New Humulane-Type Sesquiterpenes from the Liverworts *Tylimanthus tenellus* and *Marchantia emarginata* subsp. *tosana*

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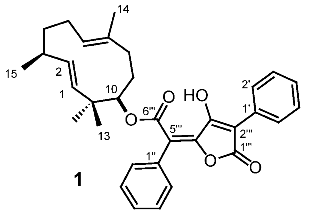
The ether extract of the New Zealand liverwort *Tylimanthus tenellus* produced three new sesquiterpenes, together with (+)-3,11-eudesmadiene and (−)-4(15),11-eudesmadiene which were enantiomeric to those isolated from higher plants. The structures of the new sesquiterpenes were established by 2D NMR spectral data and/or X-ray crystallographic analysis. Those structures were shown to be humulane type sesquiterpene alcohol, and its esters of 2,4-hexadienedioic acid 3,4-dihydroxy-2,5-diphenyl-γ-lactone. 1,6-Humuladien-10-ol was also isolated from Japanese liverwort *Marchantia emarginata* subsp. *tosana*. The absolute configuration of 1,6-humuladien-10-ol was determined by ^{1}H-NMR resolution of its diastereomeric methoxy-α-trifluoromethylphenylacetyl (MTPA) esters. It was shown to be (3*S*,10*R*)-1,6-humuladien-10-ol.

Key words *Tylimanthus tenellus*; Acrobolbaceae; *Marchantia emarginata* subsp. *tosana*; humulane; sesquiterpenoid; *ent*-selinene

In the course of an investigation of bryophyte chemistry, we have isolated many unique compounds from liverworts.[1)] The liverworts are the most primitive terrestrial plants and occasionally produce terpenoids and aromatic compounds.[1)] Although the same compounds as those isolated from higher plants have been also isolated from liverworts, many of these, especially terpenoids are the enantiomeric isomer. Thus the chemical composition of the bryophytes in natural product chemistry is quite interesting. We now report the isolation of some characteristic constituents from the New Zealand liverwort *Tylimanthus tenellus* which has not previously been investigated phytochemically, and the Japanese liverwort *Marchantia emarginata* subsp. *tosana*.

Tylimanthus tenellus (herbarium # NZ150) was collected in December, 2000 at Mt. Ruapehu (altitude 820 m), New Zealand. The ethyl ether extract of *T. tenellus* was chromatographed repeatedly and subjected to preparative HPLC to give three new sesquiterpenes **1**, **2** and **3**, together with known (+)-3,11-eudesmadiene (**5**) and (−)-4(15),11-eudesmadiene (**6**) which were enantiomeric to those found in higher plants.[2)] Pulvinic acid methyl ester (**4**) was isolated from *T. tenellus* as major constituent. It is phytochemically noteworthy that the same compound has been found in many species of lichen.[3—5)] However, as far as we are aware, no exact spectral data has been reported. Then, the structure of **4** was determined by X-ray crystallographic analysis as shown in Fig. 1. This is the first report of the isolation of pulvinic acid methyl ester (**4**) from liverworts. On the other hand, present our work also isolated 1,6-humuladien-10-ol (**2**) from *Marchantia emarginata* subsp. *tosana*.



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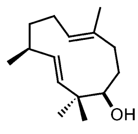

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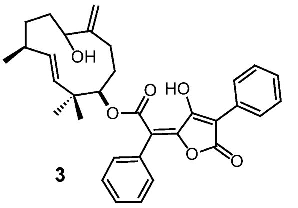

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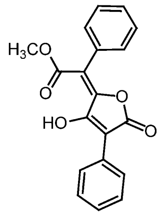

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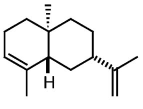

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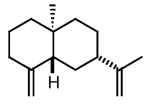

