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# Steroidal Glycosides from the Bulbs of *Ornithogalum thyrsoides*

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Phytochemical analyses have been carried out on the fresh bulbs of *Ornithogalum thyrsoides* with particular attention to the steroidal glycoside constituents, resulting in the isolation of four new spirostanol saponins and seven new cholestane glycosides, together with three known steroidal compounds. The structures of the new glycosides were determined on the basis of their spectroscopic data, including 2D NMR spectroscopy, and the results of hydrolytic cleavage. The isolated compounds were evaluated for their cytotoxic activities against HL-60 human promyelocytic leukemia cells and HSC-2 human oral squamous cell carcinoma cells.

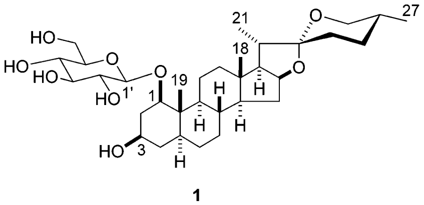
*Ornithogalum thyrsoides* (Liliaceae) is a perennial plant native to southwestern Cape Province. Although *O. thyrsoides* has no medicinal folkloric background and is now cultivated for ornamental purposes, a MeOH extract of the bulbs showed potent cytotoxic activity against HL-60 human promyelocytic leukemia cells. We have reported the structural determination of 12 cholestane glycosides isolated from *O. thyrsoides* bulbs as the cytotoxic ingredients.[1] Further phytochemical analysis of the bulb extract with particular attention to the steroidal glycoside constituents led to the isolation of four new spirostanol saponins (**1** and **3**−**5**) and seven new cholestane glycosides (**8**−**14**), along with three known steroidal compounds (**2**, **6**, and **7**). This paper deals with the structural elucidation of the new compounds on the basis of their spectroscopic data, including 2D NMR spectroscopy, and the results of hydrolytic cleavage. The cytotoxic activities of **1**−**14** against HL-60 human promyelocytic leukemia cells and HSC-2 human oral squamous cell carcinoma cells are also described.

## Results and Discussion

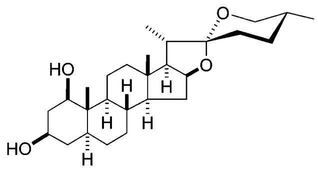
A concentrated MeOH extract of *O. thyrsoides* bulbs was passed through a porous-polymer polystyrene resin (Diaion HP-20) column eluted with 30% MeOH, 50% MeOH, and EtOH. The EtOH eluate fraction was repeatedly subjected to column chromatography over silica gel and octadecylsilanized (ODS) silica gel, as well as preparative HPLC, to give compounds **1** (71.1 mg), **2** (112 mg), **3** (33.9 mg), **4** (28.5 mg), **5** (22.1 mg), **6** (22.5 mg), **7** (50.0 mg), **8** (17.6 mg), **9** (14.3 mg), **10** (102 mg), **11** (19.7 mg), **12** (11.7 mg), **13** (26.9 mg), and **14** (14.1 mg).

Compounds **2**, **6**, and **7** were identified as (25*R*)-3*β*-hydroxyspirost-5-en-1*β*-yl *O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**2**),[2] (22*S*)-16*β*-[(*β*-D-glucopyranosyl)oxy]-3*β*,22-dihydroxy-5α-cholestan-1*β*-yl α-L-rhamnopyranoside (**6**),[3] and (22*S*)-16*β*-[(6-*O*-acetyl-*β*-D-glucopyranosyl)oxy]-3*β*,22-dihydroxy-5α-cholestan-1*β*-yl α-L-rhamnopyranoside (**7**),[3] respectively.

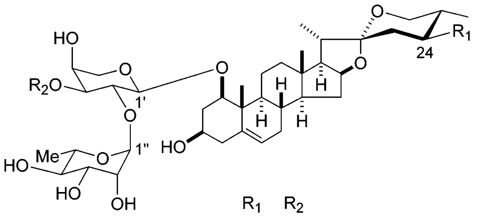
Compound **1** was obtained as an amorphous solid, $[\alpha]_D^{25}$ −68.0° (MeOH). Its molecular formula was determined as $C_{33}H_{54}O_9$ by HRESIMS, showing an $[M + H]^+$ peak at *m/z* 595.3836. The $^1H$ NMR spectrum of **1** contained signals for four steroidal methyl groups at $\delta$ 1.10 (d, $J$ =



**1**

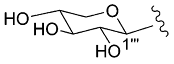


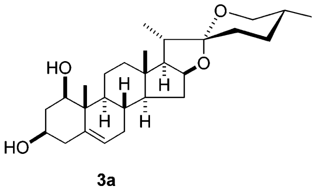
**1a**



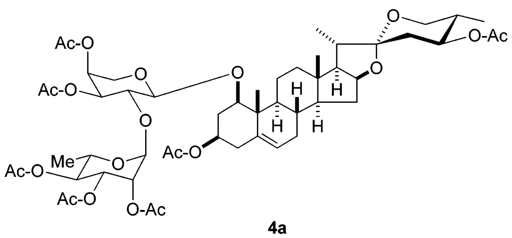
| | $R_1$ | $R_2$ |
|---|---|---|
| **2** | H | H |
| **3** | H | Xyl |
| **4** | OH | H |
| **5** | OH | Xyl |

Xyl =





**3a**



**4a**

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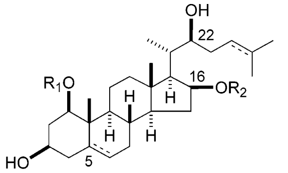
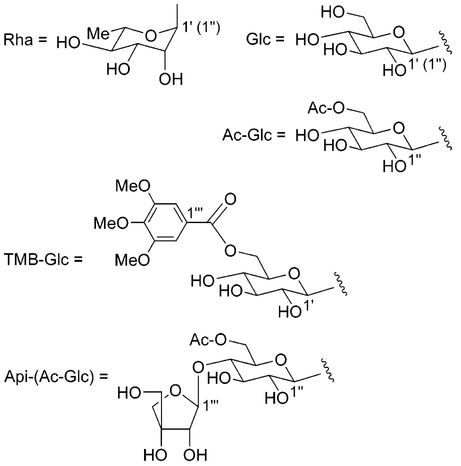
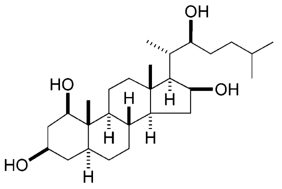
10.1021/np040108i CCC: $27.50

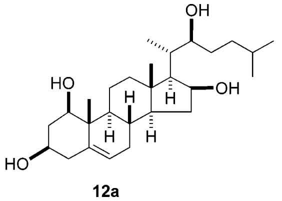


| | $R_1$ | $R_2$ | |
|---|---|---|---|
| **6** | Rha | Glc | 5α |
| **7** | Rha | Ac-Glc | 5α |
| **8** | Rha | Api-(Ac-Glc) | 5α |
| **9** | Glc | Glc | 5α |
| **10** | TMB-Glc | Glc | 5α |
| **11** | TMB-Glc | Glc | 5α, $\Delta^{24}$ |
| **12** | Rha | Rha | $\Delta^5$ |
| **13** | Rha | Rha | 5α, $\Delta^{24}$ |
| **14** | Rha | Rha | $\Delta^5$, $\Delta^{24}$ |





**8a**



**12a**

6.9 Hz), 1.03 (s), 0.85 (s), and 0.70 (d, $J = 5.3$ Hz) and one anomeric proton at $\delta$ 5.05 (d, $J = 7.7$ Hz). Acid hydrolysis of **1** with 1 M HCl in dioxane−$H_2O$ (1:1) resulted in the production of an aglycone, identified as (25*R*)-5α-spirostane-1*β*,3*β*-diol (**1a**),[4] and D-glucose as the carbohydrate component. The signal at $\delta$ 3.98 (dd, $J = 11.5$, 4.2 Hz) showed long-range correlations with C-9 ($\delta$ 54.8), C-10 ($\delta$ 40.4), and C-19 ($\delta$ 8.2) in the HMBC spectrum of **1** and was assigned to H-1. In the HMQC spectrum, the signal assigned to H-1 was correlated to the oxygen-bearing carbon signal at $\delta$ 81.2 (C-1), with which the anomeric proton of the glucosyl moiety had an HMBC correlation. Thus, the structure of **1** was elucidated as (25*R*)-3*β*-hydroxy-5α-spirostan-1*β*-yl *β*-D-glucopyranoside.

