# Sesquiterpenoids from *Saussurea laniceps*

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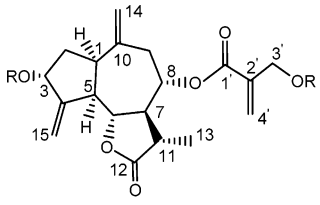
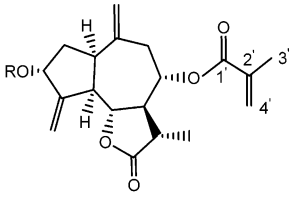
Two new guaiane-type sesquiterpenoids (**1** and **2**) and one new eudesmane-type sesquiterpenoid (**3**) were isolated from *Saussurea laniceps.* The structures of these compounds were established by spectroscopic methods, and the absolute stereochemistry of compounds **1** and **2** was determined by Mosher's method. The immunomodulatory activities of compounds **1**−**3** were evaluated. Of these, compound **3** showed significant inhibition of the proliferation of murine T and B cells in vitro.

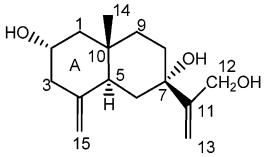
The genus *Saussurea* (Compositae) contains more than 300 species distributed in the north-temperate zone of the world. Over 200 species of the plants are found in mainland China, of which some are used in Tibetan medicine.[1] A literature survey has shown that *Saussurea* species contain flavonoids, coumarins, and sesquiterpene lactones.[2] The plant *S. laniceps* Hand-Mazz. is known as "snow-lotus" flower and is incorporated into a well-known Tibetan remedy for the treatment of rheumatic arthritis and gynopathy.[3] The compounds *n*-hentriacontane, *p*-hydroxyacetophenone, physcion, scopoletin, *β*-sitosterol, and umbelliferone have been isolated from this plant.[4] In the present study on the chemical constituents of *S. laniceps*, three new sesquiterpenoids (**1**−**3**) were obtained. This paper describes the isolation and structure elucidation of **1**−**3** as well as their in vitro immunomodulatory activities.



**1** R = H
**1a** R = (*S*)-MTPA
**1b** R = (*R*)-MTPA



**2** R = H
**2a** R = (*S*)-MTPA
**2b** R = (*R*)-MTPA



**3**

A 95% EtOH extract of the plant (3.0 kg) was extracted with petroleum ether, ethyl acetate, and *n*-butanol, successively. By a series of column chromatographic separations, **1** (60 mg), **2** (30 mg), and **3** (11 mg) were obtained from the ethyl acetate extract.

Compound **1**, a colorless gum, exhibited an ion peak at *m/z* 348.1555 in the HREIMS, consistent with a molecular formula of $C_{19}H_{24}O_6$. The IR spectrum exhibited the presence of hydroxyl (3426 $cm^{-1}$), *γ*-lactone (1766 $cm^{-1}$), conjugated ester (1716 $cm^{-1}$), and double bond (1639 $cm^{-1}$) functionalities. The $^1H$ NMR spectrum (Table 1), characteristic of a guaiane-type sesquiterpenoid,[5,6] showed the signals for a methyl doublet at *δ* 1.20 (3H, d, $J = 7.0$ Hz), two exomethylenes at *δ* 5.01, 5.12 (each 1H, br s) and 5.28, 5.34 (each 1H, br s), three oxygenated methines at *δ* 4.49 (1H, m), 4.22 (1H, t, $J = 9.9$ Hz), and 5.09 (1H, ddd, $J = 9.9$, 7.0, and 4.5 Hz), as well as the signals of a (2-hydroxymethyl)acrylate group, an oxygenated methylene at *δ* 4.29, and a terminal olefinic methylene at *δ* 5.94, 6.28 (each 1H, br s). The $^{13}C$ NMR spectrum (Table 2) exhibited signals for 19 carbons including one methyl, six methylenes (three olefinic, one oxygenated), seven methines (three oxygenated), and five quaternary carbons (three olefinic, two carbonyl). This evidence suggested that **1** is a guaiane-type sesquiterpene lactone with a C-8 ester side chain.[7] Analysis of the $^1H-^1H$ COSY, HMBC, and HMQC spectra permitted full assignments of the $^1H$ and $^{13}C$ NMR data, which revealed that **1** is very similar to 11α,13-dihydrocynaropicrin, a known sesquiterpene lactone (2-hydroxymethyl)acrylate isolated from the genus *Saussurea*.[7] Significant differences between **1** and 11α,13-dihydrocynaropicrin were the coupling constant between H-7 and H-11 and chemical shift of C-11 in their NMR spectra. Thus, 11α,13-dihydrocynaropicrin showed $J_{7,11} = 7.5$ Hz, indicating a *β*-methyl group at C-11. However, **1** revealed

