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A steroidal glycoside from *Lepisorus ussuriensis*

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Abstract

A new steroidal glycoside, 2α,3β-(22*R*)-trihydroxycholestan-6-one-22-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside, was isolated from the whole plants of *Lepisorus ussuriensis*, together with α-ecdysone and ecdysterone. Their structures were determined by means of spectroscopic and chemical methods. © 1999 Elsevier Science Ltd. All rights reserved.

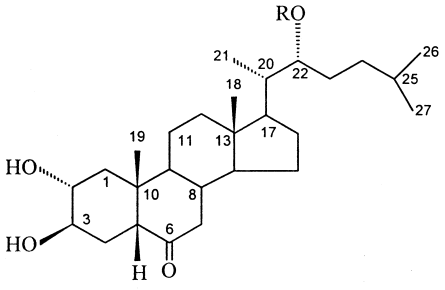
Keywords: *Lepisorus ussuriensis*; Polypodiaceae; Steroid glycoside; 2α,3β-(22*R*)-Trihydroxycholestane-6-one-22-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside; α-Ecdysone; Ecdysterone

1. Introduction

Lepisorus ussuriensis (Regel et Maack.) Ching (Polypodiaceae) is a herbal drug which is used in Korean folk medicine for its diuretic, hemostatic and antitussive activities. Previously, we reported a new flavonoid, quercetin 3-methyl ether-7-*O*-α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranoside, together with quercetin 3-methyl ether-7-*O*-β-D-glucopyranoside, vitexin, orientin and eriodyctiol-7-*O*-β-D-glucopyranoside as the constituents of *L. ussuriensis* (Choi, Lim, Yeo, & Kim, 1996). In a continuation of our investigation of *L. ussuriensis*, we report here the isolation and structural elucidation of a new steroidal glycoside.

2. Results and discussion

Compound **1** was isolated from the *n*-BuOH-soluble portion of an aqueous MeOH extract of the entire plants of *L. ussuriensis*. Its IR spectrum exhibited absorption bands at 3436 cm^{-1} (OH) and 1693 cm^{-1} (C=O). The positive FAB-mass spectrum showed peaks due to $[M+Na]^+$ at *m/z* 751 and $[M+H]^+$ at *m/z* 729.



1 R = β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl

1a R = H

The ^{1}H-NMR spectrum suggested **1** to be a steroidal derivative, showing the signals due to two angular methyl groups (3H each, s at δ 0.57, H-18 and δ 0.86, H-19), two secondary methyl groups (6H, d, *J* = 6.4 Hz at δ 0.95, H-26 and H-27) and a secondary methyl group (3H, d, *J* = 6.4 Hz at δ 1.12, H-21) (Rubinstein, Goad, Clague, & Mulheirn, 1976). In addition to these signals, two anomeric protons (1H, d, *J* = 7.6 Hz at δ 5.15 and 1H, d, *J* = 4.5 Hz at δ 5.13) were observed. Moreover, the characteristic signal at δ 2.99 (dd,

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Table 1
^{13}C-NMR data of **1** and **1a** in pyridine d_5

Position	Chemical shifts (ppm)	
	1	**1a**
1	38.1	38.3
2	67.4	67.5
3	68.7	68.8
4	33.2	33.3
5	54.6	54.7
6	214.3	214.3
7	43.4	43.5
8	41.0	40.8
9	37.1	37.2
10	40.6	40.8
11	21.6	21.7
12	39.8	39.9
13	43.1	43.1
14	56.3	56.3
15	24.2	24.2
16	27.7	27.7
17	53.5	53.6
18	12.0	12.0
19	23.9	24.0
20	40.7	40.8
21	13.6	13.1
22	83.8	72.8
23	27.1	25.2
24	36.2	36.9
25	28.6	28.5
26	23.1	23.2
27	22.8	22.8
Arabinose		
1′	103.4	
2′	80.1	
3′	72.5	
4′	67.4	
5′	63.9	
Glucose		
1″	105.5	
2″	75.5	
3″	78.1	
4″	71.8	
5″	78.1	
6″	62.9	

$J = 4.4$, 13.2 Hz) indicated that **1** was a cholestane having a carbonyl group at 6-position (Yokota, Arima, & Takahashi, 1982). Acid hydrolysis of **1** afforded arabinose and glucose by GC analysis. The above evidence suggested that **1** was a cholestan-6-one derivative having arabinose and glucose.

