Cytotoxic Hydroxylated Triterpene Alcohol Ferulates from Rice Bran

Hong-Feng Luo,[†,‡] Qinglin Li,[†,‡] Shanggong Yu,[†] Thomas M. Badger,[†,§] and Nianbai Fang*[,†,‡]

Arkansas Children's Nutrition Center, Department of Pharmaceutical Sciences, and Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, 1120 South Marshall Street, Little Rock, Arkansas 72202

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Three hydroxylated triterpene alcohol ferulates, (24*S*)-cycloart-25-ene-3β,24-diol-3β-*trans*-ferulate (**1**), (24*R*)-cycloart-25-ene-3β,24-diol-3β-*trans*-ferulate (**2**), and cycloart-23*Z*-ene-3β,25-diol-3β-*trans*-ferulate (**3**), along with known compounds cycloartenol *trans*-ferulate (**4**) and 24-methylenecycloartanol *trans*-ferulate (**5**) were isolated from rice bran. Their structures were elucidated by means of chemical and spectroscopic analysis. Compounds **2**−**5** showed moderate cytotoxicity against MCF-7 cells.

Rice bran, a byproduct of the rice milling process, contains 18−22% oil and is used in diet preparation in several countries. γ-Oryzanol, a characteristic component of rice bran oil containing triterpene alcohol and sterol ferulic acid esters,[1−4] has been reported to inhibit tumor promotion,[5,6] reduce serum cholesterol levels,[7−9] and can also be used to treat nerve imbalance and disorders of menopause.[10] Japan manufactures 7500 tons of γ-oryzanol from 150 000 tons of rice bran each year. γ-Oryzanol has been approved in Japan for several conditions including mild anxiety, stomach upset, high cholesterol, and menopausal symptoms. Two groups of γ-oryzanol components have been found in rice bran oil by LC-MS/MS.[11] Components in the relatively nonpolar major group have been extensively studied for decades, and more than 10 bioactive triterpene alcohol and sterol ferulates have been identified.[2−6] Six hydroxylated triterpene alcohol ferulates in the relatively polar minor group of γ-oryzanol were characterized by LC-MS/MS in our previous study.[11] Three minor components in the minor group were tentatively identified as 25-hydroxy-24-methylcycloartanol *trans*-ferulate and two isomers of 24-hydroxy-24-methylcycloartanol *trans*-ferulate, and three major components in the minor group were characterized only as three isomers of hydroxycycloartenol ferulate on the basis of MS/MS data.[11] There are no published data on the biological activities of hydroxylated triterpene alcohol ferulates of γ-oryzanol that could account for the bioactivities of γ-oryzanol. As part of our continuing investigation into the health benefits of the phytochemicals in human diets, the anticancer activity of γ-oryzanol components was assessed against human breast cancer MCF-7 cells by MTT in vitro bioassay in the present study. HPLC, chemical, and spectroscopic methods were used to isolate and identify the bioactive components in the bioactive minor group of γ-oryzanol.

γ-Oryzanol components in rice bran were analyzed by reverse-phase HPLC with a diode-array detector at a wavelength of 320 nm. A UV (320 nm) chromatogram is shown in Figure 1, and there were two clusters of UV peaks in the HPLC chromatogram (Figure 1A). Two fractions corresponding to the two clusters of UV peaks were collected on semipreparative HPLC and named **SH1** and **SH2**, respectively (Figure 1A). All known components of γ-oryzanol were identified from the fraction **SH2** in previous studies,[2−6] and notably, **SH2** (IC_{50} 550 ± 16 μg/mL) was much less active against MCF-7 human breast carcinoma cells than **SH1** (IC_{50} 81 ± 2 μg/mL) in the MTT bioassay (Figure 1B). Three new compounds (**1**, **2**, and **3**), which are major components of the **SH1** fraction, were isolated (Figure 1A). Structural elucidations of compounds **1**, **2**, and **3** were accomplished on the basis of their chemical and spectroscopic data and confirmed by comparison of their spectroscopic data with the known natural products: (24*R*)-cycloart-25-ene-3β,24-diol, (24*S*)-cycloart-25-ene-3β,24-diol, cycloart-23*Z*-ene-3β,24-diol, and their acetyl derivatives.[13,14]