Compound **3** had the molecular formula $C_{43}H_{68}O_{16}$ on the basis of HRESIMS, exhibiting an $[M + Na]^+$ peak at *m/z* 863.4404. The $^1H$ NMR spectrum of **3** showed three anomeric protons at $\delta$ 6.34 (br s), 4.98 (d, $J = 7.5$ Hz), and 4.73 (d, $J = 7.3$ Hz), along with four steroidal methyl protons at $\delta$ 1.43 (s), 1.09 (d, $J = 7.0$ Hz), 0.85 (s), and 0.68 (d, $J = 5.3$ Hz). Acid hydrolysis of **3** yielded a steroidal sapogenin, identified as (25*R*)-spirost-5-ene-1*β*,3*β*-diol (**3a**),[5] together with L-arabinose, L-rhamnose, and D-xylose. On comparison of the entire $^{13}C$ NMR spectrum of **3** with that of **2**, a set of five additional signals, corresponding to a terminal *β*-D-xylopyranosyl unit, appeared at $\delta$ 106.5 (CH), 74.6 (CH), 78.3 (CH), 71.0 (CH), and 67.0 ($CH_2$), and the signal due to C-3 of the arabinosyl moiety was markedly deshielded by 8.6 ppm and was observed at $\delta$ 84.6, suggesting the C-3 hydroxyl group of the arabinosyl moiety was the position to which the D-xylose unit was attached. This was confirmed by an HMBC correlation from the anomeric proton of the xylosyl unit at $\delta$ 4.98 to the arabinose C-3′ carbon at $\delta$ 84.6. Long-range correlations between the anomeric proton of the rhamnosyl at $\delta$ 6.34 and C-2′ of the arabinosyl at $\delta$ 74.2 and between the anomeric proton of the arabinosyl at $\delta$ 4.73 and C-1 of the aglycone at $\delta$ 83.8 were also noted. Accordingly, the structure of **3** was determined to be (25*R*)-3*β*-hydroxyspirost-5-en-1*β*-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside.

Compound **4** was assigned the molecular formula $C_{38}H_{60}O_{13}$ using HRESIMS (*m/z* 747.3935 $[M + Na]^+$). The $^1H$ NMR spectrum showed signals for four steroid methyl groups at $\delta$ 1.44 (s), 1.12 (d, $J = 7.0$ Hz), 1.08 (d, $J = 6.5$ Hz), and 0.86 (s) and two anomeric protons at $\delta$ 6.34 (br s) and 4.73 (d, $J = 7.3$ Hz). Acid hydrolysis of **4** with 1 M HCl gave L-arabinose and L-rhamnose, together with several unidentified artifactual sapogenols; no genuine aglycone could be obtained. Comparison of the $^1H$ and $^{13}C$ NMR spectra of **4** with those of **2** revealed that the structures of the ring A−E portion and diglycoside moiety attached at C-1 of the aglycone were identical to those of **2**. However, significant differences were recognized in the signals from the ring F portion (C-22−C-27) between **2** and **4**, and an additional oxymethine proton was observed at $\delta$ 4.01 in **4**, which was shifted downfield by 1.19 ppm on complete acetylation of **4**. These data indicated that **4** was a steroidal glycoside closely related to **2** with a hydroxyl group at ring F. The $^1H-^1H$ COSY and 2D TOCSY spectra were inspected to assign the locus of the hydroxyl group, with the methyl doublet due to Me-27 at $\delta$ 1.08 ($J = 6.5$ Hz) being used as the starting point of the analysis. The subsequent spin-coupling correlations led us to propose the F ring as $-CH_2-CH(-OH)-CH(-Me)-CH_2-O-$, giving evidence for the presence of a C-24 hydroxyl group. NOE correlations from H-26ax ($\delta$ 3.59) to H-16 ($\delta$ 4.54) and Me-27 and from H-26eq ($\delta$ 3.69) to H-25 ($\delta$ 1.82) in the PHNOESY spectrum and spin-coupling constants between $H_2$-23 and H-24 ($^3J_{H-23eq,H-24} = 4.7$ Hz, $^3J_{H-23ax,H-24} = 12.2$ Hz) and between H-25 and $H_2$-26 ($^3J_{H-25,H-26eq} = 4.8$ Hz, $^3J_{H-25,H-26ax} = 11.3$ Hz) were indicative of the 22α, 24*S*, and 25*S* configurations. Thus, the structure of **4** was defined as (24*S*,25*S*)-3*β*,24-dihydroxyspirost-5-en-1*β*-yl *O*-α-L-rhamnospyranosyl-(1→2)-α-L-arabinopyranoside.

Compound **5** was deduced as $C_{43}H_{68}O_{17}$ from its HRESIMS (*m/z* 879.4302 $[M + Na]^+$). The prominent $^1H$ NMR signals arising from the aglycone moiety of **5** at $\delta$ 5.58 (br d, $J = 5.7$ Hz, H-6), 4.01 (ddd, $J = 12.1$, 10.3, 4.7 Hz, H-24), 3.88 (m, $W_{1/2} = 20.0$ Hz, H-3), 3.83 (dd, $J = 12.0$, 3.9 Hz, H-1), 1.42 (s, Me-19), 1.12 (d, $J = 6.9$ Hz, Me-21), 1.08 (d, $J = 6.5$ Hz, Me-27), and 0.85 (s, Me-19) were superimposable on those of **4**, whereas the signals due to the anomeric protons at $\delta$ 6.34 (br s), 4.98 (d, $J = 7.5$ Hz), and 4.74 (d, $J = 7.3$ Hz) were coincident with those of **3**. Acid hydrolysis of **5** with 1 M HCl gave L-arabinose, L-rhamnose, and

D-xylose. Comparison of the $^{13}$C NMR spectrum of **5** with those of **3** and **4** and the results of acid hydrolysis, as well as the above $^{1}$H NMR data, allowed the structural assignment of **5** as (24*S*,25*S*)-3β,24-dihydroxyspirost-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside.

Compound **8** was isolated as an amorphous solid with the molecular formula $C_{46}H_{78}O_{18}$, as determined by HRESIMS data exhibiting an $[M + Na]^+$ peak at *m/z* 941.5048. The $^{1}$H NMR spectrum of **8** showed signals for five typical steroidal methyl groups at δ 1.14 (d, $J = 7.0$ Hz), 0.98 (s), 0.97 (s), 0.95 (d, $J = 6.2$ Hz), and 0.93 (d, $J = 6.2$ Hz) and suggested that the aglycone of **8** was the same as that of **7**. The presence of a 6-*O*-acetyl-β-D-glucopyranosyl moiety attached to C-16 of the aglycone and an α-L-rhamnopyraosyl attached to C-1 in **8** as in **7** was also shown by analysis of the $^{1}$H and $^{13}$C NMR spectra of **8**. However, the molecular formula of **8** was higher by $C_5H_8O_4$ than that of **7**, and three anomeric proton signals were observed at δ 5.92 (br s), 5.78 (d, $J = 3.6$ Hz), and 4.60 (d, $J = 7.8$ Hz). Acid hydrolysis of **8** with 0.2 M HCl in dioxane−$H_2O$ (1:1) gave an aglycone (**8a**), identified as (22*S*)-5α-cholestane-1β,3β,16β,22-tetraol (**8a**),[3] and D-apiose, D-glucose, and L-rhamnose as the carbohydrate moieties. In the $^{1}$H NMR spectrum of **8**, the signals at δ 5.78 and 4.76 ($J = 3.6$ Hz), 4.73 and 4.31 ($J = 9.2$ Hz), and 4.15 and 4.12 ($J = 11.3$ Hz) were assignable to the protons of the apiofuranosyl residue, and the quaternary carbon at δ 80.1 was typical of C-3 of apiofuranose. The β-furanoid anomeric form of the apiofuranosyl residue was indicated by the deshielded anomeric carbon signal at δ 111.0.[6] The apiosyl group was involved in a glycosidic linkage at C-4 of the glucosyl group, because the signal due to C-4 of the glucosyl was remakedly deshielded by 8.1 ppm, while the signals due to C-3 and C-5 were shielded by 2.1 and 1.8 ppm, respectively, when comparing the $^{13}$C NMR spectrum of **8** with that of **7**. This was confirmed by the observation of a long-range correlation from the anomeric proton of the apiosyl group at δ 5.78 to C-4″ of the glucosyl moiety at δ 80.0 in the HMBC spectrum. The anomeric proton of the substituted glucosyl group at δ 4.60 showed an HMBC correlation to C-16 of the aglycone at δ 83.0, whereas that of the rhamnosyl at δ 5.92 to C-1 of the aglycone appeared at δ 81.8. Long-range correlations from H$_2$-6″ of the glucosyl at δ 4.75 and 4.73 to the carbonyl carbon of the acetyl group at δ 170.5 were also observed. The data led to the full structure of **8** as (22*S*)-16β-[(*O*-β-D-apiofuranosyl-(1→4)-6-*O*-acetyl-β-D-glucopyranosyl)oxy]-3β,22-dihydroxy-5α-cholestan-16β-yl α-L-rhamnopyranoside.