**Table 1.** $^1H$ NMR (400 MHz) Data of Compounds **1**−**3** (ppm, *J* in Hz)[a]

| position | **1** | **2** | **3** |
|---|---|---|---|
| 1 | 2.98, m | 2.91, m | 1.25, m, 1.76, m |
| 2 | 2.20, m, 1.65, m | 1.68, m, 2.20, m | 3.77, m |
| 3 | 4.49, m | 4.48, tt (2.0, 8.0) | 2.61, dd (12.3, 5.1) |
| | | | 1.97, t (11.6) |
| 5 | 2.89, m | 2.83 br t (9.9) | 2.32, br d (10.0) |
| 6 | 4.22, t (9.9) | 4.19 t (9.9) | 1.61, m, 2.31, m |
| 7 | 2.40, ddd (9.9, 11.0) | 2.33, dd (9.9) | |
| 8 | 5.09, ddd (9.9, 7.0, 4.5) | 5.08, m | 1.81, m |
| | | | 1.54, br d (12.1) |
| 9 | 2.30, dd (7.0, 13.4) | 2.73, dd (5.1, 13.7) | 1.31, m, 0.89, m |
| | 2.78, dd (4.5, 13.4) | 2.24, dd (6.8, 13.7) | |
| 10 | | | |
| 11 | 2.65, dq (7.0, 10.8) | 2.57, dq (7.0, 10.9) | |
| 12 | | | 4.21, s |
| 13 | 1.20, d (7.0) | 1.22, d (7.0) | 5.14, 5.20, br s |
| 14 | 5.01, 5.12 br s | 4.99, 5.10 br s | 0.74, s |
| 15 | 5.28, 5.34 br s | 5.28, 5.33 br s | 4.50, 4.80, br s |
| 1′ | | | |
| 2′ | | | |
| 3′ | 4.29, br s | 1.94, s | |
| 4′ | 5.94, 6.28 br s | 5.65, 6.73 br s | |

[a] Compounds **1** and **3** were measured in $CD_3OD$ and compound **2** in $CDCl_3$.

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**Table 2.** $^{13}C$ NMR (100 MHz) Data of Compounds **1**−**3** (ppm)[a]

| position | 1 | 2 | 3 |
|---|---|---|---|
| 1 | 45.4 | 43.2 | 51.9 |
| 2 | 40.1 | 37.8 | 68.9 |
| 3 | 74.4 | 72.1 | 47.8 |
| 4 | 154.9 | 151.9 | 149.9 |
| 5 | 51.7 | 49.5 | 44.9 |
| 6 | 81.2 | 79.0 | 36.3 |
| 7 | 54.4 | 52.3 | 75.2 |
| 8 | 78.0 | 75.7 | 32.9 |
| 9 | 41.5 | 39.4 | 37.5 |
| 10 | 144.9 | 141.9 | 36.2 |
| 11 | 42.6 | 40.6 | 157.9 |
| 12 | 180.9 | 178.5 | 63.5 |
| 13 | 16.3 | 14.5 | 109.4 |
| 14 | 117.5 | 116.1 | 17.3 |
| 15 | 111.6 | 110.4 | 108.5 |
| 1′ | 166.9 | 166.1 | |
| 2′ | 142.4 | 135.5 | |
| 3′ | 62.1 | 17.3 | |
| 4′ | 126.0 | 125.8 | |