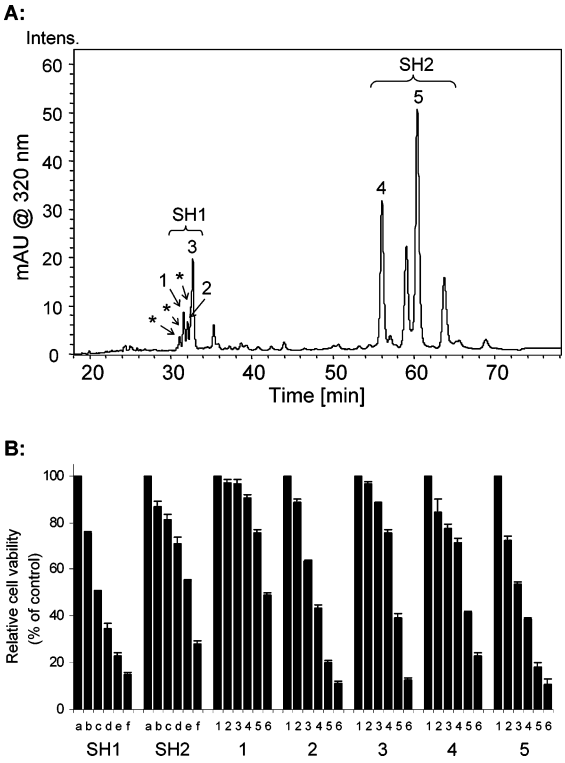

Figure 1. (A) UV (320 nm) chromatogram of a hexane extract of rice bran. (B) Cytotoxic effects of MCF-7 cell lines by five concentrations of **SH1**, **SH2**, and compounds **1**−**5** after 72 h of treatment as determined by MTT assay. a = medium as control; b = 10 μg/mL; c = 30 μg/mL; d = 100 μg/mL; e = 300 μg/mL; f = 1000 μg/mL; 1 = medium as control; 2 = 3 μM; 3 = 10 μM; 4 = 30 μM; 5 = 100 μM; 6 = 300 μM. Each value represents the mean ± SD of 3 replicates. *Three minor components in **SH1** were not identified in this study. Their structures were tentatively assigned as 25-hydroxy-24-methylcycloartanol *trans*-ferulate and two isomers of 24-hydroxy-24-methylcycloartanol *trans*-ferulate on the basis of MS/MS data in our previous study.[11]

* To whom correspondence should be addressed. Tel: (501) 364-2872. Fax: (501) 364-2818. E-mail: FangNianbai@uams.edu.
† Arkansas Children's Nutrition Center.
‡ Department of Pharmaceutical Sciences.
§ Department of Physiology and Biophysics.

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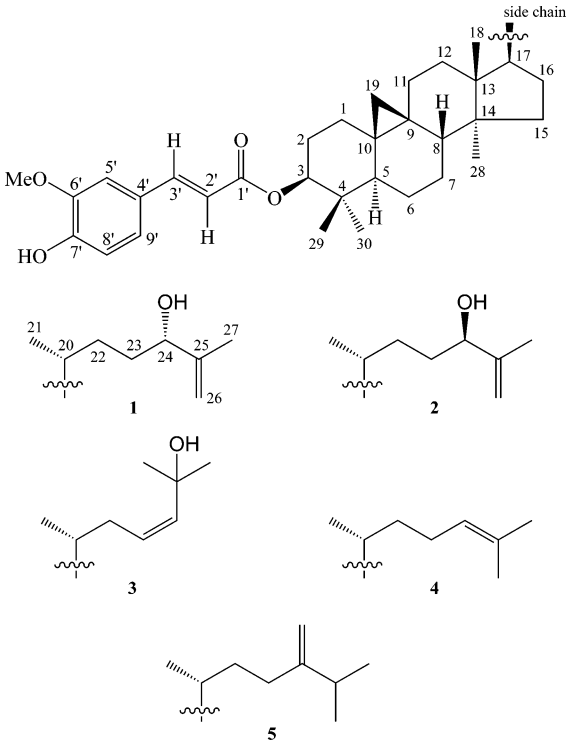


Figure 2. Structures of triterpene alcohol ferulates from rice bran.