Compound **9** analyzed for $C_{39}H_{68}O_{14}$ by HRESIMS (*m/z* 783.4490 $[M + Na]^+$). The spectroscopic data of **9** showed that **9** was a cholestane glycoside closely related to **6**. The $^{1}$H and $^{13}$C NMR spectra of **9** exhibited signals of two β-D-glucopyranosyl residues, along with signals for five steroid methyl groups at δ 1.13 (d, $J = 7.0$ Hz), 1.02 (s), 0.96 (s), 0.93 (d, $J = 6.2$ Hz), and 0.92 (d, $J = 6.2$ Hz). Acid hydrolysis of **9** yielded **8a** and D-glucose. In the HMBC spectrum of **9**, the anomeric proton signals of the two glucosyl groups at δ 5.06 and 4.75 showed long-range correlations with C-1 (δ 81.0) and C-16 (δ 82.7) of the aglycone, respectively. The structure of **9** was shown to be (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxy-5α-cholestan-1β-yl β-D-glucopyranoside.

Compound **10** exhibited the molecular formula $C_{49}H_{78}O_{18}$ on the basis of HRESIMS (*m/z* 977.5065 $[M + Na]^+$). Analysis of the $^{1}$H and $^{13}$C NMR spectra of **10** and comparison with those of **9** implied that **10** differed from **9** in the presence of an aromatic acid ester group. The aromatic acid was suggested to be 3,4,5-trimethoxybenzoic acid by the IR [1716 cm$^{-1}$ (C=O); 1590, 1504, and 1459 cm$^{-1}$ (aromatic ring)], UV [$\lambda_{max}$ 266 nm (log $\epsilon$ 4.01)], $^{1}$H NMR [δ 7.71 (2H, s), 3.97 (3H, s), and 3.85 (3H × 2, s)], and $^{13}$C NMR [δ 126.2 (C), 107.9 (CH × 2), 153.8 (C × 2), 143.1 (C), 166.3 (C=O), 60.7 (OMe), and 56.2 (OMe × 2)] spectral data. Alkaline hydrolysis of **10** with 4% KOH in EtOH yield **9** and 3,4,5-trimethoxybenzoic acid. Therefore, **10** was a 3,4,5-trimethoxybenzoyl ester of **9**. The anomeric proton of one glucosyl moiety at δ 4.75 (d, $J = 7.8$ Hz) showed a long-range correlation with C-16 of the aglycone at δ 82.9, whereas that of another glucosyl unit at δ 4.97 (d, $J = 7.8$ Hz) gave an HMBC correlation with C-1 of the aglycone at δ 82.8. The H$_2$-6′ protons due to the glucosyl group attached to C-1 of the aglycone at δ 5.15 and 5.08 were correlated to the carbonyl carbon of the 3,4,5-trimethoxybenzoyl moiety at δ 166.3. Thus, **10** was defined as (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxy-5α-cholestan-1β-yl 6-*O*-(3,4,5-trimethoxybenzoyl)-β-D-glucopyranoside.

Compound **11** analyzed for $C_{49}H_{76}O_{18}$ by HRESIMS (*m/z* 975.4915 $[M + Na]^+$). Comparison of the $^{1}$H and $^{13}$C NMR spectra of **11** with those of **10** showed their considerable structural similarity. The differences consisted only in the signals of the methyl groups assignable to Me-26 and Me-27. The two doublets for Me-26 and Me-27 observed in the $^{1}$H NMR spectrum of **10** were displaced by two three-proton singlets at δ 1.74 and 1.66 in that of **11**, suggesting that **11** was a C-24/C-25 dehydro derivative of **10**. This was confirmed by catalytic hydrogenation of **11** over PtO$_2$, resulting in the formation of **9**. The structure of **11** was formulated as (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxy-5α-cholest-24-en-1β-yl 6-*O*-(3,4,5-trimethoxybenzoyl)-β-D-glucopyranoside.

Compound **12** had the molecular formula $C_{39}H_{66}O_{12}$ on the basis of HRESIMS (*m/z* 727.4636 $[M + H]^+$). The $^{1}$H NMR spectrum of **12** exhibited signals for five steroidal methyl groups at δ 1.28 (s), 1.17 (d, $J = 6.9$ Hz), 1.00 (s), and 0.86 (3H × 2, d, $J = 6.5$ Hz), an olefinic proton at δ 5.63 (br d, $J = 5.6$ Hz), and two anomeric protons at δ 5.66 and 5.27 (each br s). Acid hydrolysis of **12** gave an aglycone, identified as (22*S*)-cholest-5-ene-1β,3β,16β,22-tetraol (**12a**),[7] and L-rhamnose. The $^{13}$C NMR spectrum of **12** showed the presence of two terminal α-L-rhamnopyranosyl units in **12**, and HMBC correlations were observed from δ 5.66 to δ 82.1 (C-1 of aglycone) and from δ 5.27 to δ 81.3 (C-16 of aglycone), allowing the assignment of the structure of **12** as (22*S*)-16β-[(α-L-rhamnopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-1β-yl α-L-rhamnopyranoside.

Compound **13** showed an accurate $[M + Na]^+$ ion at *m/z* 749.4474, corresponding to the molecular formula $C_{39}H_{66}O_{12}$. The $^{1}$H and $^{13}$C NMR spectroscopic data of **13** indicated that **13** contained two α-L-rhamnopyranosyl units, whose anomeric protons were observed at δ 5.61 (br s) and 5.25 (br s), and that the aglycone of **13** was the same as that of **11**. The linkage positions of two rhamnosyl units were revealed to be at C-1 and C-16 of the aglycone by the observation of long-range correlations from δ 5.61 to δ 81.7 (C-1 of the aglycone) and from δ 5.25 to δ 81.9 (C-16 of the aglycone). The structure of **13** was elucidated as (22*S*)-16β-[(α-L-rhamnopyranosyl)oxy]-3β,22-dihydroxycholest-24-en-1β-yl α-L-rhamnopyranoside.