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Compound **1**, a yellow crystal, mp 116 °C; $[\alpha]_D$ +3.0° (c=0.53, CHCl$_3$) was isolated from a less polar fraction of the crude ether extract of *T. tenellus*. The negative electron spray ionization mass (negative ESI-MS) spectrum of **1** gave a quasi-molecular ion peak $[M-H]^-$ at *m/z* 511. The IR spectrum of **1** showed the absorption band for a carbonyl at 1774 cm^{-1}. The presence of an aromatic ring was apparent from the absorption band at 289 and 373 nm in the UV spectrum of **1**. Its ^{1}H-NMR spectrum of **1** exhibited the presence of two tertiary methyl groups at δ 0.63 and 0.79, a secondary and vinyl methyl groups at δ 0.94 and 1.61 (Table 1). The

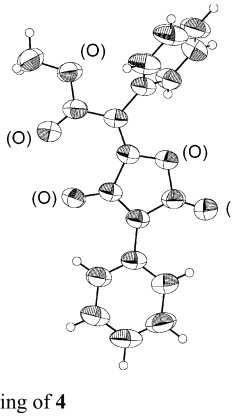

Fig. 1. ORTEP Drawing of **4**

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Table 1. Assignments of NMR Data for Compounds **1** and **3** (in $CDCl_3$)

	1		**3**	
	^1H	^{13}C	^1H	^{13}C
1	4.92 (dd, 1, 1.6)	131.3	5.24 (d, 16)	134.1
2	5.14 (dd, 16, 7)	138.0	5.14 (dd, 16, 9)	135.3
3	2.14 (m)	38.2	2.12 (m)	40.8
4	1.38 and 1.62 (each, m)	35.4	1.29 and 1.68 (each, m)	32.7
5	2.14 (2H, m)	27.0	1.48 and 1.56 (each, m)	34.5
6	4.89 (bt, 7)	128.8	4.06 (bd, 4.5)	72.9
7	—	131.6	—	151.5
8	2.01 (bt, 10) and 2.14 (m)	35.5	2.03 (bt, 9) and 2.48 (ddd, 14, 9, 9)	31.5
9	1.44 (m) and 1.79 (bt, 10)	32.2	1.45 and 1.73 (m)	30.0
10	4.54 (dd, 3, 4)	86.7	4.51 (d, 7)	84.3
11	—	39.2	—	40.5
12	0.79 (3H, s)	26.8	0.83 (3H, s)	27.6
13	0.63 (3H, s)	19.0	0.63 (3H, s)	17.2
14	1.61 (3H, s)	19.2	4.85 and 5.01 (each, s)	108.8
15	0.94 (3H, d, 7)	20.5	0.98 (3H, d, 7)	21.4
1′	—	129.1[a)]	—	129.0[a)]
2′(6′)	8.15 (2H, d, 8)	127.8	8.15 (2H, d, 8)	127.8
3′(5″)	7.38—7.45	128.0	7.38—7.46	128.1
4′	7.33 (bt, 8)	128.2	7.34 (bt, 8)	128.3
1″	—	132.2[a)]	—	132.1[a)]
2″(6″)	7.25 (2H, d, 8)	129.6	7.23 (2H, bd, 8)	129.6
3″(5″)	7.38—7.45	128.4	7.38—7.46	128.4
4″	7.38—7.45	128.4	7.38—7.46	128.4
1‴	—	166.1	—	166.0
2‴	—	104.8	—	105.0
3‴	—	160.5[b)]	—	160.4[b)]
4‴	—	154.5[b)]	—	154.7[b)]
5‴	—	116.7	—	116.5
6‴	—	170.9	—	170.9
O<u>H</u>	14.13 (s) (C-3‴)	—	14.00 (s) (C-3‴)	—

Coupling constant are give in Hz. Assignments were confirmed by HMBC, HMQC and ^1H–^1H COSY spectra. *a*, *b*) Assignments may be interchanged in each column.

characteristic signal at δ 14.13 (s, 1H) which disappeared upon addition of D_2O, showed evidence for the presence of intramolecular hydrogen bonding in **1**. The heteronuclear multiple bond correlation (HMBC) spectra of **1** showed humulane-type sesquiterpene moiety of **1**, while the aromatic partial structure of **1** could not determined. The aromatic ring has no significant proton signal for the structure determination by the HMBC spectrum. The alkaline hydrolysis of **1** gave an alcohol, whose spectral data including optical rotation were identical to those of **2** which was isolated from both the present species and *Marchantia emarginata* subsp. *tosana*, collected in Japan.