Compound **1** was obtained as a white amorphous powder, $[\alpha]^{25}_{D}$ +44.4° (*c* 0.18, $CHCl_3$). Its elementary composition was defined as $C_{40}H_{58}O_5$ by HRESIMS and confirmed by the ^{13}C NMR spectrum (Table 1). The ^1H NMR spectrum (Table 2) had two doublets at δ 0.38 (J = 3.9 Hz) and 0.61 (J = 3.9 Hz), one doublet at δ 0.90 (J = 5.9 Hz), and four singlets at δ 0.91 (6H), 0.98, 0.99, and 1.74, assignable to six methyl groups. These data associated with the occurrence of one double doublet at δ 4.72 (J = 4.7 and 11.4 Hz) were characteristic of a cycloartan-3β-ol type triterpene ester. The presence of two olefinic protons at δ 4.85 (br s) and 4.94 (br s), assignable to a terminal methylene group, and one allylic oxymethine proton at δ 4.04 (t, J = 6.4 Hz) positioned the double bond at C-25 and the hydroxyl group at C-24. The ^1H NMR spectrum also exhibited signals for a 1,3,4-trisubstituted phenyl moiety at δ 6.93 (d, J = 8.2 Hz), 7.05 (d, J = 1.4 Hz), and 7.09 (dd, J = 1.4 and 8.2 Hz), two olefinic protons at δ 6.31 (d, J = 16.0 Hz) and 7.61 (d, J = 16.0 Hz), a methoxyl at δ 3.95 (s), and a hydroxyl at δ 5.95 (s), indicating the presence of a *trans*-ferulate moiety.[6,12] The ^{13}C NMR spectrum (Table 1) indicated the presence of 40 carbons, 30 for the triterpene alcohol moiety and 10 for the ferulate moiety. It showed two oxymethine carbons at δ 80.5 and 76.7, which were assigned to C-3 and C-24, respectively. The olefinic carbon atoms C-25 and C-26 were observed at δ 147.5 and 111.5. The ^1H and ^{13}C NMR spectra of the triterpene alcohol moiety were similar to those of cycloart-25-ene-3β,24-diol and its acetate,[13,14] and the position of the *trans*-ferulate was confirmed at C-3 by comparison with the spectroscopic data of those known triterpene alcohol ferulates in rice bran.[6] The above evidence revealed the structure of **1** to be cycloart-25-ene-3β,24-diol-3β-*trans*-ferulate. The absolute configuration at C-24 was determined by application of the modified Mosher's method[15] using (*S*)-MTPA (**1*S***) and (*R*)-MTPA esters (**1*R***). As shown in Scheme 1, the $\Delta\delta$ ($\delta_S - \delta_R$) values for the H-26 ($\Delta\delta$ = 0.05, 0.08) and H-27 signals ($\Delta\delta$ = 0.13) were found to be positive, whereas those for the H-18 ($\Delta\delta$ = −0.02) and H-21 signals ($\Delta\delta$ = −0.03) were negative, which unequivocally demonstrated that **1** possesses a 24*S*-configuration.[15] Therefore, the structure of **1** was defined as (24*S*)-cycloart-25-ene-3β,24-diol-3β-*trans*-ferulate.