The ^{13}C-NMR spectrum of **1** indicated the presence of two anomeric carbon signals: δ 105.5 and 103.4. For accurate assignments of the residual signals of arabinose and glucose, ^{13}C-NMR spectra of **1** and its aglycone (**1a**), which was purified by HPLC after acid hydrolysis of **1**, were compared with each other. As a result, the signals of C-1′, C-2′, C-3′, C-4′, C-5′ of arabinose were assigned to δ 103.4, 80.1, 72.5, 67.4 and 63.9; C-1″, C-2″, C-3″, C-4″, C-5″ and C-6″ of glucose were assigned to δ 105.5, 75.5, 78.1, 71.8, 78.1 and 62.9, respectively (Joshi, Moore, & Pelletier, 1992). Moreover, these signals were assignable to a β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl residue (Joshi et al., 1992). The methyl signals of C-18, C-19, C-21, C-26 and C-27 were assigned to δ 12.0, 23.9, 13.6, 23.1 and 22.8, respectively, by analysis of ^{13}C–^{1}H COSY data. The ^{13}C-NMR chemical shift of C-19 methyl group at δ 23.9 suggested that H-5 was in a β-orientation (Blunt, & Stothers, 1977). The signal at δ 214.3 showed the presence of a saturated carbonyl group as compared with α, β-unsaturated carbonyl signal at δ 203.6 of α-ecdysone. This result was in agreement with the IR data, in which the carbonyl band of **1** (1693 cm^{-1}) was shifted from that of 1645 cm^{-1} for α-ecdysone. The signals at δ 67.4, 68.7 and 83.8 showed that **1** had three hydroxy groups at C-2, C-3 and C-22 by detailed ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY analysis. The characteristic difference ($\Delta_{C\text{-}2,3} = 1.4$ ppm) between the signals of C-2 and C-3 indicated the presence of 2α, and 3β hydroxy groups (Thakur, & Singh, 1982; Lin, Tome, & Won, 1991; Zhang, Stout, & Kubo, 1991). The C-22 signal at δ 72.8 of **1a** in the ^{13}C-NMR spectrum suggested that the configuration of C-22 was *R* since the ^{13}C-NMR signal of C-22 containing a hydroxy group in *S*-configuration was found at δ 66.8 (Blunt, & Stothers, 1977).

The site of the sugar linkage was established by comparison of the signals of C-22 of **1** and **1a** (Table 1). The downfield shift by 11 ppm of C-22 showed the 22-*O*-glycosidic linkage.

In the HMBC spectrum of **1**, correlations were observed (Fig. 1) between proton at C-5 and carbons C-6 and C-10, between protons at C-18 and carbons C-12, C-13, C-14 and C-17, between protons at C-19 and carbons C-1, C-5 and C-10, between protons at C-21 and carbons at C-17, C-20 and C-22 and between protons at C-26 and C-27 and carbons at C-24 and

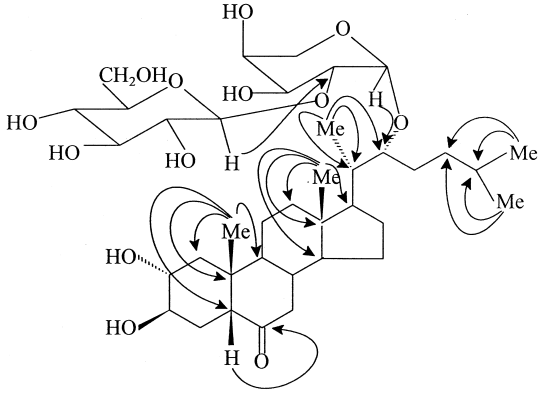

Fig. 1. HMBC correlations in compound **1**.

C-25. In addition to these correlations, the linkage position of the sugar in **1** was supported by the HMBC spectrum. Correlations between arabinose H-1 and aglycone C-22 and between glucose H-1 and arabinose C-2 were observed.

Based on the above evidence, the chemical structure of compound **1** having a new steroidal aglycone was elucidated to be 2α,3β-(22*R*)-trihydroxycholestan-6-one-22-*O*-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside. The other compounds isolated from *L. ussuriensis* were α-ecdysone and ecdysterone and they were identified by comparison of their physical and spectral data with published values (Hikino, Okuyama, Konno, & Takemoto, 1975).

3. Experimental

3.1. General

M.p.'s were determined with a DuPont 910 Differential Scanning Calorimeter. Optical rotations were determined on a JASCO DIP-1000 Digital polarimeter. IR spectra were obtained on a Perkin Elmer 1710 FT-IR spectrometer using KBr discs. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) were recorded on a JEOL GSX 400 FT-NMR spectrometer with reference to the residual solvent signal. EIMS spectra were obtained using a VG Trio-2 spectrometer at 70 eV and FAB-mass spectra were obtained using a Finnigan MAT 90 spectrometer in the positive mode. GC-FID analysis of sugar TMS ethers was carried out on an HP 5890II (Hewlett Packard) with HP 3395 integrator and helium was used as a carrier gas at a flow rate of 2 ml/min. The column was HP-5 (Hewlett Packard, 25 m×0.32 mm×0.17 μm film thickness), the oven temperature was 150 (3 min)–200°C at 5°C/min, the injector temperature was 275°C and the detector temperature was 290°C. Gravity column chromatography was performed on silica gel 60 (230–400 mesh, Art 9385, Merck) and TLC was performed on silica gel 60F_{254} plate. The HPLC instrument consisted of a Hitachi L-6200 pump and an L-4000 UV detector fixed at 205 nm (Hitachi, Tokyo).

3.2. Plant material

The whole plants of *Lepisorus ussuriensis* (Regel et Maack.) Ching (Polypodiaceae) were collected in Youngchun, Korea in August of 1991 and identified by Dr. D.S. Han, Professor Emeritus, College of Pharmacy, Seoul National University. A voucher specimen has been deposited at the Herbarium of Medicinal Plant Garden, College of Pharmacy, Seoul National University.