Compound **14** was a cholestane glycoside with the molecular formula $C_{39}H_{64}O_{12}$ (HRESIMS *m/z* 747.4266 $[M + Na]^+$), and the spectroscopic data implied that it was

**Table 1.** $^{13}$C NMR Spectroscopic Data for **1**, **3**−**5**, and **8**−**14** in $C_5D_5N$

| carbon | **1** | **3** | **4** | **5** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 81.2 | 83.8 | 83.5 | 83.8 | 81.8 | 81.0 | 82.8 | 82.7 | 82.1 | 81.7 | 82.0 |
| 2 | 37.7 | 37.5 | 37.4 | 37.5 | 37.4 | 37.5 | 38.0 | 38.0 | 35.8 | 37.2 | 35.9 |
| 3 | 67.6 | 68.2 | 68.2 | 68.2 | 67.6 | 67.7 | 67.8 | 67.8 | 68.1 | 67.6 | 68.1 |
| 4 | 39.7 | 43.9 | 43.9 | 43.8 | 39.6 | 39.7 | 39.8 | 39.8 | 43.8 | 39.6 | 43.8 |
| 5 | 42.9 | 139.5 | 139.6 | 139.5 | 42.9 | 42.9 | 43.1 | 43.1 | 139.3 | 42.9 | 139.2 |
| 6 | 28.9 | 124.7 | 124.7 | 124.7 | 29.0 | 28.6 | 28.9 | 28.9 | 125.0 | 29.0 | 125.0 |
| 7 | 32.4 | 32.4 | 32.4 | 32.4 | 32.0 | 32.1 | 32.1 | 32.1 | 31.5 | 32.0 | 31.5 |
| 8 | 36.4 | 33.1 | 33.1 | 33.1 | 36.5 | 36.4 | 36.5 | 36.5 | 33.5 | 36.6 | 33.5 |
| 9 | 54.8 | 50.3 | 50.4 | 50.3 | 55.1 | 54.8 | 54.9 | 54.9 | 50.8 | 55.0 | 50.8 |
| 10 | 40.4 | 42.9 | 42.9 | 42.9 | 41.6 | 41.4 | 41.3 | 41.3 | 42.9 | 41.6 | 42.8 |
| 11 | 23.7 | 24.1 | 24.0 | 24.0 | 24.5 | 23.8 | 23.7 | 23.7 | 24.9 | 24.6 | 24.9 |
| 12 | 40.7 | 40.3 | 40.3 | 40.3 | 40.9 | 40.7 | 40.6 | 40.6 | 40.5 | 40.9 | 40.6 |
| 13 | 41.5 | 40.2 | 40.2 | 40.2 | 42.3 | 42.2 | 42.3 | 42.3 | 42.1 | 42.2 | 42.1 |
| 14 | 56.7 | 56.8 | 56.8 | 56.8 | 55.2 | 55.0 | 54.9 | 54.9 | 55.1 | 55.1 | 55.1 |
| 15 | 32.4 | 32.0 | 32.0 | 31.9 | 37.2 | 37.3 | 37.3 | 37.2 | 35.9 | 35.9 | 35.8 |
| 16 | 81.1 | 81.1 | 81.5 | 81.5 | 83.0 | 82.7 | 82.9 | 82.9 | 81.3 | 81.9 | 81.3 |
| 17 | 63.2 | 63.0 | 62.6 | 62.6 | 58.1 | 58.2 | 58.2 | 58.2 | 57.9 | 58.0 | 57.9 |
| 18 | 16.9 | 16.7 | 16.7 | 16.7 | 13.9 | 14.0 | 14.1 | 14.1 | 13.6 | 13.7 | 13.6 |
| 19 | 8.2 | 15.0 | 15.0 | 14.9 | 8.1 | 8.2 | 8.3 | 8.3 | 14.6 | 8.2 | 14.6 |
| 20 | 42.1 | 41.9 | 42.2 | 42.2 | 36.0 | 36.0 | 35.9 | 35.5 | 36.1 | 35.3 | 35.3 |
| 21 | 14.9 | 15.0 | 15.0 | 15.0 | 12.6 | 12.5 | 12.5 | 12.4 | 12.0 | 11.8 | 11.9 |
| 22 | 109.2 | 109.2 | 111.8 | 111.8 | 73.2 | 73.2 | 73.3 | 72.9 | 72.5 | 72.0 | 72.1 |
| 23 | 31.8 | 31.8 | 41.9 | 41.8 | 33.7 | 33.8 | 33.7 | 34.7 | 34.5 | 35.4 | 35.4 |
| 24 | 29.3 | 29.3 | 70.6 | 70.6 | 36.7 | 36.7 | 36.7 | 126.2 | 36.8 | 123.1 | 125.0 |
| 25 | 30.6 | 30.6 | 40.0 | 40.0 | 28.9 | 28.9 | 28.9 | 131.3 | 28.7 | 132.3 | 132.3 |
| 26 | 66.8 | 66.8 | 65.3 | 65.3 | 23.1 | 23.1 | 23.1 | 26.0 | 22.9 | 26.0 | 26.0 |
| 27 | 17.3 | 17.3 | 13.6 | 13.6 | 23.0 | 23.0 | 23.0 | 18.2 | 22.8 | 18.1 | 18.1 |
| 1′ | 100.6 | 100.5 | 100.3 | 100.5 | 98.5 | 100.4 | 101.5 | 101.5 | 97.7 | 98.4 | 97.7 |
| 2′ | 75.6 | 74.2 | 75.1 | 74.2 | 72.8 | 75.6 | 75.3 | 75.3 | 73.0 | 73.1 | 73.0 |
| 3′ | 78.6 | 84.6 | 75.9 | 84.5 | 73.2 | 78.7 | 78.6 | 78.6 | 72.9 | 72.8 | 72.9 |
| 4′ | 72.3 | 69.7 | 70.1 | 69.6 | 73.8 | 71.7 | 72.3 | 72.2 | 73.7 | 73.8 | 73.7 |
| 5′ | 78.4 | 67.1 | 67.3 | 67.1 | 71.0 | 78.4 | 74.9 | 74.9 | 71.0 | 71.0 | 70.9 |
| 6′ | 63.6 |  |  |  | 18.7 | 63.5 | 66.0 | 66.0 | 18.7 | 18.8 | 18.7 |
| 1″ |  | 101.8 | 101.7 | 101.8 | 106.6 | 107.0 | 107.0 | 107.0 | 104.9 | 104.9 | 104.9 |
| 2″ |  | 72.6 | 72.6 | 72.5 | 75.1 | 75.7 | 75.7 | 75.7 | 72.7 | 72.6 | 72.7 |
| 3″ |  | 72.6 | 72.7 | 72.5 | 76.4 | 78.7 | 78.8 | 78.8 | 73.2 | 73.2 | 73.2 |
| 4″ |  | 74.1 | 74.2 | 74.1 | 80.0 | 71.7 | 71.7 | 71.7 | 74.0 | 74.0 | 74.0 |
| 5″ |  | 69.5 | 69.4 | 69.5 | 73.2 | 78.2 | 78.2 | 78.2 | 70.7 | 70.9 | 70.7 |
| 6″ |  | 19.2 | 19.0 | 19.2 | 64.0 | 63.5 | 62.9 | 62.9 | 18.4 | 18.4 | 18.4 |
| 1‴ |  | 106.5 |  | 106.5 | 111.0 |  |  |  |  |  |  |
| 2‴ |  | 74.6 |  | 74.6 | 77.4 |  |  |  |  |  |  |
| 3‴ |  | 78.3 |  | 78.3 | 80.1 |  |  |  |  |  |  |
| 4‴ |  | 71.0 |  | 71.0 | 64.7 |  |  |  |  |  |  |
| 5‴ |  | 67.0 |  | 67.0 | 75.2 |  |  |  |  |  |  |
| 6″ |  |  |  |  |  |  | 126.2 | 126.2 |  |  |  |
| 2‴ |  |  |  |  |  |  | 107.9 | 107.9 |  |  |  |
| 3‴ |  |  |  |  |  |  | 153.8 | 153.8 |  |  |  |
| 4‴ |  |  |  |  |  |  | 143.1 | 143.1 |  |  |  |
| 5‴ |  |  |  |  |  |  | 153.8 | 153.8 |  |  |  |
| 6‴ |  |  |  |  |  |  | 107.9 | 107.9 |  |  |  |
| 7‴ |  |  |  |  |  |  | 166.3 | 166.3 |  |  |  |
| OMe |  |  |  |  |  |  | 60.7 | 60.6 |  |  |  |
| OMe |  |  |  |  |  |  | 56.2 × 2 | 56.2 × 2 |  |  |  |
| Ac |  |  |  |  | 170.5 |  |  |  |  |  |  |
|  |  |  |  |  | 20.8 |  |  |  |  |  |  |

closely related to that of **12**. The $^1$H NMR spectrum of **14** showed two three-proton singlets at $\delta$ 1.71 and 1.68 attributable to methyl groups on a double bond, and catalytic hydrogenation of **14** over $PtO_2$ yielded **12**. Thus, **14** was defined as (22*S*)-16$\beta$-[(α-L-rhamnopyranosyl)oxy]-3$\beta$,22-dihydroxycholesta-5,24-dien-1$\beta$-yl α-L-rhamnopyranoside.

