1380, 1161, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2; HR-ESI-MS m/z 516.3310 $[M + NH_4]^+$ (calcd for $C_{30}H_{46}O_6N$, 516.3320); EIMS m/z 498 (1, $[M]^+$), 480 (1, $[M - H_2O]^+$), 265 (4, $[C_{15}H_{21}O_4]^+$), 249 (26, $[C_{15}H_{21}O_3]^+$), 233 (100, $[C_{15}H_{21}O_2]^+$).

Virgaurol Acetate C (3a): $C_{32}H_{44}O_7$; colorless gum; $[\alpha]_D^{26} = +38$ (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 2925, 2866, 1781, 1736, 1673, 1455, 1229, 1163, 992 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2; HR-ESI-MS m/z 558.3428 $[M + NH_4]^+$ (calcd for $C_{32}H_{48}O_7N$, 558.3425); EIMS m/z 540 (1, $[M]^+$), 480 (1, $[M - CH_3COOH]^+$), 307 (1, $[C_{17}H_{23}O_5]^+$), 233 (32, $[C_{15}H_{21}O_2]^+$).

Virgaurol Acetate D (4a): $C_{32}H_{44}O_7$; colorless gum; $[\alpha]_D^{26} = -58$ (c 0.4, $CHCl_3$); IR (KBr) ν_{max} 2931, 2871, 1779, 1742, 1674, 1454, 1375, 1213, 1171, 970, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2; HR-ESI-MS m/z 558.3431 $[M + NH_4]^+$ (calcd for $C_{32}H_{48}O_7N$, 558.3425); EIMS m/z 540 (1, $[M]^+$), 480 (1, $[M - CH_3COOH]^+$), 307 (1, $[C_{17}H_{23}O_5]^+$), 233 (100, $[C_{15}H_{21}O_2]^+$).

Acetylation. The mixture of compounds **3** and **4** (8 mg) was dissolved in Ac_2O/dried pyridine (1:1, 0.5 mL each), and the mixture was maintained at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure and yielded crude residue. This crude residue was purified by TLC (silica gel GF_{254}, petroleum ether/EtOAc, 9:2) to afford compounds **3a** (3 mg, R_f 0.377) and **4a** (4 mg, R_f 0.261).

Cytotoxicity Assay. Cytotoxicity assays of compounds **1** and **3** were tested according to the sulforhodamine B (SRB) method.[8]

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