[a] Compounds **1** and **3** were measured in $CD_3OD$ and compound **2** in $CDCl_3$.

a $J_{7,11}$ value of 10.8 Hz, suggesting an α-methyl group at C-11.[8] The above deduction was supported by the significant downfield shift of C-7 from $\delta$ 50.7 of 11α,13-dihydrocynaropicrin to $\delta$ 54.4 of **1**. In the ROESY NMR spectrum, correlations between H-5 and H-1 and H-7; H-7 and H-13; and H-6 and H-8 and H-11 were observed, but no correlations between H-3 and H-1 and H-5 were apparent, indicating that H-1, H-5, and H-7 were in an α-orientation, while H-3, H-6, H-8, and H-11 were $\beta$-oriented, varying from 11α,13-dihydrocynaropicrin, which has a 3$\beta$-hydroxyl group. From all of the above evidence and by biogenetic considerations, the structure of **1** was established as 3α,8α-dihydroxy-1α*H*,5α*H*,6$\beta$*H*,7α*H*,11$\beta$*H*-guai-4(15),10(14)-dien-6,12-olide 8-*O*-2-hydroxymethylacrylate.

The absolute configuration at C-3 was determined by Mosher's method.[9] Separate samples of **1** were treated with (*R*)-(+)- and (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) in anhydrous THF, respectively, to afforded the (*S*)- and (*R*)-MTPA ester derivatives (**1a** and **1b**), respectively. The chemical shift values of the (*R*)-MTPA esters were subtracted from the (*S*)-MTPA ester [$\Delta\delta$ = $\delta$(*S*)-MTPA − $\delta$(*R*)-MTPA]. The negative $\Delta\delta$ values from H-15α (−0.02 ppm) and H-15$\beta$ (−0.03 ppm) and positive $\Delta\delta$ values from H-2α (+0.02 ppm) and H-2$\beta$ (+0.03 ppm) indicated that C-3 has the *R*-configuration.

Compound **2**, a colorless gum, was assigned the molecular formula $C_{19}H_{24}O_5$ from its HREIMS (*m/z* 332.1616). The IR spectrum indicated the presence of hydroxyl (3446 $cm^{-1}$), $\gamma$-lactone (1774 $cm^{-1}$), conjugated ester (1716 $cm^{-1}$), and double bond (1637 $cm^{-1}$) units. The $^1H$ NMR spectrum (Table 1) showed characteristic signals for a guaiane-type sesquiterpenoid, constituted by a methyl doublet at $\delta$ 1.22 (3H, d, $J$ = 7.0 Hz), two exomethylenes at $\delta$ 4.99, 5.10 (each 1H, br s) and 5.28, 5.33 (each 1H, br s), and three oxygenated methines at $\delta$ 4.48 (1H, m), 4.19 (1H, t, $J$ = 9.9 Hz), and 5.08 (1H, m), as well as a 2-methylacrylate side chain, whose signals were at $\delta$ 1.94 (3H, s) for the methyl group and $\delta$ 5.65, 6.73 (each 1H, br s) for the terminal unit. The $^{13}C$ NMR spectrum (Table 2) of **2** exhibited signals for 19 carbons including two methyls, five methylenes (three olefinic), seven methines (three oxygenated), and five quaternary carbons (three olefinic and two carbonyl). Further analysis of the $^1H$ and $^{13}C$ NMR data showed that **2** possesses a very similar structure to that of **1**, with the only difference between **2** and **1** being at their side chains. The $^1H$ and $^{13}C$ NMR data of **2** were assigned by HMQC, HMBC, and ROESY spectra. Accordingly, the structure of **2** was assigned as 3α,8α-dihydroxy-1α*H*,5α*H*,6$\beta$*H*,7α*H*,11$\beta$*H*-guai-4(15),10(14)-dien-6,12-olide 8-*O*-(2-methyl)acrylate.