3.3. Extraction and isolation

Air-dried plant material (273 g) was extracted with 80% MeOH to afford an initial MeOH extract (83 g), on removal of solvent in vacuo. An aqueous suspension of this extract was partitioned successively with *n*-hexane (1.0 g), $CHCl_3$ (2.0 g) and *n*-BuOH (40.0 g), leaving a residual H_2O extract (39.0 g). The *n*-BuOH extract was dissolved in MeOH, impregnated on silica gel and subjected to CC over silica gel (700 g) using $CHCl_3$–MeOH (15:1 → 1:1) mixtures of increasing polarity as eluents. A total of 120 fractions (300 ml) were collected. Fractions showing similar TLC profiles were pooled to afford nine combined fractions. α-Ecdysone (60 mg) was obtained from fraction 5 by recrystallization with MeOH. Fraction 6 was separated using EtOAc–MeOH–AcOH (20:1:1) by CC over silica gel and the 16th fraction among 19 fractions was further purified using EtOAc–HCOOH–AcOH–H_2O (100:2:2:5) as eluent by CC over silica gel. From this separation, ecdysterone (70 mg) was isolated. Fraction 9 was separated using $CHCl_3$–MeOH–H_2O (40:10:1) by CC over silica gel. From this separation, compound **1** (60 mg) was isolated.

3.4. 2α,3β-(22*R*)-Trihydrosycholestan-6-one-22-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside (*1*)

Needles from MeOH, m.p.: 219°C, $[\alpha]_D$ −30.5° (MeOH, *c* 0.1). Found C, 61.94%; H, 8.73%, $C_{38}H_{64}O_{13}$ requires: C, 62.62%; H, 8.85%. IR ν_{max}^{KBr} cm^{-1}: 3436 (OH), 2947 (CH), 1693 (C=O), 1385, 1079. FAB-MS *m/z*: 751 $[M+Na]^+$, 729 $[M+H]^+$. ^{1}H-NMR (400 MHz, pyridine-d_5): δ 0.57 (3H, s, H-18), δ 0.86 (3H, s, H-19), δ 0.95 (6H, d, *J*=6.4 Hz, H-26 and H-27), δ 1.12 (3H, d, *J*=6.4 Hz, H-21), δ 2.99 (1H, dd, *J*=4.4 Hz, 13.2 Hz, H-5), δ 3.81 (m, H-22, H-5′ and H-5″), δ 4.1-4.4 (m, H-2, H-3, H-3′, H-4′, H-5′, H-2″, H-3″, H-4″ and H-6″), δ 5.13 (1H, d, *J*=4.5 Hz, arabinosyl H-1′), δ 5.15 (1H, d, *J*=7.6 Hz, glucosyl H-1″). ^{13}C-NMR (100 MHz, pyridine-d_5) spectral data, see Table 1.

3.5. 2α,3β-(22*R*)-Trihydrosycholestan-6-one (*1a*)

Needles from EtOAc, m.p.: 173°C, $[\alpha]_D$ −20.3° (MeOH, *c* 0.1). $C_{27}H_{46}O_4$ IR ν_{max}^{KBr} cm^{-1}: 3436 (OH), 2960 (CH), 1700 (C=O). EI-MS *m/z*: 434 $[M]^+$, 416 $[M-H_2O]^+$, 334 $[M-C_6H_{12}O]^+$, 316 $[M-C_6H_{14}O]^+$. ^{1}H-NMR (400 MHz, pyridine-d_5): δ 0.58 (3H, s, H-18), δ 0.95 (3H, s, H-19), δ 0.95 (3H, d, *J*=6.6 Hz, H-26), δ 0.96 (3H, d, *J*=6.6 Hz, H-27), δ 1.22 (3H, d, *J*=6.7 Hz, H-21), δ 3.00 (1H, dd, *J*=4.6 Hz, 13.2 Hz, H-5), δ 3.91 (1H, td, *J*=3.2, 3.6 Hz, H-22), δ 4.16

(1H, br d, $J=11$ Hz, H-2), δ 4.46 (1H, br s, H-3). ^{13}C-NMR (100 MHz, pyridine-d_5) spectral data, see Table 1.

3.6. Hydrolysis of compound *1*

Compound **1** (20 mg) was dissolved in 2 ml of 2 N HCl–dioxane (1:1) and heated at 70°C in a water bath for 3 h. The reaction mixture was dried under N_2 gas and partitioned between EtOAc and H_2O. For GC analysis of sugars, the H_2O fraction was evaporated under N_2 stream and silylated with 100 μl of HMDS–TMCS–pyridine (1:1:1). The EtOAc fraction was evaporated in vacuo and purified by HPLC to provide 3 mg of aglycone (**1a**). HPLC column was a YMC-Pack, ODS-A (250×4.6 mm, S-5 μm) (YMC, Kyoto, Japan); mobile phase system: acetonitrile–water (60:40); flow rate: 1.0 ml/min. The retention time of aglycone (**1a**) was 6.88 min.

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