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Chemical Constituents of the Moss *Hylocomium splendens*

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Abstract – Investigation of the chemical constituents of the dichloromethane extract from the moss *Hylocomium splendens* has led to the isolation of 5α,8α-epidioxy-24(*S*)-ethylcholesta-6,22-dien-3β-ol (**1**), diploptene (**2**), β-sitosterol (**3**), and 1-hexacosanol (**4**). The chemical structures of **1** - **4** were established by spectroscopic methods including extensive 1D and 2D NMR analysis. This is the first isolation of compound **1** from the mosses, although it has been isolated from marine sponge.
Keywords - *Hylocomium splendens*, 5α,8α-epidioxy-24(*S*)-ethylcholesta-6,22-dien-3β-ol, diploptene, sterols, 1-hexacosanol

Introduction

The bryophytes, taxonomically placed between the algae and the pteridophytes, are the simplest land plants and more than 20,000 species are known in the world. The bryophytes are morphologically classified into three classes, mosses (Musci, 14,000 species), liverworts (Hepaticae, 6,000 species), and hornworts (Anthocerotae, 300 species) (Koponen, *et al.*, 1990; Asakawa, 1995, 2001). Among the bryophytes, the liverworts has been subjected to intensive phytochemical investigations over the last decades because liverworts have oil bodies in their cells and produce mono-, sesqui-, and diterpenoids with a variety of carbon skeletons and aromatic compounds as major constituents. However, the other two classes, hornworts and mosses, have no oil bodies in their leaf cells and contain fatty acids, sterols, and triterpenoids and aromatic compounds. Mono-, sesqui-, and diterpenoids are very rare constituents of the mosses (Asakawa, 1982, 1995, 2001; Saritas, *et al.*, 2001). Previous phytochemical study have shown that triterpenoids such as ursane-, fernane-, friedelane-, hopane-, lupine-, taraxane-, cycloeucalane-, cycloartane-, cycloludane-, 24-methylenecycloartane-, norcyclolaudane-, obstusifolane-types are widely distributed in mosses (Asakawa, 1995, 2001). Campesterol, stigmasterol, sitosterol, together with cholesterol, rassicasterol and 24-methyl-5,7,22-cholestatrienol were also found in the mosses (Matsuo and Sato, 1991; Asakawa, 1995). Apigenin, luteolin, kaempferol, orobol and biflavonoid derivatives are the usual flavonoids found in mosses (Mues and Zinsmeister, 1988; Basile, *et al.*, 1999).

Hylocomium splendens (Hylocomiaceae) is one of the most common and widespread mosses of the boreal forest, which covers huge areas of Alaska, Canada, northern Europe, and Siberia (Vitt, *et al.*, 1988). Previous phytochemical investigations on this plant have resulted in the isolation of 5',3''-dihydroxyrobustaflavone, apigenin 7-rahmaoglucoside, and 3,4,5-trimethyl-5-pentyl-2(5*H*)-furanone (Becker, *et al.*, 1986; Ron, *et al.*, 1990; Saritas, *et al.*, 2001).

We report herein the isolation of 5α,8α-epidioxy-24(*S*)-ethylcholesta-6,22-dien-3β-ol (**1**), diploptene [hop-22(29)-ene] (**2**), β-sitosterol (**3**), and 1-hexacosanol (**4**) from *H. splendens*. This is the first report on the isolation of 5α,8α-epidioxy-24(*S*)-ethylcholesta-6,22-dien-3β-ol (**1**) from the mosses and diploptene (**2**) from *H. splendens*.

Experimental

Plant material – *Hylocomium splendens* was collected from Squamish in July 2004 and Chilliwack in December 2004 and identified in the Herbarium of University of British Columbia. Voucher specimens are kept at Department of Microbiology and Institute of Basic Sciences,

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General experimental procedure – The melting points were measured on a Buchi model B-540 without correction. The UV and IR spectra were obtained on a JASCO UV-550 and JASCO Report-100 spectrometer, respectively. 1D and 2D NMR spectra were taken on a Bruker AMX 500 MHz NMR spectrometer with TMS as an internal standard, and chemical shifts are expressed in δ values. EI-MS was recorded on Hewlett-Packard MS 5988 mass spectrometer. Semipreparative HPLC was performed on a Waters HPLC system (515 pump and 2996 PDA detector) with a ODS column (Xterra Prep RP_{18}, 50 × 20 mm). Column chromatography was performed using a silica gel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, Merck), and thin layer chromatography (TLC) using a pre-coated silica gel $60F_{254}$ (0.25 mm, Merck).

