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EUDESMANE-TYPE SESQUITERPENOIDS FROM THE LIVERWORT *LEPIDOZIA VITREA*

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Key Word Index—*Lepidozia vitrea*; *Lepidozia* (Dum.) Dum.; Lepidoziaceae; Jungermanniales; Hepaticae; eudesmane-type sesquiterpenoids.

Abstract—Four new eudesmane-type sesquiterpenoids have been isolated from the ether extract of *Lepidozia vitrea*. Their structures were elucidated by spectral means and were shown to be eudesm-3-ene-6α-acetoxy-7α-ol, eudesm-3-ene-7α-ol, eudesm-4(15)-ene-6β,7α-diol and eudesm-4(15)-ene-7α-ol. These compounds were isolated together with the previously known sesquiterpenes (−)-isobicyclogermacrenal, (−)-lepidozenal, maalian-5-ol and (+)-eudesm-3-ene-6β,7α-diol.

INTRODUCTION

In the genus *Lepidozia*, six epiphytic species, *Lepidozia vitrea*, *L. reptans*, *L. fauriana*, *L. mamillosa*, *L. wallichiana* and *L. subtransversa* which grow on the trunks of deciduous plants or rock are known in Japan. *Lepidozia vitrea* Steph. of the genus is a common species. The liverworts, including the Jungermanniales, are rich sources of terpenoids with a variety of carbon skeletons [1]. Liverworts occasionally produce their own peculiar constituents such as the sacculatane- and pinguisane-type terpenoids and bisbibenzyl derivatives. These have not been found in higher plants, fungi or marine organisms. Classification of liverworts belonging to the Jungermanniales is extremely difficult and a study of liverwort chemical constituents is necessary from a chemosystematical point of view. Even when the same species is investigated, the constituents of liverworts are often different.

Previous work on the genus *Lepidozia* (Dum.) Dum. has led to the isolation of (−)-isobicyclogermacrenal (**8**) [2, 3], (−)-lepidozenal (**9**) [3, 4] and (+)-vitrenal [5, 6] from Japanese *L. vitrea* and of (+)-eudesm-3-ene-6β,7α-diol (**5**) [7] from Scottish *L. reptans*. More recent work on *Lepidozia* species resulted in the isolation of 5β-hydroperoxylepidozenolide and 6β-acetoxyvitranoxide from Taiwanese *L. vitrea*, and of (+)-lepidozenolide from Taiwanese *L. fauriana* [8]. Also, steroids [9] and terpenoid hydrocarbons [10–12] of the genus have been identified by GC-MS analysis. In this paper, we report the isolation and structural elucidation of four new eudesmane-type sesquiterpenoids from *Lepidozia vitrea* Steph. Additionally, two prenylated bibenzyls have been isolated from the ether extract of this species.

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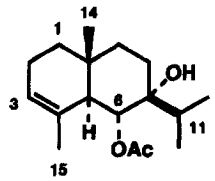
RESULTS AND DISCUSSION

The ether extract of *Lepidozia vitrea* Steph. was chromatographed on Sephadex LH-20 and silica gel to give four new sesquiterpenoids **1**–**4**, in addition to (+)-eudesm-3-ene-6β,7α-diol (**5**) [7], (−)-isobicyclogermacrenal (**8**) [2, 3], (−)-lepidozenal (**9**) [3, 4], maalian-5-ol (**10**) [13], 3,5-dihydroxy-2-(3-methyl-2-butenyl)- (**11**) and 2-geranyl-3,5-dihydroxy-bibenzyls (**12**) [14].