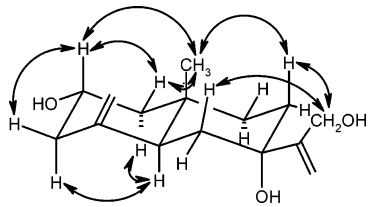


**Figure 1.** Key ROESY correlations of **3**.

The absolute configuration of C-3 was determined by Mosher's method in a manner similar to **1**. The chemical shift values of the (*R*)-MTPA ester (**2b**) were subtracted from the (*S*) -MTPA ester (**2a**) [$\Delta\delta$ = $\delta$(*S*)-MTPA − $\delta$(*R*)-MTPA]. Like **1**, negative $\Delta\delta$ values from H-15α (−0.04 ppm) and H-15$\beta$ (−0.04 ppm) and positive $\Delta\delta$ values from H-2α (+0.03 ppm) and H-2$\beta$ (+0.02 ppm) again indicated that C-3 has the *R*-configuration.

Compound **3**, a colorless gum, gave a molecular ion at *m/z* 252.1726 in the HREIMS, corresponding to the molecular formula of $C_{15}H_{24}O_3$. The IR spectrum exhibited the presence of hydroxyl (3425 $cm^{-1}$) and double bond (1648 $cm^{-1}$) moieties. The $^1H$ NMR spectrum showed signals for one methyl group ($\delta$ 0.74, s), two exomethylenes ($\delta$ 5.14, 5.20, each 1H, br s; $\delta$ 4.50, 4.80, each 1H, br s), one oxygenated methylene ($\delta$ 4.21, 2H, s), and one oxygenated methine ($\delta$ 3.77, m). The $^{13}C$ NMR spectrum revealed 15 carbons including one methyl, eight methylenes (two olefinic, one oxygenated), two methines (one oxygenated), and four quaternary carbons. The above NMR data were similar to those of 7-hydroxycostol, a known eudesmane-type sesquiterpenoid.[10] However, **3** had one more hydroxyl group than 7-hydroxycostol. The $^1H-^1H$ COSY study showed that a $CH_2CH(OH)CH_2$ unit in ring A of **3** replaced the $CH_2CH_2CH_2$ unit in 7-hydroxycostol, indicating that the additional hydroxyl group of **3** was at C-2 ($\delta_H$ 3.77, 1H, m; $\delta_C$ 68.9 d). The ROESY correlation (Figure 1) between $CH_3$-14 and H-2 indicated that the C-2 hydroxyl group in **3** has an α-configuration. Further, the HMBC, HMQC, ROESY, and $^1H-^1H$ COSY spectra permitted assignments of all $^1H$ and $^{13}C$ NMR data of **3**. Therefore, compound **3** could be established as 3α,7α,12-trihydroxyeudesm-4(15),-11(13)-diene.

Guaianolide 2-methylacrylate and 2-hydroxymethylacrylate esters have been isolated from many *Saussurea* species.[7,11] Compounds **1** and **2** are additional examples of compounds with such ester groups. Compounds **1**−**3** were tested in an in vitro murine lymphocyte proliferation assay induced by concanavalin A (ConA) and lipopolysaccharide (LPS). The results in Table 3 show that compounds **1** and **2** revealed weak activity, while compound **3** had strong inhibition on proliferation of murine T and/or B cells. Previously, a guaiane-type sesquiterpene lactone, cynaropicrin, isolated from *Saussurea lappa*, was reported to potently inhibit the proliferation of leukocyte cancer cell lines through pro-apoptotic activity.[12] The immunosuppressive activity of **3** demonstrated in the present study supports the practice of using *S. laniceps* in Tibetan medicine for the treatment of rheumatic arthritis.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured with a Horiba Sepa-300 polarimeter. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. NMR