The isolated compounds (**1**−**14**) were evaluated for their cytotoxic activities against HL-60 luekemia cells and HSC-2 cells, using a modified MTT assay method. Compounds **1**−**4**, **7**, **10**, and **14** showed moderate cytotoxic activity against HL-60 cells with $IC_{50}$ values ranging from 1.6 to 5.3 $\mu$g/mL, when that of etoposide used as positive control was 0.23 $\mu$g/mL. As for cytotoxicty against HSC-2 cells, which were resistant to etoposide (24.4 $\mu$g/mL), **1**−**3** were also cytotoxic, with $IC_{50}$ values ranging from 19.9 to 34.6 $\mu$g/mL.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer and UV spectra on a JASCO V-520 instrument. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for $^1$H NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as $\delta$-values with reference to tetramethylsilane (TMS) as internal standard. ESIMS data were obtained on a Micromass LCT mass spectrometer (Manchester, UK). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 $F_{254}$ (0.25 mm, Merck, Darmstadt, Germany) and RP-18 $F_{254}$ S (0.25 mm thick, Merck) plates, and spots were visualized

**Table 2.** Cytotoxic Activities of **1**−**14** and Etoposide against HL-60 and HSC-2 Cells

| | IC$_{50}$ ($\mu$g/mL) | |
|---|---|---|
| compound | HL-60 | HSC-2 |
| **1** | 1.6 | 34.6 |
| **2** | 2.2 | 19.9 |
| **3** | 1.9 | 21.2 |
| **4** | 5.2 | >100 |
| **5** | >10 | >100 |
| **6** | >10 | >100 |
| **7** | 5.3 | >100 |
| **8** | >10 | >100 |
| **9** | >10 | >100 |
| **10** | 5.0 | >100 |
| **11** | >10 | >100 |
| **12** | >10 | >100 |
| **13** | >10 | >100 |
| **14** | 5.1 | >100 |
| etoposide | 0.23 | 24.4 |

by spraying with 10% $H_2SO_4$ followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh) or a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port. A Capcell Pak C$_{18}$ UG120 column (10 mm i.d. × 250 mm, 5 $\mu$m, Shiseido, Tokyo, Japan) was employed for preparative HPLC. The following reagents were obtained from the indicated companies: RPMI 1640 medium and Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY); fetal bovine serum (FBS) (Bio-Whittaker, Walkersville MD or JRH Biosciences, Lenexa, KS); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma, St. Louis, MO); penicillin and streptomycin (Sigma or Meiji-Seika, Tokyo, Japan). All other chemicals used were of biochemical reagent grade.

**Plant Material.** The bulbs of *O. thyrsoides* were purchased from a nursery in Heiwaen, Nara, Japan. The bulbs were cultivated, and the flowered plant was identified by one of the authors (Y.S.). A voucher specimen of the plant has been deposited in our laboratory (99-4-OT).

**Extraction and Isolation.** The plant material (fresh weight, 15.4 kg) was extracted with hot MeOH (twice). The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (702 g) was passed through a Diaion HP-20 column, successively eluted with 30% MeOH, 50% MeOH, and EtOH. Column chromatography of the EtOH eluate portion on silica gel and elution with a stepwise gradient mixture of $CHCl_3$−MeOH−$H_2O$ (90:10:0; 40:10:1; 20:10:1) and finally with MeOH alone gave four fractions (I, II, III, and IV). Fraction II was subjected to a silica gel column eluted with $CHCl_3$−MeOH−$H_2O$ (60:10:1) to collect two subfractions (IIa and IIb). Fraction IIa was chromatographed on silica gel eluted with $CHCl_3$−MeOH−$H_2O$ (80:10:1; 70:10:1) and ODS silica gel with MeOH−$H_2O$ (4:1) to yield **1** (71.1 mg) and **2** (112 mg). Fraction IIb was subjected to an ODS silica gel column eluted with MeCN−$H_2O$ (1:2) and a silica gel column with $CHCl_3$−MeOH−$H_2O$ (60:10:1) and was divided into four further fractions (IIb-1, IIb-2, IIb-3, and IIb-4). Fraction IIb-1 was subjected to a silica gel column eluted with $CHCl_3$−MeOH−$H_2O$ (60:10:1) and preparative HPLC using MeCN−$H_2O$ (2:3) to give **4** (28.5 mg). Fraction IIb-2 was chromatographed on silica gel eluted with $CHCl_3$−MeOH−$H_2O$ (80:10:1) and ODS silica gel with MeCN−$H_2O$ (2:5) to give a mixture of **7** and **14**, which was then separated by preparative HPLC using MeCN−$H_2O$ (1:2) to yield **7** (50.0 mg) and **14** (14.1 mg). Fraction IIb-3 was subjected to a silica gel column eluted with $CHCl_3$−MeOH−$H_2O$ (80:10:1) and an ODS silica gel column with MeCN−$H_2O$ (2:5) to give **10** (102 mg) and **13** (26.9 mg) in pure form and a mixture of **11** and **12**. The mixture was separated by preparative HPLC using MeCN−$H_2O$ (1:2) to furnish **11** (19.7 mg) and **12** (11.7 mg). Fraction IIb-4 was subjected to an ODS silica gel column eluted with MeCN−$H_2O$ (2:5) and preparative HPLC using MeCN−$H_2O$ (2:5) to yield **6** (22.5 mg) and **8** (17.6 mg). Fraction III was separated by silica gel column chromatography eluted with $CHCl_3$−MeOH−$H_2O$ (40:10:1) and ODS silica gel column chromatography with MeCN−$H_2O$ (1:2) to afford four subfractions (IIIa, IIIb, IIIc, and IIId). Fraction IIIb was subjected to a silica gel column eluted with $CHCl_3$−MeOH−$H_2O$ (60:10:1) and an ODS silica gel column with MeCN−$H_2O$ (1:2) to give **3** (33.9 mg). Fraction IIId was separated by a silica gel column eluted with $CHCl_3$−MeOH−$H_2O$ (40:10:1; 30:10:1) and an ODS silica gel column with MeCN−$H_2O$ (2:3; 2:5) to give **5** and **9** with a few impurities. Final purification of **5** and **9** was established by preparative HPLC using MeCN−$H_2O$ (2:3; 2:5) to yield **5** (22.1 mg) and **9** (14.3 mg).

**Compound 1:** amorphous solid; $[\alpha]_D^{25}$ −68.0° (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3363 (OH), 2950, 2926, and 2875 (CH), 1073, 1055, 1025 cm$^{-1}$; $^1$H NMR ($C_5D_5N$) $\delta$ 5.05 (1H, d, $J$ = 7.7 Hz, H-1′), 4.59 (1H, dd, $J$ = 11.5, 3.3 Hz, H-6′a), 4.54 (1H, m, H-16), 4.36 (1H, dd, $J$ = 11.5, 5.8 Hz, H-6′b), 4.24 (1H, dd, $J$ = 8.9, 8.9 Hz, H-3′), 4.15 (1H, dd, $J$ = 8.9, 8.9 Hz, H-4′), 4.03 (1H, dd, $J$ = 8.9, 7.7 Hz, H-2′), 3.98 (1H, dd, $J$ = 11.5, 4.2 Hz, H-1), 3.90 (1H, br m, $W_{1/2}$ = 22.3 Hz, H-3), 3.88 (1H, ddd, $J$ = 8.9, 5.8, 3.3 Hz, H-5′), 3.58 (1H, dd, $J$ = 10.6, 2.9 Hz, H-26eq), 3.51 (1H, dd, $J$ = 10.6, 10.6 Hz, H-26ax), 1.10 (3H, d, $J$ = 6.9 Hz, Me-21), 1.03 (3H, s, Me-19), 0.85 (3H, s, Me-18), 0.70 (3H, d, $J$ = 5.3 Hz, Me-27); HRESIMS *m/z* 595.3836 $[M + H]^+$ (calcd for $C_{33}H_{55}O_9$, 595.3846).