The EI mass spectrum of **1** resembled that of **5** which has been isolated from *L. reptans* [7]. The IR spectrum of **1** showed absorption bands for acetoxyl (1703, 1263 cm^{-1}) and hydroxyl groups (3450 cm^{-1}). The ^1H NMR spectrum exhibited signals for five methyl groups (δ0.84, 2.10 (both *s*), 1.63 (*br s*), 0.95, 0.96 (both *d*, $J = 7$ Hz)), an olefinic proton (δ5.38) and a methine group bearing an oxygen (δ5.20). The ^1H NMR spectral data of **1** was similar to that of **5** except for the appearance of an acetyl group at δ2.10 and a methine proton at δ5.20. The latter signal was shifted downfield (Δδ1.3) in comparison with that of **5**. The HMBC spectrum (Table 1) showed a cross peak between the methyl proton signal at δ2.10 and the carbonyl carbon at δ170.5 which further correlated with the proton signal at δ5.20. Furthermore, the signal at δ5.20 is a doublet with $J = 11$ Hz confirming the stereochemistry of the acetoxyl group is equatorial at C-6 in **1**. The NOE observed between the tertiary methyl proton at δ0.84 and H-6 at δ5.20 in the difference NOE spectra of **1** provided further evidence for the relative configuration of the acetoxyl group in **1**. The above evidence enabled the conclusion that compound **1** differed from **5** only by the replacement of an axial hydroxyl group by an equatorial oriented acetoxyl group. Lithium aluminium hydride reduction of **1** yielded a diol **6**, the ^1H NMR data of which showed an upfield shift (Δδ1.49) to δ3.71 in comparison with that of **1**. The



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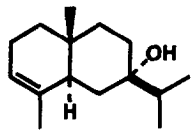

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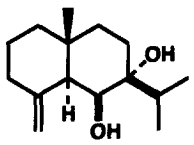

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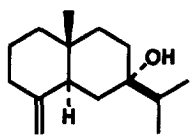

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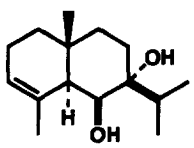

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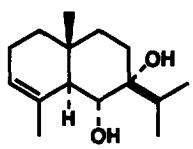

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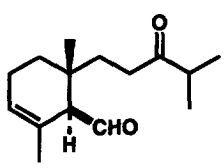

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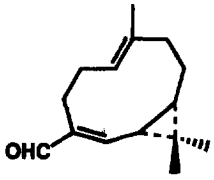

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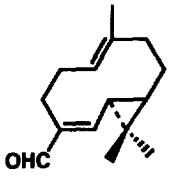

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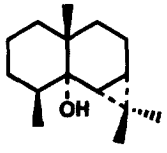

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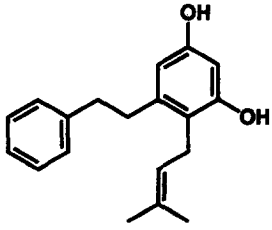

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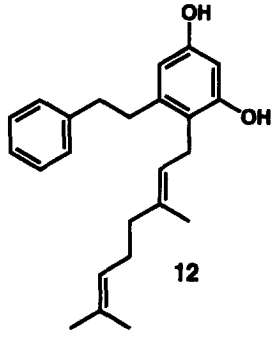

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Table 1. The long range C/H correlations of compound **1***

^{1}H	Long range C/H correlations (^{1}H to ^{13}C)
H-15 (δ1.63)	C-3 (δ124.6)
	C-4 (δ133.2)
	C-5 (δ45.0)
H-12 (H-13) (δ0.96, 0.95)	C-7 (δ73.8)
	C-11 (δ34.0)
H-14 (δ0.84)	C-1 (δ37.9)
	C-5 (δ45.0)
	C-9 (δ34.2)
	C-10 (δ34.3)
H-1α (δ1.35)	C-5
H-1β (δ1.47)	C-9
H-6 (δ5.20)	C-4
	C-5
	C=O (δ170.5)

*Confirmed by HMBC spectrum in $CDCl_3$.

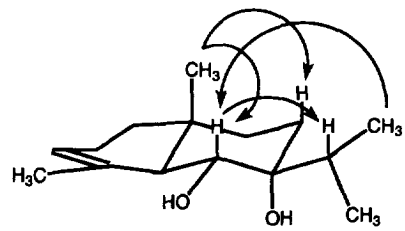

Fig. 1. The arrows display NOE correlations of compound **6**.