**Table 3.** Effect of Compounds **1**−**3** on Murine Lymphocyte Proliferation Induced by Concanavalin A (ConA) (5 μg/mL) or Lipopolysaccharide (LPS) (10 μg/mL)

| compound | concentration (M) | [H³] TdR incorporation × $10^{-3}$ (cpm)[a] ConA-induced T cell proliferation | LPS-induced B cell proliferation |
|---|---|---|---|
| negative control | | 3.40 ± 0.86 | 2.60 ± 0.50 |
| positive control (ConA or LPS) | | 43.96 ± 0.37 | 34.72 ± 0.54 |
| **1** | $1 \times 10^{-7}$ | 46.33 ± 0.52 | 24.27 ± 0.36 ↓ |
| | $1 \times 10^{-6}$ | 43.75 ± 0.69 | 29.80 ± 0.54 |
| | $1 \times 10^{-5}$ | 13.44 ± 1.02 | 34.94 ± 0.62 |
| | $1 \times 10^{-4}$ | 0.03 ± 0.02 | 0.03 ± 0.01 |
| **2** | $1 \times 10^{-7}$ | 33.78 ± 4.09 ↓ | 34.78 ± 0.91 |
| | $1 \times 10^{-6}$ | 36.13 ± 2.23 ↓ | 31.12 ± 0.88 |
| | $1 \times 10^{-5}$ | 30.59 ± 2.39 | 20.58 ± 0.32 |
| | $1 \times 10^{-4}$ | 0.04 ± 0.02 | 0.08 ± 0.04 |
| **3** | $1 \times 10^{-7}$ | 39.78 ± 3.36 | 36.12 ± 3.33 |
| | $1 \times 10^{-6}$ | 31.53 ± 1.29 ↓↓ | 33.65 ± 2.98 |
| | $1 \times 10^{-5}$ | 28.32 ± 3.85 ↓ | 30.83 ± 0.59 |
| | $1 \times 10^{-4}$ | 12.13 ± 2.65 ↓↓ | 17.21 ± 0.32 ↓↓ |

[a] Results are represented as means ± SD based on three independent experiments ($n = 6$; ↑, ↓ $P < 0.05$; ↑↑, ↓↓ $P < 0.01$, compared with control group).

spectra were run in $CDCl_3$, $CD_3OD$, or $D_2O$ on a Bruker AM-400 spectrometer with TMS as internal standard. EIMS and HREIMS experiments were carried out on a VG-ZAB-HS and JEOL JMX-HX110 mass spectrometer, respectively. LRESIMS were measured using a Finnigan LCQ-DECA instrument, and HRESIMS data were obtained on Micromass LCT and Mariner spectrometers. Column chromatographic separations were carried out on silica gel H-60 (Qingdao Haiyang Chemical Group Corporation, Qingdao, People's Republic of China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), and LiChroprep RP-18 (40−63 μm, Merck). HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, People's Republic of China) were used for analytical TLC.

**Plant Material.** The dried whole plant material was collected in February 2002 in the Zhong Dian district of Yunnan Province, People's Republic of China. It was identified by Prof. Shao-Qing Cai of the College of Pharmacy, Beijing University, Beijing, People's Republic of China. A voucher specimen (No. 02-02-19) is deposited at the herbarium of the same university.

**Extraction and Isolation.** The dried whole plants of *Saussurea laniceps* (3.0 kg) were percolated with 95% EtOH (5 L × 3) at room temperature. The filtrate was concentrated in vacuo. The residue was partitioned with $H_2O$ and petroleum ether, EtOAc, and *n*-BuOH (500 mL × 3), successively. The EtOAc extract (50.0 g) was subjected to column chromatography over silica gel H-60 eluted with $CHCl_3$−MeOH (50:1 to 5:1) to afford five fractions (A−E). Fraction B was subjected to column chromatographic separation over silica gel H-60 eluted with $CHCl_3$−MeOH (30:1 to 5:1), LiChroprep RP-18 eluted with MeOH−$H_2O$ (1:1 to 2:1), and Sephadex LH-20 (MeOH) successively to yield compound **1** (60 mg, 0.0020%). Fraction C afforded compounds **2** (30 mg, 0.0010%) and **3** (11 mg, 0.0004%) using the same method.

**3α,8α-Dihydroxy-1α*H*,5α*H*,6β*H*,11β*H*-guai-4(15),10(14)-dien-12,6-olide 8-*O*-2-hydroxymethylacrylate (1):** colorless gum; $[\alpha]^{20}_D$ +52.0° (*c* 0.25, MeOH); IR $\nu_{max}$ 3426 (OH), 1766 (γ-lactone), 1716 (C=$CCO_2$R), 1639 (double bond) $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; HREIMS *m/z* 348.1555 $[M]^+$ (calcd for $C_{19}H_{24}O_6$, 348.1573).