Table 1. ^{13}C NMR Spectroscopic Data of Compounds **1**−**3** (δ ppm) (100 MHz, $CDCl_3$)

C	1	2	3
1	31.7	31.6	31.7
2	27.0	27.0	27.0
3	80.5	80.5	80.5
4	39.7	39.7	39.7
5	47.2	47.2	47.2
6	21.0	21.0	21.0
7	28.1	28.2	28.1
8	47.9	47.9	47.9
9	20.2	20.2	20.1
10	26.0	26.0	26.0
11	25.9	25.9	25.9
12	35.5	35.5	35.6
13	45.3	45.3	45.3
14	48.8	48.8	48.8
15	32.9	32.9	32.8
16	26.5	26.5	26.5
17	52.2	52.1	52.0
18	18.0	18.0	18.1
19	29.8	29.8	30.0
20	36.0	35.9	36.4
21	18.4	18.3	18.3
22	31.9	31.9	39.1
23	31.5	31.6	139.4
24	76.7	76.4	125.6
25	147.5	147.8	70.8
26	111.5	111.0	29.8[a]
27	17.2	17.6	29.9[a]
28	19.3	19.3	19.3
29	15.4	15.4	15.4
30	25.5	25.5	25.5
1′	167.1	167.1	167.1
2′	116.3	116.3	116.3
3′	144.4	144.4	144.4
4′	127.1	127.2	127.1
5′	109.3	109.2	109.3
6′	146.8	146.8	146.8
7′	147.8	147.8	147.9
8′	114.7	114.7	114.7
9′	123.1	123.1	123.1
OMe	56.0	56.0	56.0

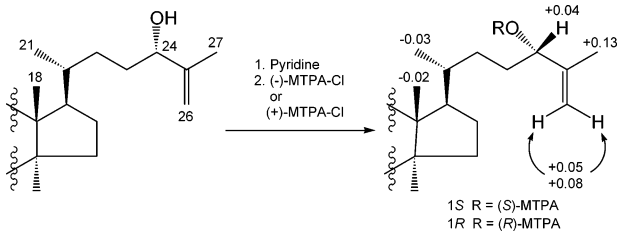
[a] Assignment may be interchangeable.

Table 2. ^1H NMR Spectrocopic Data of Compounds **1**−**3** (δ ppm, J = Hz) (400 MHz $CDCl_3$)

H	1	2	3
3	4.72 (dd, 4.7, 11.4)	4.72 (dd, 4.7, 11.4)	4.72 (dd, 4.3, 10.9)
18	0.99 (s)[a]	0.98 (s)	0.98 (s)
19	0.38 (d, 3.9)	0.37 (d, 4.3)	0.37 (d, 3.9)
	0.61 (d, 3.9)	0.61 (d, 4.3)	0.60 (d, 3.9)
21	0.90 (d, 5.9)	0.89 (d, 6.2)	0.87 (d, 6.2)
23			5.61 (m)
24	4.04 (t, 6.4)	4.03 (t, 6.2)	5.61 (m)
26	4.85 (br s)	4.84 (br s)	1.33 (s)[a]
	4.94 (br s)	4.95 (br s)	
27	1.74 (s)	1.74 (s)	1.32 (s)[a]
28	0.91 (s)	0.91 (s)	0.90 (s)
29	0.98 (s)[a]	0.98 (s)	0.98 (s)
30	0.91 (s)	0.90 (s)	0.90 (s)
2′	6.31 (d, 16.0)	6.31 (d, 16.0)	6.30 (d, 16.0)
3′	7.61 (d, 16.0)	7.60 (d, 16.0)	7.60 (d, 16.0)
5′	7.05 (d, 1.4)	7.05 (d, 2.0)	7.04 (d, 1.8)
8′	6.93 (d, 8.2)	6.92 (d, 8.2)	6.92 (d, 8.2)
9′	7.09 (dd, 1.4, 8.2)	7.09 (dd, 2.0, 8.2)	7.08 (dd, 1.8, 8.2)
OH	5.95 (s)	5.88 (s)	6.00 (s)
OMe	3.95 (s)	3.94 (s)	3.93 (s)

[a] Assignment may be interchangeable.