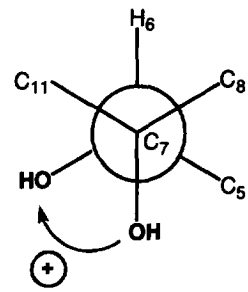

Fig. 2. The Newman's projection formula of diol **6**.

disappearance of a 7 Hz coupling from the secondary hydroxyl methine proton at δ3.71 (*dd*, J = 11, 7 Hz) and the absence of the hydroxyl proton at δ2.00 were observed after shaking with D_2O. The relative configuration of **6** was determined by difference NOE spectra as shown in Fig. 1. Pyridinium dichromate (PDC) oxidation of **6** afforded the aldehyde **7**, confirming the presence of a vicinal glycol moiety in **6**. Thus the absolute configuration at C-6 was determined by means of the 1,2-diol complexation method [15, 16]. The CD spectrum ($\Delta\varepsilon_{302}$ + 25.6 in CCl_4 employing Eu(FOD)$_3$ as the complexing reagent) of **6** revealed a 6*R*-configuration as shown in Fig. 2.

The structure of **2** was deduced by comparing its spectral data with those of **1** and **5**. The resonances for an

acetoxyl group and a methine group bearing an oxygen were missing in the ^{1}H NMR of **2** and the presence of an extra methylene carbon was confirmed in the DEPT spectra of **2**. The ^{1}H NMR data of **3** were similar to those of **5** except for the absence of a trisubstituted double bond and for the appearance of an exocyclic methylene proton at δ4.87 (2H, *m*). The ^{1}H NMR data of **4** showed the absence of a methine group bearing an oxygen in comparison with that of **3**. Accordingly, the structures of **2**–**4** were elucidated as shown.

Determination of the absolute configuration of **5** was attempted, but PDC oxidation of **5** to **7** did not work due to the lack of reactivity of the axial secondary hydroxyl group at C-6 in **5**.

Although previous work reported the isolation of (+)-vitrenal from *L. vitrea* Steph. [5], this compound was not present in this species. Even when the same species are investigated liverwort constituents are often different. The species *L. vitrea* is divided into at least two chemotypes, one of which contains (+)-vitrenal while the other species contains mainly eudesmane-type sesquiterpenoids. Taiwanese *L. vitrea* contains eudesmane-type sesquiterpenoids, and no (+)-vitrenal [8], therefore Taiwanese *L. vitrea* and our species are related chemosystematically.

The prenylated bibenzyls **11** and **12** are constituents so far only found in the *Radula* species of liverwort.

EXPERIMENTAL

General. TLC was carried out on silica gel precoated glass plates with *n*-hexane–EtOAc (1 : 1 and 4 : 1). Detection was with Godin reagent [17]. For normal phase column chromatography (CC), silica gel 60 (40–63 μm) was used. The mix. of CH_2Cl_2–MeOH (1 : 1) was used for CC on Sephadex LH-20 as solvent.

Spectral data. NMR spectra were recorded at 100 MHz for ^{13}C and 400 or 200 MHz for ^{1}H. EIMS were measured at 70 eV.

Plant material. Lepidozia vitrea Steph. (345.5 g) was collected in April, 1993 at Kainan-cho (Altitude 70 m), Kaifu-gun, Tokushima, Japan. A voucher specimen (#93025) is deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University. The liverwort was dried for 2 days, impurities removed, ground mechanically and then extracted with Et_2O for 1 month.