**Acid Hydrolysis of 1.** A solution of **1** (20.0 mg) in 1 M HCl (dioxane−$H_2O$, 1:1, 5 mL) was heated at 95 °C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and chromatographed on Diaion HP-20 eluted with $H_2O$−MeOH (3:2) followed by $Me_2CO$−EtOH (1:1) to give an aglycone fraction and a sugar fraction (5.0 mg). The aglycone fraction was chromatographed on silica gel eluted with $CHCl_3$−MeOH (19:1) to give **1a** (12.1 mg). The sugar fraction was passed through a Sep-Pak C$_{18}$ cartridge (Waters, Milford, MA) and a Toyopak IC-SP-M cartridge (Tosoh), which was then analyzed by HPLC under the following conditions: column, a Capcell Pak C$_{18}$ UG80 (4.6 mm i.d. × 250 mm, 5 $\mu$m, Shiseido); solvent, MeCN−$H_2O$ (17:3); flow rate, 0.9 mL/min; detection, RI and OR. Identification of D-glucose present in the sugar fraction was carried out by the comparison of its retention time and polarity with that of an authentic sample; $t_R$ (min) 17.57 (D-glucose, positive polarity).

**Compound 3:** amorphous solid; $[\alpha]_D^{25}$ −54.0° (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3377 (OH), 2950, 2928 and 2905 (CH), 1090, 1050 cm$^{-1}$; $^1$H NMR ($C_5D_5N$) $\delta$ 6.34 (1H, br s, H-1″), 5.58 (1H, br d, $J$ = 5.7 Hz, H-6), 4.98 (1H, d, $J$ = 7.5 Hz, H-1‴), 4.73 (1H, d, $J$ = 7.3 Hz, H-1′), 4.52 (1H, q-like, $J$ = 7.4 Hz, H-16), 3.89 (1H, m, $W_{1/2}$ = 21.2 Hz, H-3), 3.82 (1H, dd, $J$ = 12.0, 3.9 Hz, H-1), 3.56 (1H, dd, $J$ = 10.5, 2.6 Hz, H-26eq), 3.48 (1H, dd, $J$ = 10.5, 10.5 Hz, H-26ax), 1.74 (3H, d, $J$ = 6.2 Hz, Me-6″), 1.43 (3H, s, Me-19), 1.09 (3H, d, $J$ = 7.0 Hz, Me-21), 0.85 (3H, s, Me-18), 0.68 (3H, d, $J$ = 5.3 Hz, Me-27); HRESIMS *m/z* 863.4404 $[M + Na]^+$ (calcd for $C_{43}H_{68}O_{16}Na$, 863.4405).

**Acid Hydrolysis of 3.** A solution of **3** (25.0 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone fraction and a sugar fraction (11.2 mg). The aglycone fraction was chromatographed on silica gel eluted with $CHCl_3$−MeOH (19:1) to give **3a** (10.1 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of L-arabinose, L-rhamnose, and D-xylose; $t_R$ (min) 8.71 (L-rhamnose, negative polarity); 10.43 (L-arabinose, positive polarity); 11.04 (D-xylose, positive polarity).

**Compound 4:** amorphous solid; $[\alpha]_D^{23}$ −72.0° (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3386 (OH), 2951, 2927 and 2906 (CH), 1084, 1054, 1037 cm$^{-1}$; $^1$H NMR ($C_5D_5N$) $\delta$ 6.34 (1H, br s, H-1″), 5.58 (1H, br d, $J$ = 5.7 Hz, H-6), 4.73 (1H, d, $J$ = 7.3 Hz, H-1′), 4.54 (1H, q-like, $J$ = 7.4 Hz, H-16), 4.01 (1H, ddd, $J$ = 12.2, 10.5, 4.7 Hz, H-24), 3.87 (1H, m, $W_{1/2}$ = 19.5 Hz, H-3), 3.84 (1H, dd, $J$ = 12.1, 3.9 Hz, H-1), 3.69 (1H, dd, $J$ = 11.3, 4.8 Hz, H-26eq), 3.59 (1H, dd, $J = 11.3$, 11.3 Hz, H-26ax), 2.31 (1H, dd, $J = 12.7$, 4.7 Hz, H-23eq), 1.98 (1H, dd, $J = 12.7$, 12.2 Hz, H-23ax), 1.82 (1H, m, H-25), 1.75 (3H, d, $J = 6.1$ Hz, Me-6″), 1.44 (3H, s, Me-19), 1.12 (3H, d, $J = 7.0$ Hz, Me-21), 1.08 (3H, d, $J = 6.5$ Hz, Me-27), 0.86 (3H, s, Me-18); HRESIMS *m/z* 747.3935 $[M + Na]^+$ (calcd for $C_{38}H_{60}O_{13}Na$, 747.3932).

**Acid Hydrolysis of 4.** A solution of **4** (5.0 mg) was subjected to acid hydrolysis as described for **1** to give a mixture of several decomposed sapogenols and a sugar fraction (1.5 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of L-arabinose and L-rhamnose.

**Acetylation of 4.** Compound **4** (8.5 mg) was treated with $Ac_2O$ (2 mL) and pyridine (1 mL) in the presence of 4-(dimethylamino)pyridine (8.0 mg) as catalyst at room temperature for 12 h. After addition of $H_2O$ (3 mL) into the reaction mixture followed by evaporation to dryness, it was chromatographed on silica gel eluted with hexane−$Me_2CO$ (5:2) to give the corresponding heptaacetate (**4a**, 8.3 mg).

**Compound 4a**: amorphous solid; IR (film) $\nu_{max}$ 2952 (CH), 1739 (C═O), 1038 $m^{-1}$; $^1H$ NMR ($C_5D_5N$) $\delta$ 5.63 (1H, br d, $J = 5.6$ Hz, H-6), 5.60 (1H, br s, H-1″), 5.20 (1H, ddd, $J = 10.9$, 10.9, 4.8 Hz, H-24), 4.87 (1H, m, $W_{1/2} = 19.8$ Hz, H-3), 4.63 (1H, d, $J = 7.6$ Hz, H-1′), 4.51 (1H, t-like, $J = 7.3$ Hz, H-16), 3.68 (1H, dd, $J = 12.0$, 4.0 Hz, H-1), 3.63 (1H, dd, $J = 11.3$, 5.0 Hz, H-26eq), 3.55 (1H, dd, $J = 11.3$, 11.3 Hz, H-26ax), 2.20, 2.18, 2.15, 2.09, 2.08, 2.03, and 2.02 (each 3H, s, Ac × 7), 1.48 (3H, d, $J = 6.2$ Hz, Me-6″), 1.29 (3H, s, Me-19), 1.13 (3H, d, $J = 6.9$ Hz, Me-21), 0.89 (3H, s, Me-18), 0.80 (3H, d, $J = 6.5$ Hz, Me-27).