Scheme 1. $\Delta\delta$ ($= \delta_S - \delta_R$) Values Obtained from the ^{1}H NMR Spectra of the MTPA Esters **1*S*** and **1*R***



Compound **2** was a white amorphous powder, $[\alpha]^{25}_D$ +24.3° (*c* 0.21, $CHCl_3$). HRESIMS of **2** exhibited a deprotonated molecular ion $[M - H]^-$ at *m/z* 617.4160 corresponding to $C_{40}H_{58}O_5$, and this molecular formula was supported by ^{13}C NMR data (Table 1). The ^{1}H and ^{13}C NMR spectra of **2** showed almost identical chemical shifts and the same multiplicity to all carbon atoms as in **1** with little difference of chemical shifts of C-24, C-25, C-26, and C-27 between compounds **1** and **2**. These results suggested the opposite configuration for C-24 in **2** relative to that defined for **1**. This was confirmed by the ^{1}H and ^{13}C NMR spectra of the triterpene alcohol moiety that were similar to those of (24*R*)-cycloart-25-ene-3β,24-diol and its acetate,[13,14] which indicated that **2** also possessed a 24*R*-configuration. Therefore **2** was defined as (24*R*)-cycloart-25-ene-3β,24-diol-3β-*trans*-ferulate.

Compound **3** was obtained as a white amorphous powder, $[\alpha]^{25}_D$ +22.4° (*c* 0.25, $CHCl_3$). Its elementary composition was found to be $C_{40}H_{58}O_5$, as revealed by HRESIMS and ^{13}C NMR spectrum (Table 1). The ^{1}H NMR spectrum (Table 2) had two doublets at δ 0.37 (J = 3.9 Hz) and 0.60 (J = 3.9 Hz), one doublet at δ 0.87 (J = 6.2 Hz), and four singlets at δ 0.90 (6H), 0.98 (6H), 1.32, and 1.33, assignable to seven methyl groups. These data associated with the occurrence of one double doublet at δ 4.72 (J = 4.3 and 10.9 Hz) were characteristic of a cycloartan-3β-ol type triterpene ester. Two olefinic protons at δ 5.61 (m) and two olefinic carbon atoms at δ 139.4 and 125.6 indicated the presence of one double bond. The similarity of the carbon chemical shifts for the four-ring moiety to those of other cycloartanes (compounds **1**, **2**, **4**, and **5**) indicated the double bond in the side chain, and the doublet was assigned at C-23 on the basis of the ^{1}H–^{1}H COSY. In the ^{1}H–^{1}H COSY spectrum of **3**, the olefinic protons gave cross-peaks with both the H-22 protons at δ 1.77 and 2.17, which, in turn, were correlated to H-20 at δ 1.60, with the latter finally to the H-21 methyl doublet. The configuration of the double bond was assigned as *Z* since the olefinic protons appeared as narrow multiplets with a half bandwidth of 7.4 Hz in the ^{1}H NMR spectrum. The ^{1}H NMR spectrum also exhibited signals for a 1,3,4-trisubstituted phenyl moiety at δ 6.92 (d, J = 8.2 Hz), 7.04 (d, J = 1.8 Hz), and 7.08 (dd, J = 1.8 and 8.2 Hz), two olefinic protons at δ 6.30 (d, J = 16.0 Hz) and 7.60 (d, J = 16.0 Hz), a methoxyl at δ 3.93 (s), and a hydroxyl at δ 6.00 (s), indicating the presence of a *trans*-ferulate unit.[6,12] The positive-ion spectrum of ESI-MS/MS exhibited two prominent cations, $[M + H - 194]^+$ at *m/z* 425 and $[M + H - 194 - H_2O]^+$ at *m/z* 407. CID of the cation $[M + H - 194]^+$ yielded the $[M + H - 194 - H_2O]^+$ as the most abundant ion, and CID of the cation $[M + H - 194 - H_2O]^+$ gave a multipeak spectrum silimar to those of cycloartenol *trans*-ferulate (**4**). Formation of the ion $[M + H - 194 - H_2O]^+$ by loss of H_2O from the intact alcohol unit suggested a hydroxyl group in the triterpene alcohol moiety,[11] and the resonances at δ 1.32 and 1.33 of two methyl singlets justified its location at C-25. The ^{13}C NMR spectrum (Table 1) indicated the presence of 40 carbons, 30 for the triterpene alcohol moiety and 10 for the ferulate moiety. It showed two oxymethine carbons at δ 80.5 and 70.8, which were assigned to C-3 and C-25, respectively. The ^{1}H and ^{13}C NMR spectra of the triterpene alcohol moiety were similar to those of cycloart-23*Z*-ene-3β,25-diol and its acetates,[13,14] and the position of the *trans*-ferulate was confirmed at C-3 by comparison of the spectroscopic data with those of triterpene alcohol ferulates in rice bran.[6] Therefore **3** was defined as cycloart-23*Z*-ene-3β,25-diol-3β-*trans*-ferulate.