**3α,8α-Dihydroxy-1α*H*,5α*H*,6β*H*,11β*H*-guai-4(15),10(14)-dien-12,6-olide 8-*O*-2-methylacrylate (2):** colorless gum; $[\alpha]^{20}_D$ +38.0° (*c* 0.25, MeOH); IR $\nu_{max}$ 3446 (OH), 1774 (γ-lactone), 1716 (C=$CCO_2$R), 1637 (double bond) $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; HREIMS *m/z* 332.1616 $[M]^+$ (calcd for $C_{19}H_{24}O_5$, 332.1624).

**3α,7α,12-Trihydroxyeudesm-4(15),11(13)-diene (3):** colorless gum; $[\alpha]^{20}_D$ +0.1° (*c* 0.40, MeOH); IR $\nu_{max}$ 3425 (OH), 1648 (double bond) $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; HREIMS *m/z* 252.1726 $[M]^+$ (calcd for $C_{15}H_{24}O_3$, 252.1714).

**(*R*)- and (*S*)-MTPA Esters of 1 and 2.** To an anhydrous THF solution (0.5 mL) of either compound **1** or **2** (1.0 mg, respectively) were added (*R*)- or (*S*)-MTPA-Cl (10 μL) and triethylamine (5 μL) under a nitrogen atmosphere, and the resulting mixture was allowed to stand at room temperature for 8 h. After evaporation of solvent, the residue was passed through a silica gel column ($CHCl_3$−MeOH, 50:1) to afford the corresponding (*S*)- or (*R*)-MTPA ester derivative.

**(*S*)-MTPA ester of 1 (1a):** $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.58 (2H, m, MTPA-ArH), 7.44 (3H, m, MTPA-ArH), 3.56 (3H, s, MTPA-$OCH_3$), 7.56 (2H, m, MTPA-ArH), 7.42 (3H, m, MTPA-ArH), 3.55 (3H, s, MTPA-$OCH_3$), 3.03 (1H, m, H-1), 1.92 (1H, m, H-2α), 2.49 (1H, m, H-2β), 5.73 (1H, m, H-3), 2.79 (1H, t, $J = 9.9$ Hz, H-5), 4.24 (1H, t, $J = 9.9$ Hz, H-6), 2.35 (1H, dd, $J = 9.9$ Hz, H-7), 4.99 (1H, m, H-8), 2.01 (1H, dd, $J = 6.5$, 13.6 Hz, H-9α), 2.62 (1H, dd, $J = 5.2$, 13.6 Hz, H-9β), 2.60 (1H, dq, $J = 6.9$, 10.8 Hz, H-11), 1.25 (3H, d, $J = 6.9$ Hz, H-13), 4.91 (1H, br s, H-14α), 4.99 (1H, br s, H-14β), 5.33 (1H, br s, H-15α), 5.41 (1H, br s, H-15β), 5.23 (3H, s, H-3′), 5.99 (1H, br s, H-4′), 6.34 (1H, br s, H-4′).

**(*R*)-MTPA Ester of 1 (1b):** $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.57 (2H, m, MTPA-ArH), 7.44 (3H, m, MTPA-ArH), 3.56 (3H, s, MTPA-$OCH_3$), 7.55 (2H, m, MTPA-ArH), 7.40 (3H, m, MTPA-ArH), 3.55 (3H, s, MTPA-$OCH_3$), 3.01 (1H, m, H-1), 1.90 (1H, m, H-2α), 2.46 (1H, m, H-2β), 5.72 (1H, m, H-3), 2.80 (1H, t, $J = 9.9$ Hz, H-5), 4.25 (1H, t, $J = 9.9$ Hz, H-6), 2.38 (1H, dd, $J = 9.9$ Hz, H-7), 5.01 (1H, m, H-8), 2.00 (1H, dd, $J = 6.4$, 13.6 Hz, H-9α), 2.60 (1H, dd, $J = 5.2$, 13.6 Hz, H-9β), 2.61 (1H, dq, $J = 6.9$, 10.8 Hz, H-11), 1.26 (3H, d, $J = 6.9$ Hz, H-13), 4.90 (1H, br s, H-14α), 4.98 (1H, br s, H-14β), 5.35 (1H, br s, H-15α), 5.44 (1H, br s, H-15β), 5.22 (3H, s, H-3′), 5.97 (1H, br s, H-4′), 6.33 (1H, br s, H-4′).

