# *cis*-Eudesmane Sesquiterpene Glycosides from *Liriope muscari* and *Ophiopogon japonicus*

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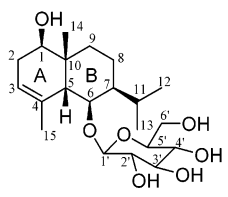
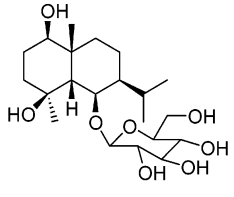
Two new *cis*-eudesmane sesquiterpene glycosides, liriopeoside A (**1**) and ophiopogonoside A (**2**), were extracted and purified from tubers of *Liriope muscari* and *Ophiopogon japonicus*, respectively, along with three known compounds. Their structures were elucidated as 1*β*,6*β*-dihydroxy-*cis*-eudesm-3-ene-6-*O*-*β*-D-glucopyranoside (**1**) and 1*β*,4*β*,6*β*-trihydroxy-*cis*-eudesmane-6-*O*-*β*-D-glucopyranoside (**2**) by spectral data analysis. The structure and the relative configuration of compound **1** were confirmed by X-ray crystallographic analysis. This is the first time that *cis*-eudesmane-type sesquiterpenes have been reported from the genera *Ophiopogon* and *Liriope*.

Ophiopogon japonicus (Thunb.) Ker.-Gawl. (Liliaceae) is an evergreen perennial widely distributed in mainland China, especially in Zhejiang and Sichuan Provinces, where it is known as "Zhemaidong" and "Chuanmaidong", respectively. Its tubers have been employed in traditional Chinese medicine as an expectorant, antitussive, and tonic agent as well as showing pharmacological effects on the cardiovascular system.[1] *Liriope muscari* (Decn.) Bailey (Liliaceae), the second plant studied, is used as a substitute for *O. japonicus* and is distributed in Fujian Province, People's Republic of China.[2] A number of studies have shown that steroidal saponins are present in both plants, while homoisoflavonoids are found only in *O. japonicus*.[3−7] As part of our continued investigation of the constituents of these two plants, phytochemical studies of the ether extracts were carried out. In this paper, we report the isolation and structure elucidation of two new *cis*-eudesmane sesquiterpene glycosides, liriopeoside A (**1**) and ophiopogonoside A (**2**), along with three known compounds, oleanolic acid, ursolic acid, and *N*-*p*-coumaroyltyramine,[8] from *L. muscari* and oleanolic acid and *N*-*p*-coumaroyltyramine from *O. japonicus*, on the basis of 1D and 2D NMR and HRESIMS analysis. The relative configuration of **1** was further confirmed by X-ray crystallographic analysis.




**1**




**2**

Liriopeoside A (**1**) was obtained as colorless needles and analyzed for $C_{21}H_{36}O_7$ by $^{13}C$ NMR (DEPT) and positive-ion HRESIMS. Its IR spectrum exhibited absorption bands for hydroxyl (3431 $cm^{-1}$) and olefinic (1627 $cm^{-1}$) groups.

**Table 1.** $^{13}C$ NMR Spectral Data of Compounds **1** and **2** (125 MHz, $C_5D_5N$, $\delta$ values)

| C | **1** | **2** |
|---|---|---|
| 1 | 74.3 | 74.8 |
| 2 | 31.8 | 27.9 |
| 3 | 120.4 | 37.5 |
| 4 | 133.5 | 71.5 |
| 5 | 46.2 | 52.6 |
| 6 | 78.1 | 77.4 |
| 7 | 45.2 | 45.9 |
| 8 | 20.3 | 21.4 |
| 9 | 32.1 | 33.6 |
| 10 | 36.3 | 38.2 |
| 11 | 27.6 | 28.1 |
| 12 | 20.7 | 20.9 |
| 13 | 21.4 | 21.8 |
| 14 | 24.8 | 25.4 |
| 15 | 22.1 | 28.5 |
| 1′ | 106.2 | 106.6 |
| 2′ | 75.5 | 75.7 |
| 3′ | 77.6 | 77.7 |
| 4′ | 71.9 | 72.4 |
| 5′ | 78.3 | 78.5 |
| 6′ | 62.9 | 63.4 |