Extraction and purification – The whole parts of *H. splendens* (3.2 kg) were extracted with 80% MeOH at the room temperature to obtain 297.44 g of the solid extract. The 80% MeOH extract was successively partitioned to give *n*-hexane (65.8 g), CH_2Cl_2 (9.1 g), EtOAc (15.1 g) and BuOH (26.1 g). The CH_2Cl_2 extract was subjected to a silica gel column using gradient system of hexane-CH_2Cl_2 (10 : 0 → 0 : 10) and CH_2Cl_2-acetone (100 : 1 → 10 : 1) to give 30 fractions (D1-D30). D1 was diploptene (**2**, 15 mg). D7 was rechromatographied with hexane-acetone (100 : 4) to give 1-hexacosanol (**4**, 25 mg). D14 was rechromatographed over silica gel eluting CH_2Cl_2-acetone (100 : 0 → 100 : 1) to give β-sitosterol (**3**, 30 mg). D20 was subjected to a silica gel column using CH_2Cl_2-acetone (100 : 1 → 100 : 4) to obtain three fractions. Among them D20-2 fraction was rechromatographed with CH_2Cl_2-acetone (100 : 1) to give white needle crystals. Purification of these crystals with Semipreparative HPLC eluting with acetonitrile-H_2O (75 : 25) at the flow rate of 6.5 ml/min afforded 5α,8α-epidioxy-24(*S*)-ethylcholesta-6,22-dien-3β-ol (**1**, 6.0 mg).

5α,8α-Epidioxy-24(*S*)-ethylcholesta-6,22-dien-3β-ol (1) – White needles crystal; $[\alpha]^{24}_D$ –10° (c 0.05, $CHCl_3$); EIMS *m/z* : 442 $[M]^+$, 424 $[M-H_2O]^+$, 410 $[M-O_2]^+$, 377 $[M-H_2O-O_2-CH_3]^+$, 351 $[M-O_2-C_3H_7O]^+$, 303 [M-side chain]$^+$, 301 [M-side chain-2H]$^+$; ^{1}H-NMR (pyridine-d_5, 500 MHz) δ : 6.52 (1H, d, 8.5, H-7), 6.31 (1H, d, 8.5, H-6), 5.15 (1H, dd, 15.0, 8.6, H-22), 5.04 (1H, dd, 15.0, 8.9, H-23), 4.37 (1H, m, H-3), 1.03 (3H, d, 6.5, H-21), 0.89 (3H, s, H-19), 0.88 (3H, d, 6.5, H-27), 0.86 (3H, t, 7.3, H-29), 0.83 (3H, d, 6.5, H-26), 0.78 (3H, s, H-18); ^{13}C-NMR (pyridine-d_5, 125 MHz) δ : 35.5 (C-1), 31.2 (C-2), 65.8 (C-3), 38.3 (C-4), 82.3 (C-5), 136.2 (C-6), 130.9 (C-7), 79.3 (C-8), 51.9 (C-9), 37.5 (C-10), 23.7 (C-11), 39.6 (C-12), 44.7 (C-13), 52.1 (C-14), 21.2 (C-15), 29.4 (C-16), 56.3 (C-17), 12.5 (C-18), 18.3 (C-19), 40.3 (C-20), 21.3 (C-21), 138.4 (C-22), 129.9 (C-23), 51.4 (C-24), 32.2 (C-25), 21.4 (C-26), 19.2 (C-27), 25.7 (C-28), 13.0 (C-29)

Diploptene [Hop-22(29)-ene] (2) – White long needle crystals, m.p. 214 - 216 °C; $[\alpha]^{24}_D$ 210 - 211° (c 0.5, CH_2Cl_2), IR(Kbr) ν max 1602, 883 cm^{-1}; UV (MeOH) λ_{max} (log) 240 (4.32); EIMS *m/z* 410 $[M]^+$ (100), 395, 367, 341, 299, 231, 218, 205, 191, 177, 161, 149, 137, 123, 121, 109, 95, 81, 69, 55; ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 4.79 (2H, s, H-29), 2.69 (1H, m, H-21), 1.76 (3H, s, H-30), 0.97 (3H, s, H-26), 0.95 (3H, s, H-27), 0.85 (3H, s, H-23), 0.82 (3H, s, H-25), 0.80 (3H, s, H-24), 0.73 (3H, s, H-28); ^{13}C-NMR (125 MHz, $CDCl_3$) δ : 40.3 (C-1), 18.7 (C-2), 42.1 (C-3), 33.2 (C-4), 56.1 (C-5), 18.7 (C-6), 33.2 (C-7), 41.9 (C-8), 50.3 (C-9), 37.4 (C-10), 20.9 (C-11), 24.0 (C-12), 49.4 (C-13), 42.0 (C-14), 33.6 (C-15), 21.6 (C-16), 54.8 (C-17), 44.8 (C-18), 41.9 (C-19), 27.4 (C-20), 46.4 (C-21), 148.8 (C-22), 33.4 (C-23), 21.6 (C-24), 15.8 (C-25), 16.7 (C-26), 16.7 (C-27), 16.1 (C-28), 110.1 (C-29), 25.0 (C-30).