**Compound 5:** amorphous solid; $[\alpha]_D^{26}$ −48.0° (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3386 (OH), 2950, 2928 and 2905 (CH), 1047 $cm^{-1}$; $^1H$ NMR ($C_5D_5N$) $\delta$ 6.34 (1H, br s, H-1″), 5.58 (1H, br d, $J = 5.7$ Hz, H-6), 4.98 (1H, d, $J = 7.5$ Hz, H-1‴), 4.74 (1H, d, $J = 7.3$ Hz, H-1′), 4.54 (1H, q-like, $J = 7.4$ Hz, H-16), 4.01 (1H, ddd, $J = 12.1$, 10.3, 4.7 Hz, H-24), 3.88 (1H, m, $W_{1/2} = 20.0$ Hz, H-3), 3.83 (1H, dd, $J = 12.0$, 3.9 Hz, H-1), 3.71 (1H, dd, $J = 11.3$, 3.3 Hz, H-26eq), 3.59 (1H, dd, $J = 11.3$, 11.3 Hz, H-26ax), 2.30 (1H, dd, $J = 12.7$, 4.7 Hz, H-23eq), 1.97 (1H, dd, $J = 12.7$, 12.1 Hz, H-23ax), 1.82 (1H, m, H-25), 1.74 (3H, d, $J = 6.2$ Hz, Me-6″), 1.42 (3H, s, Me-19), 1.12 (3H, d, $J = 6.9$ Hz, Me-21), 1.08 (3H, d, $J = 6.5$ Hz, Me-27), 0.85 (3H, s, Me-18); HRESIMS *m/z* 879.4302 $[M + Na]^+$ (calcd for $C_{43}H_{68}O_{17}Na$, 879.4354).

**Acid Hydrolysis of 5.** A solution of **5** (6.0 mg) was subjected to acid hydrolysis as described for **1** to a give a mixture of several decomposed sapogenols and a sugar fraction (1.5 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of L-arabinose, L-rhamnose, and D-xylose.

**Compound 8:** amorphous solid; $[\alpha]_D^{26}$ −18.0° (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3376 (OH), 2929 and 2874 (CH), 1731 (C═O), 1036 $cm^{-1}$; $^1H$ NMR ($C_5D_5N$) $\delta$ 5.92 (1H, br s, H-1′), 5.78 (1H, d, $J = 3.6$ Hz, H-1‴), 4.76 (1H, d, $J = 3.6$ Hz, H-2‴), 4.75 (1H, dd, $J = 9.6$, 2.1 Hz, H-6″a), 4.73 (1H, dd, $J = 9.6$, 9.2 Hz, H-6″b), 4.73 (1H, d, $J = 9.2$ Hz, H-5‴a), 4.60 (1H, d, $J = 7.8$ Hz, H-1″), 4.50 (1H, m, H-16), 4.31 (1H, d, $J = 9.2$ Hz, H-5‴b), 4.25 (1H, br d, $J = 8.6$ Hz, H-22), 4.15 (1H, d, $J = 11.3$ Hz, H-4‴a), 4.12 (1H, d, $J = 11.3$ Hz, H-4‴b), 3.88 (1H, m, H-3), 3.72 (1H, dd, $J = 11.4$, 4.2 Hz, H-1), 2.05 (3H, s, Ac), 1.68 (3H, d, $J = 6.0$ Hz, Me-6′), 1.14 (3H, d, $J = 7.0$ Hz, Me-21), 0.98 (3H, s, Me-19), 0.97 (3H, s, Me-18), 0.95 (3H, d, $J = 6.2$ Hz, Me-26), 0.93 (3H, d, $J = 6.2$ Hz, Me-27); HRESIMS *m/z* 941.5048 $[M + Na]^+$ (calcd for $C_{46}H_{78}O_{18}Na$, 941.5086).

**Acid Hydrolysis of 8.** A solution of **8** (6.0 mg) in 0.2 M HCl (dioxiane−$H_2O$, 1:1, 2 mL) was heated at 95 °C for 1 h under an Ar atmosphere, and the hydrolysate was treated as described for **1** to give an aglycone fraction and a sugar fraction (1.9 mg). The aglycone fraction was chromatographed on silica gel eluted with $CHCl_3$−MeOH (19:1) to give **8a** (1.2 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-apiose, D-glucose, and L-rhamnose; $t_R$ (min) 8.01 (D-apiose, positive polarity), 8.69 (L-rhamnose, negative polarity), 17.55 (D-glucose, positive polarity).

**Compound 9:** amorphous solid; $[\alpha]_D^{24}$ −20.0° (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3357 (OH), 2926 and 2870 (CH), 1074 $cm^{-1}$; $^1H$ NMR ($C_5D_5N$) $\delta$ 5.06 (1H, d, $J = 7.6$ Hz, H-1′), 4.75 (1H, d, $J = 7.8$ Hz, H-1″), 4.51 (1H, m, H-16), 4.30 (1H, br d, $J = 8.9$ Hz, H-22), 3.98 (1H, dd, $J = 11.5$, 4.2 Hz, H-1), 3.93 (1H, m, $W_{1/2} = 23.9$ Hz, H-3), 1.13 (3H, d, $J = 7.0$ Hz, Me-21), 1.02 (3H, s, Me-19), 0.96 (3H, s, Me-18), 0.93 (3H, d, $J = 6.2$ Hz, Me-26), 0.92 (3H, d, $J = 6.2$ Hz, Me-27); HRESIMS *m/z* 783.4490 $[M + Na]^+$ (calcd for $C_{39}H_{68}O_{14}Na$, 783.4507).

**Acid Hydrolysis of 9.** A solution of **9** (5.0 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone fraction and a sugar fraction (1.1 mg). The aglycone fraction was chromatographed on silica gel eluted with $CHCl_3$−MeOH (19:1) to give **8a** (1.8 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose.

**Compound 10:** amorphous solid; $[\alpha]_D^{22}$ +2.0° (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3386 (OH), 2935 and 2876 (CH), 1716 (C═O), 1590, 1504 and 1459 (aromatic ring), 1076, 1045 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 266 nm (log $\epsilon$ 4.01); $^1H$ NMR ($C_5D_5N$) $\delta$ 7.71 (2H, s, H-2‴ and H-6‴), 5.15 (1H, dd, $J = 11.4$, 7.3 Hz, H-6′a), 5.08 (1H, d, $J = 11.4$, 2.1 Hz, H-6′b), 4.97 (1H, d, $J = 7.8$ Hz, H-1′), 4.75 (1H, d, $J = 7.8$ Hz, H-1″), 4.52 (1H, m, H-16), 4.28 (1H, m, H-22), 3.97 (3H, s, OMe), 3.89 (1H, m, H-3), 3.85 (3H × 2, s, OMe), 3.82 (1H, dd, $J = 11.6$, 4.0 Hz, H-1), 1.07 (3H, d, $J = 7.0$ Hz, Me-21), 1.00 (3H, s, Me-19), 0.97 (3H, s, Me-18), 0.93 (3H × 2, d, $J = 6.2$ Hz, Me-26 and Me-27); HRESIMS *m/z* 977.5065 $[M + Na]^+$ (calcd for $C_{49}H_{78}O_{18}Na$, 977.5086).

**Alkaline Hydrolysis of 10.** Compound **10** (20.0 mg) was treated with 4% KOH in EtOH (7 mL) at room temperature for 1 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B (Organo) column and chromatographed on silica gel eluted with $CHCl_3$−MeOH−$H_2O$ (40:10:1) to give **9** (11.5 mg) and 3,4,5-trimethoxybenzoic acid (1.1 mg).

**Compound 11:** amorphous solid; $[\alpha]_D^{24}$ +10.0° (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3377 (OH), 2959, 2927 and 2857 (CH), 1715 (C═O), 1591, 1505 and 1457 (aromatic ring), 1077, 1044 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 266 nm (log $\epsilon$ 3.99); $^1H$ NMR ($C_5D_5N$) $\delta$ 7.58 (2H, s, H-2‴ and H-6‴), 5.86 (1H, br t, $J = 8.5$ Hz, H-24), 5.15 (1H, dd, $J = 11.4$, 6.9 Hz, H-6′a), 5.08 (1H, d, $J = 11.4$, 2.1 Hz, H-6′b), 4.97 (1H, d, $J = 7.7$ Hz, H-1′), 4.75 (1H, d, $J = 7.8$ Hz, H-1″), 4.54 (1H, m, H-16), 4.13 (1H, m, H-22), 3.97 (3H, s, OMe), 3.89 (1H, m, H-3), 3.84 (3H × 2, s, OMe), 3.83 (1H, dd, $J = 11.5$, 3.9 Hz, H-1), 1.74 (3H, s, Me-26), 1.66 (3H, s, Me-27), 1.10 (3H, d, $J = 7.0$ Hz, Me-21), 0.99 (3H, s, Me-19), 0.95 (3H, s, Me-18); HRESIMS *m/z* 975.4915 $[M + Na]^+$ (calcd for $C_{49}H_{76}O_{18}Na$, 975.4929).