Preliminary inspection of the $^1H$ NMR spectrum of **1** led to the identification of the following representative signals: two methyl singlet protons at $\delta$ 1.43 and 1.63; two methyl doublet signal protons at $\delta$ 0.71 (d, $J = 6.8$ Hz) and 0.90 (d, $J = 6.5$ Hz); an olefinic proton at $\delta$ 5.17 (br s); an anomeric proton at $\delta$ 4.74 (d, $J = 7.7$ Hz); and two hydroxyl protons at $\delta$ 3.39 and 4.49 (each br s). The $^{13}C$ NMR spectrum displayed 21 signals, of which eight resonated in the region 60−80 ppm, and a methine signal at $\delta$ 106.2 suggested the presence of an anomeric carbon of a hexose residue. All the information mentioned above was in support of compound **1** being a sesquiterpene glycoside.[9] Acid hydrolysis of **1** with 1 M HCl produced a monosaccharide and an aglycon. The monosaccharide was determined to be glucose by high-performance TLC analysis comparison with an authentic sample. Complete $^1H$ and $^{13}C$ NMR assignments of **1** (Table 1) were made from its HMQC, HMBC, and DEPT spectra. The NMR data were found to be similar to those of the aglycon of a known eudesmane sesquiterpene, 6*β*-cinnamoyloxy-1*β*-hydroxy-eudesm-3-ene.[10] The diagnostic change of C-6 of **1** from the

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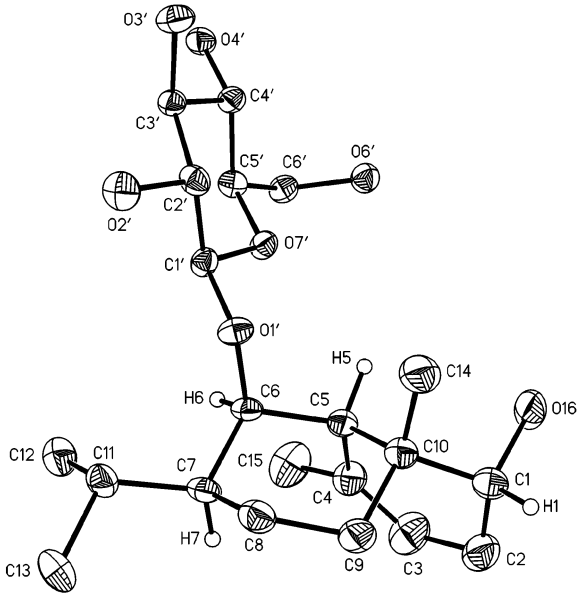


**Figure 1.** X-ray crystal structure of **1** showing the *cis*-A/B fusion and the relative stereochemistry of the hydroxyl and isopropyl groups.

reported data of 6$\beta$-cinnamoyloxy-1$\beta$-hydroxyeudesm-3-ene[10] indicated that the glucose of **1** was attached to C-6. The anomeric proton ($\delta$ 4.74) of the glucose giving a three-bond connection to the C-6 ($\delta$ 78.1) in the HMBC spectra confirmed the position of the glycosidic linkage.

A single-crystal X-ray diffraction study of **1** was carried out, from which its relative stereochemistry was determined. The ORTEP perspective drawing depicted in Figure 1 supports the deduction concerning this structure made by both NMR and mass spectrometry. The configurations at H-1, H-6, H-7, and C-14 (methyl) are α-, α-, α-, and $\beta$-oriented in **1**, which is consistent with those of 6$\beta$-cinnamoyloxy-1$\beta$-hydroxyeudesm-3-ene. The structure in Figure 1 clearly shows that H-5 and the C-14 (methyl) are on the same side of an A/B *cis*-decalin moiety, whereas in 6$\beta$-cinnamoyloxy-1$\beta$-hydroxyeudesm-3-ene the H-5 and the C-14 methyl group are on a different side of an A/B *trans*-decalin unit.[10] It is worthwhile mentioning that, in the NOESY spectrum of **1**, a correlation between H-5 and Me-14, characteristic of the *cis*-fusion of the A/B rings, was observed. Clear NOEs between H-1α and Me-14$\beta$, H-6α and Me-14$\beta$, and H-5$\beta$ and H-6α were also observed due to the *cis*-fusion of A/B, otherwise they should be absent in the *trans*-fusion as in 6$\beta$-cinnamoyloxy-1$\beta$-hydroxyeudesm-3-ene. In addition, the chemical shift of C-14 in **1** is 12.6 ppm ($\Delta_{cis-trans}$) downfield from the *trans*-eudesmane 6$\beta$-cinnamoyloxy-1$\beta$-hydroxyeudesm-3-ene, which indicated that the diagnostic difference of the $^{13}$C NMR data at C-14 could be used to distinguish *cis*- and *trans*-eudesmane. Therefore, we have established that **1** is 1$\beta$,6$\beta$-dihydroxy-*cis*-eudesm-3-ene-6-*O*-$\beta$-D-glucopyranoside. The *cis*-eudesmanes are not commonly found and so far have been reported only from *Parepigynum funingense*[11] and *Verbesina virginica*.[12] This is the first report of the isolation of a eudesmane-type skeleton from the genus *Liriope*.