β-Sitosterol (3) – White powder, m.p. 136 - 137 °C; $[\alpha]^{24}_D$ –36° (c 0.1, $CHCl_3$); EIMS 414 $[M]^+$, 396, 329, 303, 273, 255, 213, 199, 159, 145; ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 5.34 (1H, br d, *J* = 5.1 Hz,H-6), 3.52 (2H, m, H-3), 1.00 (3H, s, Me-19), 0.93 (3H, d, *J* = 6.6, Me-21), 0.84 (3H, d, *J* = 7.3 Hz, Me-26), 0.82 (3H, d, *J* = 7.3 Hz, Me-26), 0.79 (3H, d, *J* = 6.8 Hz, Me-27), 0.68 (3H, s, Me-18); ^{13}C-NMR (100 MHz, $CDCl_3$) δ : 37.9 (C-1), 32.6 (C-2), 72.5 (C-3), 43.0 (C-4), 141.5 (C-5), 122.4 (C-6), 32.3 (C-7), 32.4 (C-8), 50.8 (C-9), 37.2 (C-10), 21.8 (C-11), 40.5 (C-12), 43.0 (C-13), 57.5 (C-14), 25.0 (C-15), 28.9 (C-16), 56.7 (C-17), 12.6 (C-18), 19.7 (C-19), 36.8 (C-20), 19.5 (C-21), 35.6 (C-22), 26.8 (C-23), 46.5 (C-24), 29.8 (C-25), 20.5 (C-26), 20.1 (C-27), 23.8 (C-28), 12.6 (C-29).

1-Hexacosanol (4) – White poweder, m.p. 80 °C; EIMS 382 $[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 3.59 (2H, m), 1.53 (2H, s), 1.44 ~ 1.26 (46 H, m), 0.89 (3H, t, *J* = 6.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ : 63.1 (C-1), 32.9 (C-2), 32.0 (C-24), 29.7 (C-6~21), 29.5 (C-22~23), 29.4 (C-4~5), 25.8 (C-3), 22.7 (C-25), 14.1 (C-26).

Results and Discussion

Phytochemical investigation of the CH_2Cl_2-soluble extract of *H. splendens* by repeated column chromatography using silica gel and preparative HPLC afforded 5α,8α-epidioxy-24(*S*)-ethylcholesta-6,22-dien-3β-ol (**1**), diploptene (**2**), β–sitosterol (**3**), and 1-hexacosanol (**4**). The compound

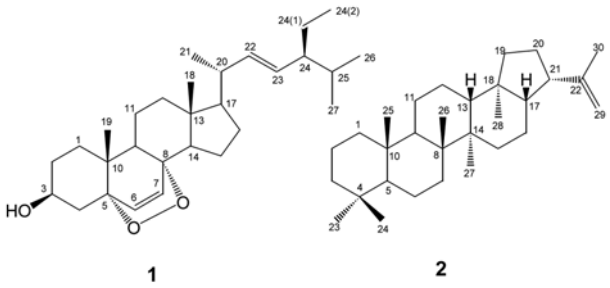

Fig. 1. Structures of compounds **1** and **2**.

3 and **4** were identified by comparing their physical and spectral data with the literature values (Ulubelen and Halfon, 1976; Garg and Nes, 1984; Do, *et al.*, 1988).

Compound **1** was isolated as white needles crystal. In the EI-MS spectrum, the molecular ion was observed at *m/z* 442 $[M]^+$ accompanied by fragment ions at *m/z* 424 $[M-H_2O]^+$, 410 $[M-O_2]^+$, 377 $[M-H_2O-O_2-CH_3]^+$, and 351 $[M-O_2-C_3H_7O]^+$. The ^{1}H-NMR spectrum showed signals due to two tertiary methyl groups [δ_H 0.78 (3H, s, H-18) and 0.89 (3H, s, H-19)], three secondary methyl groups [δ_H 0.83 (3H, d, J = 6.5 Hz, H-26), 0.88 (3H, d, J = 6.5 Hz, H-27), and 1.03 (3H, d, J = 6.5 Hz, H-21)], one primary methyl group [δ_H 0.86 (3H, t, J = 7.3 Hz, H-29], one oxygenated methine proton [δ_H 4.37 (1H, m, H-3], four disubstituted olefinic protons [δ_H 6.52 (1H, d, J = 8.5 Hz, H-7), 6.31 (1H, d, J = 8.5 Hz, H-6), 5.15 (1H, dd, J = 15.0, 8.6 Hz, H-22), and 5.04 (1H, dd, J = 15.0, 8.9 Hz, H-23)]. The ^{13}C-NMR and DEPT spectra of **1** disclosed 29 carbon signals, which were indicative of an oxygenated methine carbon [δ_C 65.8 (C-3)], two oxygenated quaternary carbons [δ_C 82.3 (C-5), 79.3 (C-8)], four olefinic carbons [δ_C 136.2 (C-6), 130.9 (C-7), 138.4 (C-22), 129.9 (C-23)] in addition to six methyls, eight methylenes, six methines, and two quaternary carbons.