Finally, the structure of **1** was clarified by an X-ray crystallographic analysis on crystals obtained from a *n*-hexane–ethyl acetate solution. The stereoscopic view of the crystal of **1** is shown in Fig. 2 and the structure of **1** was established as an ester of 1,6-humuladien-10-ol and (3-hydroxy-5-oxo-4-phenyl-5*H*-furan-2-ylidene)-phenyl acetic acid.

The absolute configuration of **1** was determined by the adaptation of a modified Mosher's method[6)] to (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) and (*S*)-(−)-MTPA esters of sesquiterpene alcohol **2**, which was isolated from *M. emarginata* subsp. *tosana*. The result of the determination is shown in Fig. 3, which in addition gives the $\Delta\delta$ values in Hz. The $\Delta\delta$ values of the protons showed that the absolute configuration of C-10 was an *R*-configuration, while three irregular protons (H-2, 3, 8) were also observed in the structure. The reason why the irregular $\Delta\delta$ value protons appeared, remains to be clarified. We attempted to reconfirm the absolute configuration of **1**, using a lanthanide

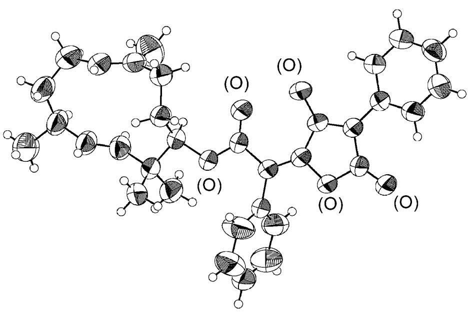

Fig. 2. ORTEP Drawing of **1**

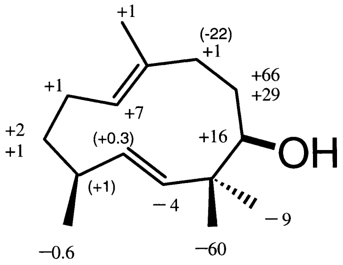

Fig. 3. $\Delta\delta$ Values are shown in Hz and Irregular Protons are in Parenthesis

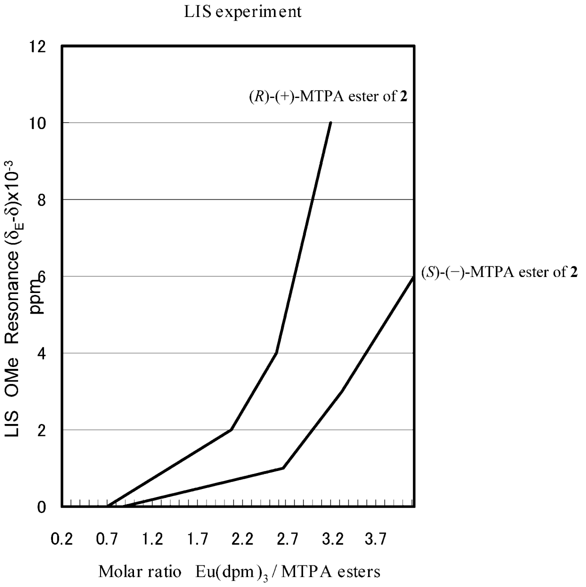

Fig. 4. Plotting of Lanthanide Induced Shift (LIS) of the Methoxyl Proton Resonance *vs.* Molar Ratio of $Eu(dpm)_3$ for the Diastereomeric MTPA Esters

induced shift (LIS) experiment of (*R*)-(+)- and (*S*)-(−)-MTPA esters of **2**. The LIS values of the ^{1}H-NMR of both esters are summarized in Fig. 4. The *R*-configuration at C-10 was apparent from the magnitude of the value of *R*-(+)-MTPA ester of **1**.[7] The absolute configuration of **1** was confirmed as shown.