Ophiopogonoside A (**2**) was obtained as a white amorphous powder, and its molecular formula was deduced as $C_{21}H_{38}O_8$ from its HRESIMS and $^{13}$C NMR data. The $^{13}$C NMR spectrum of **2** showed signals for a glucose group along with 15 carbon signals assigned to the aglycon moiety (Table 1). The $^{13}$C and $^1$H NMR assignments of an isopropyl group (C-11, C-12, and C-13) at C-7 and the fused cyclohexyl rings (C-1 to C-10) indicated **2** was also a eudesmane glycoside.[13] A comparison between the $^{13}$C NMR data of **2** and **1** revealed that the carbon signals of the aglycon of the two eudesmanes were almost identical, except that a carbon signal corresponding to a tertiary alcohol carbon ($\delta$ 71.5, C-4) in **2** was observed instead of an olefinic group in **1**. The NMR spectral data corresponding to the aglycon of **2** was also found similar to the reported eudesma-1$\beta$,4$\beta$,6$\beta$-triol.[13] The glycosidic linkage at C-6 was confirmed by the observed downfield shift in C-6 by 8.4 ppm ($\Delta_{cis-trans}$) with respect to the corresponding signal in eudesma-1$\beta$,4$\beta$,6$\beta$-triol. The relative configuration of **2** was deduced by the NOESY spectra and by comparing its $^{13}$C NMR spectral data with **1**. The cross-peak of H-5 and Me-14 observed in the NOESY spectrum and the downfield shift of C-14 in **2** by 10.8 ppm ($\Delta_{cis-trans}$) compared with eudesma-1$\beta$,4$\beta$,6$\beta$-triol strongly suggested it is an A/B ring *cis*-fusion, as in **1**. The chemical shifts of C-1, C-6, and C-7 in **2** and those in **1** are similar, indicating that the H-1, H-6, and H-7 are all α-oriented (Table 1). The agreement of C-15 in **2** ($\delta$ 28.5) and eudesma-1$\beta$,4$\beta$,6$\beta$-triol ($\delta$ 28.8) showed that the methyl group at C-4 is α-substituted.[13] Therefore compound **2** was elucidated as 1$\beta$,4$\beta$,6$\beta$-trihydroxy-*cis*-eudesmane-6-*O*-$\beta$-D-glucopyranoside. Again, this is the first sesquiterpene with a eudesmane skeleton found in the genus *Ophiopogon*.

## Experimental Section

**General Experimental Procedures.** Melting points were obtained on an X4 micro-melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. IR spectra were recorded on an Impact-410 (Nicolet) instrument. The $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectra were recorded on a Bruker AM-500 spectrometer, and chemical shifts are reported in ppm using the residual solvent peak as reference. The HRESIMS were obtained on a Bruker APIII MS instrument. Column chromatography was carried out on silica gel (200−300 mesh, Qingdao Marine Chemistry Company, Qingdao, People's Republic of China) and Sephadex LH-20 (Pharmacia, Sweden). High-performance TLC (HPTLC) was conducted on Si gel 60 $F_{254}$ plates (Merck, Germany).

**Plant Material.** The tubers of *O. japonicus* were collected from Hangzhou, Zhejiang Province, People's Republic of China, in August 2001. The tubers of *L. muscari* were collected in Quanzhou, Fujian Province, People's Republic of China, in September 2000. Both of these specimens were identified by Prof. Luo-Shan Xu, Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (Herbarium No. 1109) for *O. japonicus* and a voucher specimen (Herbarium No. 1123) for *L. muscari* both have been deposited at the Herbarium of the Department of Traditional Chinese Medicine, China Pharmaceutical University.