In addition, two known triterpene alcohol ferulates were isolated from the **SH2** fraction and identified as cycloartenol *trans*-ferulate (**4**) and 24-methylenecycloartanol *trans*-ferulate (**5**), respectively, on the basis of the comparison of their spectroscopic data with those in the literature.[5]

SH1, **SH2**, and compounds **1**–**5** were evaluated for their cytotoxicity against human breast cancer MCF-7 cells. After 72 h of treatment, IC_{50} values of **SH1** and **SH2** are 81 ± 2 and 550 ± 16 μg/mL and IC_{50} values of compounds **1**–**5** are 283 ± 11, 42 ± 1, 79 ± 1, 80 ± 2, and 27 ± 1 μM, respectively (Figure 1B). Compounds **2**–**5** showed moderate cytotoxicity. All samples were evaluated for their cytotoxicity after 24, 48, and 72 h of treatment. Three treatments with different times showed a similar trend, and samples after 72 h treatment were most active. (The data of 24 and 48 h are not shown.)

The MTT bioassay indicated that the cytotoxicity of **SH1** was 7 times more potent than that of **SH2**, but the cytotoxicities of compounds **2** and **3** from **SH1** were similar to those of compounds **4** and **5** from **SH2**. There are two possible explanations for these findings: (1) some minor constituents with strong cytotoxicity in **SH1** have not been identified; (2) there is a synergistic effect among the components in **SH1**.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a DigiPol-781-T6S automatic polarimeter in $CHCl_3$. ^{1}H and ^{13}C NMR spectra were recorded on a Varian MercuryPlus 400 MHz spectrometer in $CDCl_3$. Chemical shifts were referenced to the residual solvent peaks (δ_H 7.27 and δ_C 77.0). LC-MS/MS was performed using an Agilent 1100 series liquid chromatograph interfaced to a Bruker model Esquire-LC multiple-ion trap mass spectrometer equipped with an atmospheric pressure interface electrospray (API-ES) chamber. HRESIMS measurements were carried out on a Micromass Q-Tof micromass spectrometer. Analytical and preparative HPLC were carried out on a normal-phase column (Phenomenex Luna Silica (2) 5 μm) using CH_2Cl_2–EtOAc and hexanes–EtOAc solvent systems, and a reverse-phase column (Phenomenex Luna C8 (2) 5 μm) using MeOH–H_2O and MeCN–H_2O solvent systems. Silica gel (32–63 μm) and precoated plates of silica gel 60 F_{254} (Selecto) were used for column chromatography and for TLC, respectively.

Plant Materials. Full-fat stabilized rice bran was obtained from Arkansas-grown long-grain rice (Riceland Foods, Inc., Stuttgart, AR). According to Riceland Foods, Inc., Arkansas-grown rice consist of 85% long-grain rice from three varieties (Drew, Cypress, and Cocodrie), and the remaining 15% is made up by 10 varieties including Alan, Kaybonnet, XL-6, Wells, Jefferson, Lagrue, Lemont, Madison, Millie, and Priscilla.