Compound **2**, $[\alpha]_D$ +56.4° (c=0.2, $CHCl_3$), was isolated from a mixture of **4**. The spectral data including $[\alpha]_D$ of **2** were identical to those of the sesquiterpene alcohol which was obtained by alkaline hydrolysis of **1**. On the other hand, 1,6-humuladien-10-ol (**2**), $[\alpha]_D$ +63.7° (c=0.75, $CHCl_3$), was also isolated from Japanese *Marchantia emarginata* subsp. *tosana*.

Compound **3**, an oil; $[\alpha]_D$ −87.5° (c=0.4, $CHCl_3$) was isolated from the mixture of **4**. The electron impact (EI)-MS of **3** indicated molecular ion peak at *m/z* 528 which different from that of **1** by 16 mass units. The IR spectrum of **3** showed the absorption bands for a hydroxyl group and a carbonyl group at 3526 and 1774 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra (Table 1) of **3** were similar to those of **1**, except for the appearance of proton signals at δ 4.85 and 5.01 (each 1H, s), and for the absence of the vinyl methyl group signal of **1**. As a result of the HMBC spectral analysis, it was proven that the structure of **3** was similar to that of **1**. The HMBC correlation between the signals of sp^2 methylene proton and carbinyl carbon at δ 72.9 and a sp^3 methylene carbon at δ 31.5 indicated the presence of allylic secondary hydroxyl group in **3**. The configuration of the hydroxyl group at C-6 was analyzed by nuclear Overhauser enhancement spectroscopy (NOESY). The correlation between δ 4.06 (H-6) and 1.29 (H-4), 5.14 (H-2), and δ 1.73 (H-9) apparently indicated that H-6 oriented on the same planar surface with H-2, axial H-4 and H-9. In order to obtain further evidence to establish the configuration at C-6 of **3**, conformational analysis of **3** was performed for an optimized structure by Molecular Operating Environment (MOE) software program with Merck molecular force field 94 (MMFF94) parameter.[8] However, many optimized conformers, whose difference of the formation energies were within 10 kcal/mol, were obtained. It was difficult to compare the result of NOE with that of conformational analysis. Consideration of the spectral data led to the conclusion that the structure of **3** was established as shown, although the stereochemistry of **3** remained to be determined.

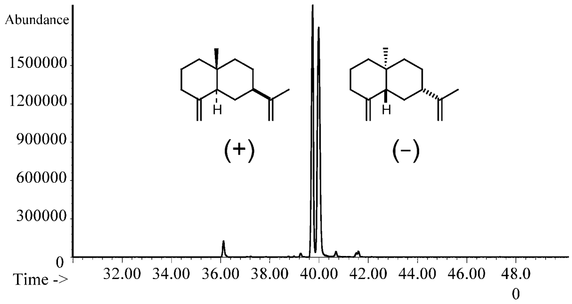

Fig. 5. The Total Ion Chromatogram (TIC) of Co-injected (+)- and (−)-4(15),11-Eudesmadiene

(−)-Enantiomer was isolated from *T. tenellus*. (+)-Isomer was obtained from (+)-β-eudesmol by dehydration with $POCl_3$.

The ether extract of *T. tenellus* afforded two unique sesquiterpene esters **1** and **3**. The non polar fraction of the extract gave enantiomeric sesquiterpene hydrocarbons **5** and **6** as major compounds of the fraction. Those absolute configurations were determined by optical rotation and/or chiral separation by GC-MS analysis (Fig. 5). The liverworts often elaborate sesquiterpenes morphologically enantiomeric to those found in the higher plants.[2]

Tylimanthus species belonging to the Acrobolbaceae superficially rather resemble *Plagiochila* species (Plagiochilaceae).[9] However, there is a chemical affinity between Acrobolbus and *Marchantia* belonging to the Marchantiales. *Marchantia emarginata* subsp. *tosana* produces humulane-type sesquiterpene, but neither eudesmane- nor the esters with aromatic γ-lactone acid have been found in this species.

Experimental

TLC was carried out on silica gel precoated glass plates with *n*-hexane–EtOAc (1 : 1 and 4 : 1). Detection was with Godin reagent.[10] For normal phase column chromatography, silica gel 60 (40—63 μm) was used. A mixture of CH_2Cl_2–MeOH (1 : 1) was used as the solvent for column chromatography on Sephadex LH-20.