**Extraction and Isolation.** The dried tubers (50 kg) of *O. japonicus* were extracted with hot methanol (3 × 250 L). The extract was combined and evaporated to dryness in vacuo. The residue (1.0 kg) was dissolved in water and extracted with ether to afford a diethyl ether-soluble fraction, which was then chromatographed on a silica gel column and eluted with gradient mixtures of petroleum ether and EtOAc (10:1 to 2:1) to afford three fractions (I−III). Each fraction was further subjected to repeated silica gel column chromatography using mixtures of petroleum ether−EtOAc of increasing polarity (50:1 to 20:1) as eluents and then passed over a Sephadex LH-20 column eluted with MeOH−$CHCl_3$ (1:1). Fraction I (14.78 g) afforded oleanolic acid (8 mg). Fraction II (12.1 g) yielded *N*-*p*-coumaroyltyramine (50 mg), and fraction III (9.0 g) gave **2** (9 mg).

The dried tubers (50 kg) of *L. muscari* were extracted with hot methanol (3 × 250 L). The extract was combined and evaporated to dryness in vacuo. The residue (1.2 kg) was dissolved in water and extracted with diethyl ether. The ether-soluble fraction was concentrated in vacuo to afford a brown

residue (39.5 g), which was then chromatographed on a silica gel column and eluted with petroleum ether−EtOAc (25:1, 10:1, and 5:1) to afford three fractions (I−III). Fractions I and II were further subjected to repeated silica gel (200−300 mesh) column chromatography using mixtures of petroleum ether−EtOAc of increasing polarity (50:1 to 20:1) as eluents. Fraction I afforded oleanolic acid and ursolic acid (7 mg, as a mixture). Fraction II yielded *N*-*p*-coumaroyltyramine (43 mg). Fraction III was passed over Sephadex LH-20 eluted with MeOH−$CHCl_3$ (1:1) to give **1** (32 mg).

**Liriopeoside A (1):** colorless needles (MeOH); mp 169−171 °C; $[\alpha]^{25}_D$ −7.3° (*c* 1.00, MeOH); IR (KBr) $\nu_{max}$ 3431, 1627, 1055 $cm^{-1}$; $^1H$ NMR ($C_5D_5N$, 500 MHz) $\delta$ 3.70 (1H, t, $J$ = 3.2 Hz, H-1), 2.34 (1H, d, $J$ = 18.1 Hz, H-2), 1.34 (1H, m, H-2), 5.17 (1H, br s, H-3), 3.14 (1H, s, H-5), 4.79 (1H, t, $J$ = 2.6 Hz, H-6), 0.79 (1H, m, H-7), 1.54 (1H, m, H-8), 1.35 (1H, m, H-8), 2.09 (1H, d, $J$ = 18.0 Hz, H-9), 1.01 (1H, m, H-9), 1.99 (1H, m, H-11), 0.71 (3H, d, $J$ = 6.8 Hz, H-12), 0.90 (3H, d, $J$ = 6.5 Hz, H-13), 1.43 (3H, s, H-14), 1.63 (3H, s, H-15), 4.74 (1H, d, $J$ = 7.7 Hz, H-1′), 3.83 (1H, t, $J$ = 8.5 Hz, H-2′), 3.75 (1H, m, H-3′), 3.91 (1H, t, $J$ = 9.4 Hz, H-4′), 4.03 (1H, m, H-5′), 4.27 (1H, dd, $J$ = 2.8, 11.5 Hz, H-6′), 4.03 (1H, m, H-6′); $^{13}C$ NMR ($C_5D_5N$, 125 MHz), see Table 1; positive-ion HRESIMS *m*/*z* 423.2368 $[M + Na]^+$ (calcd for $C_{21}H_{36}O_7Na$, 423.2359); EIMS *m*/*z* 238 $[M - C_6H_{10}O_5]^+$ (2), 220 $[M - C_6H_{12}O_6]^+$ (97), 202 $[M - C_6H_{12}O_6 - H_2O]^+$ (14), 177 $[M - C_6H_{12}O_6 - C_3H_7]^+$ (100), 159 $[M - C_6H_{12}O_6 - C_3H_7 - H_2O]^+$ (37), 121 $[M - C_6H_{12}O_6 - C_3H_7 - H_2O - C_3H_2]^+$ (31), 107 $[M - C_6H_{12}O_6 - C_3H_7 - H_2O - C_4H_4]^+$ (47).