Extraction and isolation. The Et_2O extract (6.0 g) of *L. vitrea* was chromatographed on silica gel using an *n*-hexane–EtOAc gradient, giving 10 frs (I-X). Fraction II (1.7 g) was rechromatographed on Sephadex LH-20 to give a mixt. containing **2**, **3**, **4**, **8** and **9**. The mixture was further purified by CC on silica gel using a *n*-hexane–EtOAc gradient to afford eudesm-3-ene-7α-ol (**2**) (58.8 mg; 0.98% of the total extract), eudesm-4 (15)-ene-6β,7α-diol (**3**) (8.6 mg; 0.14%), eudesm-4(15)-ene-7α-ol (**4**) (8.6 mg; 0.14%), (−)-isobicyclogermacrenal (**8**) (63.7 mg; 1.06%) and (−)-lepidozenal (**9**) (96 mg; 1.6%), respectively. Fraction III (0.94 g) was rechromatographed on silica gel and prep. HPLC to give eudesm-3-ene-6α-acetoxy-7α-ol (**1**) (114.2 mg; 1.9%), (+)-eudesm-3-ene-6β,7α-diol (**5**) (71.5 mg; 1.19%) and maalian-5-ol (**10**) (10.6 mg; 0.2%) respectively. Fr. VII (0.76 g) was subjected to CC on silica gel and then purified by MPLC on silica gel to give 3,5-dihydroxy-2-(3-methyl-2-butenyl)-bibenzyl (**11**) (3.2 mg; 0.05%) and 2-geranyl-3,5-dihydroxy-bibenzyl (**12**) (8.1 mg; 0.14%).

Compound **1**. $[\alpha]_D$ −43.8 ($CHCl_3$; *c* 0.7), IR ν_{max}^{neat} cm^{-1}: 3450, 1703, 1377, 1263, 1152, 1061, 1032, 990, 968, 920, 831; ^{1}H NMR (400 MHz, $CDCl_3$): δ0.84 and 2.10 (each 3H, *s*), 0.95 and 0.96 (each 3*H*, *d*, *J* = 6.4 Hz), 1.63 (3H, *br s*), 2.61 (1H, *br d*, *J* = 11 Hz), 5.20 (1H, *d*, *J* = 11 Hz), 5.38 (1H, *br s*); ^{13}C NMR (50 MHz, $CDCl_3$); δ15.8 (*q*), 16.2 (*q*), 17.8 (*q*), 21.8 (*q*), 22.8 (*t*), 22.9 (*t*), 23.1 (*q*), 34.0 (*d*), 34.2 (*t*), 34.3 (*s*), 37.9 (*t*), 45.0 (*d*), 73.8 (*d*), 76.9 (*s*), 124.6 (*d*), 133.2 (*s*), 170.5 (*s*); EIMS *m/z* (rel. int.): 262 $[M - H_2O]^+$ (2), 237 $[M - Ac]^+$ (6), 220 (40), 205 (9), 187 (6), 178 (16), 177 (100), 159 (10), 149 (8), 135 (5), 133 (5), 121 (15), 119 (7), 109 (6), 107 (10), 105 (6), 97 (7), 95 (6), 93 (10), 91 (5), 81 (7), 79 (5), 43 (18).

Compound **2**. $[\alpha]_D$ +14.3 ($CHCl_3$; *c* 0.73), IR ν_{max}^{neat} cm^{-1}: 3440, 1615, 1453, 1377, 1175, 1026, ^{1}H NMR (400 MHz, $CDCl_3$): δ0.75 (3H, *s*), 0.94 and 0.95 (each 3H, *d*, *J* = 7 Hz), 1.59 (3H, *br s*), 5.32 (1H, *br s*), ^{13}C NMR (100 MHz, $CDCl_3$); δ14.4 (*q*), 16.8 (*q*), 16.9 (*q*), 21.0 (*q*), 22.9 (*t*), 29.2 (*t*), 32.1 (*s*), 32.3 (*t*), 35.6 (*t*), 37.5 (*t*), 39.1 (*d*), 40.9 (*d*), 74.2 (*s*), 121.2 (*d*), 134.9 (*s*); EIMS *m/z* (rel. int.): 204 $[M - H_2O]^+$ (21), 162 (15), 161 (100), 149 (17), 121 (19), 119 (16), 105 (19), 95 (13), 93 (13), 91 (11), 81 (22), 69 (11).