**(*S*)-MTPA Ester of 2 (2a):** $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.56 (2H, m, MTPA-ArH), 7.43 (3H, m, MTPA-ArH), 3.56 (3H, s, MTPA-$OCH_3$), 3.09 (1H, m, H-1), 1.98 (1H, m, H-2α), 2.53 (1H, m, H-2β), 5.76 (1H, m, H-3), 2.81 (1H, t, $J = 9.9$ Hz, H-5), 4.23 (1H, t, $J = 9.9$ Hz, H-6), 2.30 (1H, dd, $J = 9.9$ Hz, H-7), 4.89 (1H, m, H-8), 2.23 (1H, dd, $J = 6.6$, 13.8 Hz, H-9α), 2.73 (1H, dd, $J = 5.2$, 13.8 Hz, H-9β), 2.58 (1H, dq, $J = 6.9$, 10.8 Hz, H-11), 1.20 (3H, d, $J = 6.9$ Hz, H-13), 4.97 (1H, br s, H-14α), 5.05 (1H, br s, H-14β), 5.38 (1H, br s, H-15α), 5.43 (1H, br s, H-15β), 1.96 (3H, s, H-3′), 5.63 (1H, br s, H-4′), 6.13 (1H, br s, H-4′).

**(*R*)-MTPA Ester of 2 (2b):** $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.55 (2H, m, MTPA-ArH), 7.43 (3H, m, MTPA-ArH), 3.56 (3H, s, MTPA-$OCH_3$), 3.07 (1H, m, H-1), 1.95 (1H, m, H-2α), 2.51 (1H, m, H-2β), 5.73 (1H, m, H-3), 2.83 (1H, t, $J = 9.9$ Hz, H-5), 4.24 (1H, t, $J = 9.9$ Hz, H-6), 2.31 (1H, dd, $J = 9.9$ Hz, H-7), 4.90 (1H, m, H-8), 2.21 (1H, dd, $J = 6.6$, 13.8 Hz, H-9α), 2.72 (1H, dd, $J = 5.2$, 13.8 Hz, H-9β), 2.58 (1H, dq, $J = 6.9$, 10.8 Hz, H-11), 1.20 (3H, d, $J = 6.9$ Hz, H-13), 4.96 (1H, br s, H-14α), 5.02 (1H, br s, H-14β), 5.42 (1H, br s, H-15α), 5.47 (1H, br s, H-15β), 1.95 (3H, s, H-3′), 5.63 (1H, br s, H-4′), 6.13 (1H, br s, H-4′).

**Lymphocyte Proliferation Test.** The prepared spleen cells of mice ($4 \times 10^6$) were seeded into each well of a 96-well microplate, and 5 μg/mL of concanavalin A (Con A, from *Canavalia ensiformis* Type III, Sigma) or lipopolysaccharide (LPS, from *Escherichia coli*, Sigma) was added to various concentrations of compounds **1**−**3**. The plates were cultured at 37 °C with 5% $CO_2$ in a humidified atmosphere for 48 h. For the last 6 h, each well was pulsed with 0.25 μCi/well $^3H$-TdR (thymidine, [methyl-$^3H$], ICN Pharmaceuticals, Inc., Irvine, CA). The cells were harvested and the radioactivity incorporated was counted using a liquid scintillation counter. All counts/min values shown were the mean of triplicate sample ± SD. Statistical analysis was carried out by the Student *t*-test. ConA or LPS was used as a positive control. Inhibition was evaluated only for cells that grew well and were not damaged by toxicity during the test procedure.[13,14]

## References and Notes

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