**Ophiopogonoside A (2):** white amorphous powder; mp 217−219 °C; $[\alpha]^{25}_D$ −3.4° (*c* 0.30, MeOH); $^1H$ NMR ($C_5D_5N$, 500 MHz) $\delta$ 3.68 (1H, t, $J$ = 3.1 Hz, H-1), 1.73 (1H, m, H-2), 2.53 (1H, ddd, $J$ = 3.4 Hz, H-3), 1.45 (1H, m, H-3), 3.06 (1H, s, H-5), 4.92 (1H, d, $J$ = 2.0 Hz, H-6), 1.31 (1H, m, H-7), 1.03 (1H, m, H-8), 1.72 (1H, m, H-9), 1.39 (1H, m, H-9), 2.13 (1H, m, H-11), 0.94 (3H, d, $J$ = 6.5 Hz, H-12), 0.75 (3H, d, $J$ = 6.8 Hz, H-13), 1.48 (3H, s, H-14), 1.32 (3H, s, H-15), 4.83 (1H, d, $J$ = 7.8 Hz, H-1′), 3.84 (1H, t, $J$ = 7.8 Hz, H-2′), 3.81 (1H, m, H-3′), 3.66 (1H, t, $J$ = 8.7 Hz, H-4′), 3.96 (1H, t, $J$ = 8.8 Hz, H-5′), 4.30 (1H, dd, $J$ = 15.8, 7.4 Hz, H-6′), 3.75 (1H, m, H-6′); $^{13}C$ NMR ($C_5D_5N$, 125 MHz), see Table 1; positive-ion HRESIMS *m*/*z* 441.2475 $[M + Na]^+$ (calcd for $C_{21}H_{38}O_8Na$, 441.2464); EIMS *m*/*z* 238 $[M - C_6H_{12}O_6]^+$ (8), 220 $[M - C_6H_{12}O_6 - H_2O]^+$ (25), 203 $[M - C_6H_{12}O_6 - 2H_2O + H]^+$ (33), 177 $[M - C_6H_{12}O_6 - H_2O - C_3H_7]^+$ (13), 101 (100).

**Acid Hydrolysis of 1 and 2.** Compounds **1** (2 mg) and **2** (1 mg) were heated with 1 M HCl at 80 °C for 4 h, respectively. After cooling, the two reaction mixtures were neutralized with 8% NaOH and partitioned between $CHCl_3$ and $H_2O$, respectively. The $H_2O$-soluble phase was concentrated and examined by HPTLC ($CHCl_3$−MeOH−$H_2O$, 65:35:10, lower layer) with $\beta$-D-glucose.

**X-ray Crystal Structure Analysis of Liriopeoside A (1).** Suitable crystals for X-ray diffraction were obtained by recrystallization from a methanol solution of **1**. A colorless crystal was mounted on a glass fiber using Paratone N oil. The X-ray intensity data were collected on a Siemens P4 diffractometer using graphite-monochromated Mo Kα radiation using a $\omega-2\theta$ scan. The data were corrected for Lorentz and polarization effects, and equivalent reflections were merged. The structure was solved by direct methods, and refinement was based on $F^2$.[15] The hydrogen atoms of the hydroxyl groups and the water of crystallization were located from a difference Fourier map and were included in structure factor calculations without refinement, the displacement parameters being set equal to $1.5U_{eq}$ of the oxygen atom. The remaining hydrogen atoms were placed in idealized positions with displacement parameters set equal to $1.2U_{eq}$ of the parent carbon atoms. In the final cycles of full-matrix least-squares refinement the nonhydrogen atoms were assigned anisotropic displacement parameters. The crystallogaphic data are summarized in Table S1 (Supporting Information), and the final fractional coordinates are listed in Table S2 (Supporting Information).[16]

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**Supporting Information Available:** Tables of crystallographic data and final fractional coordinates for **1**. These data are available free of charge via the Internet at http://pubs.acs.org.

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(16) Crystallographic data for compound **1** have been deposited at the Cambridge Crystallographic Data Center, Cambridge, U.K. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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