Extraction and Isolation. Full-fat stabilized rice bran (4 kg) was pulverized and extracted by macerating with EtOH at room temperature. The solvent was evaporated under reduced pressure at room temperature followed by lyophilization to yield 635 g of extract. The extract was partitioned between H_2O and hexanes. The hexane extract was dried under reduced pressure at room temperature followed by lyophilization. The hexane extract (5.0 g) was subjected to a

reverse-phase preparative HPLC using MeCN−H_2O (20−70% in 15 min, 70−85% from 15 to 20 min, 85−90% from 20 to 30 min, 90−100% from 30 to 70 min, 100−100% to 75 min, 100−20% from 75 to 80 min) as solvent. Two fractions, **SH1** (310 mg) and **SH2** (1930 mg), were collected on the basis of two clusters of UV peaks (320 nm) (Figure 1A). The large portion of hexane extract (562 g) was subjected to a silica gel column eluted with a hexanes−EtOAc gradient solvent system to afford 82 fractions. Fractions 51−55 (520 mg) were combined and further purified by a normal-phase preparative HPLC using CH_2Cl_2−EtOAc (95.2:4.8) and hexanes−EtOAc (79:21) to give **1** (22 mg) and **2** (27 mg). Fractions 60−63 (410 mg) were combined and further purified by a normal-phase preparative HPLC using CH_2Cl_2−EtOAc (95:4) and hexanes−EtOAc (75:25) to give **3** (32 mg). Fractions 8 (740 mg) was purified by a normal-phase preparative HPLC using hexanes−EtOAc (88:12) and a reverse-phase preparative HPLC using MeOH−H_2O (94:6) to give **4** (14 mg) and **5** (20 mg). Finally, compounds **1**−**5** were analyzed with reverse-phase HPLC and are shown in Figure 1A by comparing their retention times. All extraction and isolation procedures for ferulates were carried out in "white" fluorescent light to prevent isomerization of the ferulates.[16−18]

(24*S*)-Cycloart-25-ene-3*β*,24-diol-3*β*-*trans*-ferulate (1): white powder; $[\alpha]^{25}_D$ +44.4° (*c* 0.18, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; ESI-MS/MS, negative-ion mode *m/z* 617 $[M - H]^-$, 602 $[M - H - Me]^-$ (100); positive-ion mode *m/z* 425 $[M + H - 194]^+$ (100), 407 $[M + H - 194 - H_2O]^+$ (54.8); HRESIMS *m/z* 617.4177 (calcd for $C_{40}H_{57}O_5$ $[M - H]^-$, 617.4206).

(24*R*)-Cycloart-25-ene-3*β*,24-diol-3*β*-*trans*-ferulate (2): white powder; $[\alpha]^{25}_D$ +24.3° (*c* 0.21, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; ESI-MS/MS, negative-ion mode *m/z* 617 $[M - H]^-$, 602 $[M - H - Me]^-$ (100); positive-ion mode *m/z* 425 $[M + H - 194]^+$ (70.3), 407 $[M + H - 194 - H_2O]^+$ (100); HRESIMS *m/z* 617.4160 (calcd for $C_{40}H_{57}O_5$ $[M - H]^-$, 617.4206).

Cycloart-23*Z*-ene-3*β*,25-diol-3*β*-*trans*-ferulate (3): white powder; $[\alpha]^{25}_D$ +22.4° (*c* 0.25, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; ESI-MS/MS: negative-ion mode *m/z* 617 $[M - H]^-$, 602 $[M - H - Me]^-$ (100); positive-ion mode *m/z* 425 $[M + H - 194]^+$ (100), 407 $[M + H - 194 - H_2O]^+$ (16.8); HRESIMS *m/z* 617.4170 (calcd for $C_{40}H_{57}O_5$ $[M - H]^-$, 617.4206).