**Catalytic Hydrogenation of 11.** A mixture of **11** (5.5 mg) and $PtO_2$ (6.0 mg) was stirred in an $H_2$ atmosphere at room temperature for 12 h. The reaction mixture, after removal of the catalyst by filtration, was subjected to a silica gel column eluted with $CHCl_3$−MeOH−$H_2O$ (40:10:1) to give **9** (1.8 mg).

**Compound 12:** amorphous solid; $[\alpha]_D^{24}$ −50.0° (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3376 (OH), 2933 and 2878 (CH), 1071, 1048 $cm^{-1}$; $^1H$ NMR ($C_5D_5N$) $\delta$ 5.66 (1H, br s, H-1′), 5.63 (1H, br d, $J = 5.6$ Hz, H-6), 5.27 (1H, br s, H-1″), 4.39 (1H, m, H-16), 4.01 (1H, br d, $J = 9.2$ Hz, H-22), 3.89 (1H, m, $W_{1/2} = 21.5$ Hz, H-3), 3.82 (1H, dd, $J = 11.5$, 3.6 Hz, H-1), 1.70 (3H, d, $J = 4.8$ Hz, Me-6″), 1.67 (3H, d, $J = 6.0$ Hz, Me-6′), 1.28 (3H, s, Me-19), 1.17 (3H, d, $J = 6.9$ Hz, Me-21), 1.00 (3H, s, Me-18), 0.86 (3H × 2, d, $J = 6.5$ Hz, Me-26 and Me-27); HRESIMS *m/z* 727.4636 $[M + H]^+$ (calcd for $C_{39}H_{67}O_{12}$, 727.4633).

**Acid Hydrolysis of 12.** A solution of **12** (5.0 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone fraction and a sugar fraction (1.1 mg). The aglycone fraction was chromatographed on silica gel eluted with $CHCl_3$−MeOH (19:1) to give **12a** (1.8 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of L-rhamnose.

**Compound 13:** amorphous solid; $[\alpha]_D^{26}$ $-36.0°$ (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3376 (OH), 2968, 2926 and 2856 (CH), 1052 cm$^{-1}$; $^1$H NMR ($C_5D_5N$) $\delta$ 5.61 (1H, br s, H-1′), 5.55 (1H, br t, $J$ = 7.1 Hz, H-24), 5.25 (1H, br s, H-1″), 4.38 (1H, m, H-16), 4.11 (1H, br t, $J$ = 6.8 Hz, H-22), 3.89 (1H, m, $W_{1/2}$ = 22.3 Hz, H-3), 3.73 (1H, dd, $J$ = 11.4, 4.1 Hz, H-1), 1.70 (3H, s, Me-26), 1.69 (3H, d, $J$ = 5.8 Hz, Me-6′), 1.67 (3H, s, Me-27), 1.66 (3H, d, $J$ = 6.0 Hz, Me-6″), 1.20 (3H, d, $J$ = 6.9 Hz, Me-21), 1.03 (3H, s, Me-19), 0.95 (3H, s, Me-18); HRESIMS $m/z$ 749.4474 [M + Na]$^+$ (calcd for $C_{39}H_{66}O_{12}Na$, 749.4452).

**Acid Hydrolysis of 13.** A solution of **13** (8.0 mg) was subjected to acid hydrolysis as described for **1** to a give a mixture of several decomposed sapogenols and a sugar fraction (2.5 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of L-rhamnose.

**Compound 14:** amorphous solid; $[\alpha]_D^{30}$ $-30.0°$ (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3322 (OH), 2922, 2881 and 2854 (CH), 1066, 1043 cm$^{-1}$; $^1$H NMR ($C_5D_5N$) $\delta$ 5.65 (1H, br s, H-1′), 5.62 (1H, br d, $J$ = 6.2 Hz, H-6), 5.55 (1H, br t, $J$ = 7.1 Hz, H-24), 5.25 (1H, d, $J$ = 0.7 Hz, H-1″), 4.38 (1H, m, H-16), 4.11 (1H, br t, $J$ = 6.7 Hz, H-22), 3.89 (1H, m, $W_{1/2}$ = 23.5 Hz, H-3), 3.81 (1H, dd, $J$ = 11.5, 3.6 Hz, H-1), 1.71 (3H, s, Me-26), 1.69 (3H, d, $J$ = 6.3 Hz, Me-6′), 1.68 (3H, s, Me-27), 1.66 (3H, d, $J$ = 6.1 Hz, Me-6″), 1.30 (3H, s, Me-19), 1.19 (3H, d, $J$ = 6.9 Hz, Me-21), 0.98 (3H, s, Me-18); HRESIMS $m/z$ 747.4266 [M + Na]$^+$ (calcd for $C_{39}H_{64}O_{12}Na$, 747.4295).

**Catalytic Hydrogenation of 14.** A mixture of **14** (4.8 mg) and $PtO_2$ (5.0 mg) was stirred in an $H_2$ atmosphere at room temperature for 12 h. The reaction mixture, after removal of the catalyst by filtration, was subjected to an ODS silica gel column eluted with $MeCN-H_2O$ (1:1) to give **12** (1.8 mg).

**HL-60 Cell Culture Assay.** HL-60 cells, which were obtained from Human Science Research Resources Bank (JCRB 0085, Osaka, Japan), were maintained in the RPMI 1640 medium containing heat-inactivated 10% FBS supplemented with L-glutamine, 100 units/mL penicillin, and 100 $\mu$g/mL streptomycin. The leukemia cells were washed and resuspended in the above medium to $4 \times 10^4$ cells/mL, and 196 $\mu$L of this cell suspension was placed in each well of a 96-well flat-bottom plate (Iwaki Glass, Chiba, Japan). The cells were incubated in 5% $CO_2$ air for 24 h at 37 °C. After incubation, 4 $\mu$L of $EtOH-H_2O$ (1:1) solution containing the sample was added to give the final concentrations of 0.1−20 $\mu$g/mL; 4 $\mu$L of $EtOH-H_2O$ (1:1) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated by an MTT assay procedure.[8] At the end of incubation, 10 $\mu$L of 5 mg/mL MTT in phosphate-buffered saline (PBS) was added to every well and the plate was further incubated in 5% $CO^2$/air for 4 h at 37 °C. The plate was then centrifuged at 1500*g* for 5 min to precipitate cells and formazan. An aliquot of 150 $\mu$L of the supernatant was removed from every well, and 175 $\mu$L of DMSO was added to dissolve the MTT formazan crystals. The plate was mixed on a microshaker for 10 min and then read on a microplate reader (Spectra Classic, Tecan, Salzburg, Austria) at 550 nm. Each assay was done in triplicate, and cytotoxicity was expressed as $IC_{50}$ value, which reduced the viable cell number by 50%.

**HSC-2 Cell Culture Assay.** HSC-2 cells were maintained as monolayer cultures at 37 °C in DMEM supplemented with 10% heat-inactivated FBS in a humidified 5% $CO_2$ atmosphere. Cells were trypsinized, inoculated at $6 \times 10^3$ per each 96-microwell plate (Falcon, flat bottom, treated polystyrene, Becton Dickinson, San Jose, CA), and incubated for 24 h. After washing once with PBS, they were treated for 24 h without or with test compounds. They were washed once with PBS and incubated for 4 h with 0.2 mg/mL MTT in DMEM supplemented with 10% FBS. After the medium was removed, the cells were lysed with 0.1 mL of DMSO and the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate, using Labsystems Multiskan (Biochromatic, Helsinki, Finland) connected to a Star dot matrix printer JL-10.[9,10]

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## References and Notes

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