The presence of a peroxide was confirmed by the EI-MS fragment ion peak at *m/z* 410 $[M-O_2]^+$, which was due to the loss of O_2 from the molecule, presumably by a retro-Diels-Alder fragmentation (Gunatilaka, *et al.*, 1981; Sheu, *et al.*, 2000). The chemical shift values of two oxygenated quaternary carbons of δ_C 82.5 (C-5), 79.3 (C-8) and the characteristic signals for H-6 and H-7 at δ_H 6.31 (d, J = 8.5 Hz) and 6.52 (d, J = 8.5 Hz) was further suggested the 5α,8α-epidioxy group in the molecule (Iguchi, *et al.*, 1993; Sera, *et al.*, 1999; Gauvin, *et al.*, 2000). The HMBC spectrum revealed correlations between H-6 to C-4, C-5, C-7 and C-8; H-7 to C-6, C-8 and C-9; H-22 to C-20 and C-24; H-23 to C-20; H_3-18 to C-12, C-13, C-14 and C-17; H_3-19 to C-1, C-5, C-9 and C-10; H_3-21 to C-17, C-20 and C-22; H_3-26 and H_3-27 to C-24 and C-25; H_3-29 to C-28. The above data suggested that **1** is a 5α,8α-epidioxysterol containing a 22-en-24-ethyl moiety. The geometry of the Δ^{22}-double bond was deduced to be *E* from the ^{1}H-^{1}H coupling constant (J = 15.0 Hz) between H-22 and H-23. The absolute configuration at C-24 for **1** was deduced as *S* configuration by comparing the chemical shifts of their side chain carbons with those given by Goad and Akihisa for several C-24 alkyl 3â-hydroxy sterols (Goad and Akihisa, 1997). Thus, the structure of **1** was determined to be 5α,8α-epidioxy-24(*S*)-ethylcholesta-6,22-dien-3β-ol (Gauvin, *et al.*, 2000). This is the first report on the isolation of **1** from the mosses, although it has isolated from marine sponge *Luffariella cf. variabilis*.

Compound **2** was obtained as white long needle crystal. The EI-MS spectrum showed molecular ion peak at *m/z* 410 $[M]^+$ and a base ion peak at *m/z* 191 indicated of hopene or lupene skeleton (Ogunkoya, 1981). The ^{1}H-NMR spectrum showed signals due to seven tertiary methyl groups [δ_H 1.76 (3H, s, H-30), 0.97 (3H, s, H-26), 0.95 (3H, s, H-27), 0.85 (3H, s, H-23), 0.82 (3H, s, H-25), 0.80 (3H, s, H-24), 0.73 (3H, s, H-28)] and an exomethylene group [δ_H 4.79 (2H, s, H-29)]. The ^{13}C-NMR spectrum of compound **2** revealed 30 carbon signals, including 7 methyl, 12 methylene, 5 methine groups, and 6 quaternary carbons. The hopane nature of **2** was indicated by the ^{13}C-NMR data, particularly, the resonances of carbons C-17, C-18, and the isopentenyl group in comparison with hopene, 21αH-hop-22(29)-ene, and lupene derivatives (Wilkins, *et al.*, 1987; Rowan and Russell, 1992; Mahato and Kundu, 1994; Chavez, *et al.*, 1997). Furthermore, the C-21 resonance of **2** (δ_C 46.4) was similar to that of hop-22(29)-ene (δ_C 46.5) and different from 21αH-hop-22(29)-ene (δ_C 48.0), an upfield shift consistent with a pseudo-axial configuration of the C-21 isopropenyl group (Rowan and Russell, 1992). Thus the structure of compound **2** was determined to be diploptene [hop-22(29)-ene] (Ageta, *et al.*, 1993; Arai, *et al.*, 1994). Diploptene (**2**) was already reported from the liverwort *Adelanthus lindenbergianus*, *Plagiochila bispinosa*, and the moss *Floribundaria aurea* supsp. *nipponica*. However, this is the first report on the isolation of **2** in the *Hylocomium* species.

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