Preparation of the (*S*)- and (*R*)-MTPA Esters of 1. A solution of **1** (3 mg in 200 μL of dried pyridine, 5 μmol) was treated with (−)-MTPA chloride (13 μL, 67 μmol), and the mixture was kept overnight at room temperature. *N*,*N*-Dimethyl-1,3-propanediamine (13 μL, 101 μmol) was added, and the solution was left for 10 min at room temperature. The residue obtained after evaporation of the solvent under a stream of N_2 was purified by HPLC (silica gel with hexanes−EtOAc, 88:12) to afford the (*S*)-MTPA ester (**1*S***, 2.1 mg, 52%). Treatment of **1** with (+)-MTPA chloride in the same manner gave the (*R*)-MTPA ester (**1*R***, 2.4 mg, 59%).

Compound 1*S*: colorless amorphous solid; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.60 (1H, d, J = 16.0 Hz, H-3′), [7.52 (2H, dd-like), 7.40 (3H, m), Ph-H], 7.09 (1H, dd, J = 1.8, 8.2 Hz, H-9′), 7.05 (1H, d, J = 1.8 Hz, H-5′), 6.92 (1H, d, J = 8.2 Hz, H-8′), 6.31 (1H, d, J = 16.0 Hz, H-2′), 5.84 (1H, s, OH), 5.40 (1H, t, J = 6.8 Hz, H-24), 5.07 (1H, br s, H-26), 4.99 (1H, br s, H-26), 4.72 (1H, dd, J = 4.7, 10.2 Hz, H-3), 3.95 (3H, s, OMe), 3.55 (3H, s, OMe), 1.74 (3H, s, H-27), 0.98 (3H, s, H-29), 0.95 (3H, s, H-18), 0.90 (6H, s, H-28, 30), 0.85 (3H, d, J = 6.3 Hz, H-21), 0.61 (1H, d, J = 4.1 Hz, H-19), 0.37 (1H, d, J = 4.1 Hz, H-19).

Compound 1*R*: colorless amorphous solid; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.60 (1H, d, J = 16.0 Hz, H-3′), [7.52 (2H, dd-like), 7.40 (3H, m), Ph-H], 7.09 (1H, dd, J = 1.8, 8.2 Hz, H-9′), 7.05 (1H, d, J = 1.8 Hz, H-5′), 6.93 (1H, d, J = 8.2 Hz, H-8′), 6.31 (1H, d, J = 16.0 Hz, H-2′), 5.84 (1H, s, OH), 5.36 (1H, t, J = 6.8 Hz, H-24), 4.99 (1H, br s, H-26), 4.94 (1H, br s, H-26), 4.72 (1H, dd, J = 4.5, 11.1 Hz, H-3), 3.95 (3H, s, OMe), 3.58 (3H, s, OMe), 1.61 (3H, s, H-27), 0.98 (3H, s, H-29), 0.97 (3H, s, H-18), 0.90 (6H, s, H-28, 30), 0.88 (3H, d, J = 6.3 Hz, H-21), 0.61 (1H, d, J = 4.2 Hz, H-19), 0.37 (1H, d, J = 4.2 Hz, H-19).

Assay of Cytotoxicity. Cytotoxicity of fractions **SH1** and **SH2** and compounds **1**−**5** were measured by MTT assays as described previously.[19] In brief, MCF-7 cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD), and cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. MCF-7 cells were plated (10^4/well/100 μL) in a 96-well plate and incubated 48 h and then incubated for 24 h with DMEM without fetal bovine serum. Prior to the addition of test compounds, serial dilutions of samples were dissolved in DMSO followed by dilution with medium, and the final DMSO concentration in the assay was 0.2%. The various compounds and concentrations were treated for 24, 48, and 72 h. Subsequently, the wells were incubated with 100 μL $well^{-1}$ of MTT at 1 mg/mL, a tetrazolium dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma, St. Louis, MO). Plates were incubated in the presence of MTT dye for 4 h at 37 °C. The supernatant was removed, and 200 μL of DMSO was added. The absorbance was read using an enzyme-linked immunosorbent assay plate reader at 570 nm. The results were assayed in triplicate experiments.

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