Compound **3**. $[\alpha]_D$ +4.6 ($CHCl_3$; *c* 0.6); IR ν_{max}^{neat} cm^{-1}: 3480, 1639, 1454, 1383, 1190, 1097, 997, 909, 816; ^{1}H NMR (200 MHz, $CDCl_3$): δ0.92 and 0.96 (each 3H, *d*, *J* = 7 Hz), 0.97 (3H, *s*), 2.02 (1H, *sept*, *J* = 7 Hz), 3.97 (1H, *br s*). 4.87 (2H, *m*): EIMS *m/z* (rel. int.): 238 $[M]^+$ (1), 220 $[M - H_2O]^+$ (25), 205 (20), 191 (41), 178 (33), 177 (100), 149 (45), 137 (20), 135 (20), 123 (21), 122 (42), 121 (53), 109 (43), 107 (31), 100 (31), 95 (30), 93 (42), 81 (28).

Compound **4**. $[\alpha]_D$ +8.7 ($CHCl_3$; *c* 0.14); IR ν_{max}^{neat} cm^{-1}: 3464, 1454, 1377, 1173, 1026, 972, 799; ^{1}H NMR (400 MHz, $CDCl_3$): δ0.67 (3H, *s*), 0.95 and 0.94 (each 3H, *d*, *J* = 7 Hz), 4.40 (1H, *d*, *J* = 1.5 Hz), 4.70 (1H, *d*, *J* = 1.5 Hz); EIMS *m/z* (rel. int.) 204 $[M - H_2O]^+$ (48), 189 (14), 180 (9), 179 (61), 162 (17), 161 (100), 133 (28), 123 (19), 121 (19), 119 (23), 109 (12), 107 (13), 105 (32), 95 (23), 93 (21), 91 (19), 81 (26).

Reduction of compound **1** *with* $LiAlH_4$. Compound **1** (38.8 mg) in dry Et_2O was added dropwise to a suspension of $LiAlH_4$ (12 mg) in dry Et_2O (5 ml) and stirred for 3 hr at room temp. An alcohol (38.4 mg) was obtained. **6**; $[\alpha]_D$ −60.6 ($CHCl_3$; *c* 0.71); IR ν_{max}^{neat} cm^{-1}: 3420, 1644; ^{1}H NMR (400 MHz, $CDCl_3$): δ0.78 (3H, *s*), 0.93 and 0.95 (each 3H, *d*, *J* = 7 Hz), 1.66 (1H, *ddd*, *J* = 14, 14, 4 Hz), 1.89 (3H, *br s*), 2.12 (1H, *sept*, *J* = 7 Hz), 3.71 (1H, *dd*, *J* = 11, 7 Hz), 5.39 (1H, *br s*); EIMS *m/z* (rel. int.): 220 $[M - H_2O]^+$ (5), 205 (8), 195 (9), 177 (100), 159 (12), 149 (12), 135 (8), 121 (24), 107 (19), 81 (10), 71 (9). CD: $\Delta\varepsilon_{302}$ +25.6, $\Delta\varepsilon_{280}$ −15.2 [employing 2×10^{-4} M $Eu(FOD)_3$, CCl_4; *c* 0.0054].

Oxidation of compound **6** *with pyridinium dichromate (PDC).* To PDC (200 mg) in CH_2Cl_2 (3 ml) was added

6 (29.7 mg) in CH_2Cl_2 (2 ml) and the mixt. stirred for 1 hr at room temp. The resulting mixt. was filtered and gave, after removal of solvent, a residue that was purified by CC on silica gel using a *n*-hexane–EtOAc gradient to yield **7** (24.2 mg).

Compound **7**. $[\alpha]_D$ +302 ($CHCl_3$; *c* 0.55); IR ν_{max}^{neat} cm^{-1}: 1715; ^1H NMR (200 MHz, $CDCl_3$): δ0.95 (3H, *s*), 1.09 (6H, *d*, *J* = 7 Hz), 1.58 (3H, *br s*), 2.61 (1H, *sept*, *J* = 7 Hz), 5.73 (1H, *br s*), 9.49 (1H, *d*, *J* = 5 Hz).

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