NMR spectra were recorded at 150 for ^{13}C and 600 or 200 MHz for ^{1}H. EI-MS were measured at 70 eV. The temperature programming of GC-mass analysis performed from 50 °C isothermal for 3 min, then 50—230° at 3 °C min^{-1}, and finally isothermal at 230 °C for 20 min. Injection temperature was 250 °C. A fused silica column coated with β-DEX120 (30 m×0.25 mm i.d., film thickness 0.25 μm) was used.

Plant Materials and Isolation *Tylimanthus tenellus* (herbarium # NZ150) was collected in December, 2000 at Mt. Ruapehu (altitude 840 m), New Zealand. The ethyl ether extract (813 mg) was chromatographed repeatedly and subjected to preparative HPLC [column: 5SL-II SiO_2, i.d. 6.0×250 mm; mobile phase, EtOAc–*n*-hexane; flow rate, 2.0 ml/min] to give three new sesquiterpene **1** (61.5 mg; 7.6% in yield of the total extract), **2** (2 mg; 0.2%) and **3** (8 mg; 1.0%), together with **4** (48.9 mg; 6.0%). The hydrocarbon fraction was purified by 1% $AgNO_3$ impregnated SiO_2 column to give (+)-3,11-eudesmadiene (**5**), $[\alpha]_D$ +19.6° (c=3.4, $CHCl_3$) (34.9 mg; 4.3%) and (−)-4(15),11-eudesmadiene (**6**), $[\alpha]_D$ −30.9° (c=1.9, $CHCl_3$) (18.5 mg; 2.3%).

Marchantia emarginata subsp. *tosana* was collected in April, 2001 at Kamiyama, Tokushima in Japan. The ethyl ether extract (3.7 g) of *M. emarginata* subsp. *tosana* was chromatographed repeatedly and subjected to preparative HPLC [column: 5SL-II SiO_2, i.d. 6.0×250 mm; mobile phase, EtOAc–*n*-hexane; flow rate, 2.0 ml/min] to give a new sesquiterpene **2** (88 mg; 2.4% in yield of the total extract).

(3-Hydroxy-5-oxo-4-phenyl-5*H*-furan-2-ylidene)-phenyl Acetic Acid 1,6-Humuladien-10-yl Ester (**1**): Yellow crystals; mp. 116 °C; $[\alpha]_D$ +3.0° (c=0.53, $CHCl_3$); Negative ESI-MS *m/z* 511; HR-EI-MS *m/z*: 512.2567: $[M]^+$ (Calcd for $C_{33}H_{36}O_5$: 512.2563); IR ($CHCl_3$) cm^{-1}: 1774, 1441; UV λ_{max} (EtOH) nm (log ε): 373 (3.95), 289 (4.30).

1,6-Humuladien 10-ol (**2**): An oil, $[\alpha]_D$ +56.4° (c=0.20, $CHCl_3$) (from *T. tenellus*); $[\alpha]_D$ +63.7° (c=0.75, $CHCl_3$) (from *M. emarginata* subsp. *tosana*). FT-IR (neat): 3615 (OH) cm^{-1}. EI-MS *m/z* (rel. int.): 222 $(M)^+$(10), 204 (22), 189 (49), 161 (91), 122 (56), 109 (50), 107 (100), 105 (60), 95 (52), 93 (61), 81 (52). ^{1}H-NMR (600 MHz; $CDCl_3$): δ 0.94, 1.02 (each 3H, s), 0.98 (3H, d, J=7 Hz), 1.58 (3H, d, J=1 Hz), 3.18 (bs), 4.95 (bt, J=7 Hz), 5.05 (1H, dd, J=16, 1 Hz), 5.15 (1H, dd, J=16, 7 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$): δ 18.9 (C-12), 19.6 (C-14), 20.6 (C-15), 26.7 (C-13), 27.1 (C-5), 32.7 (C-9), 35.7 (C-4), 35.7 (C-8), 38.3 (C-3), 40.4 (C-11), 80.4 (C-10), 127.9 (C-6), 132.7 (C-7), 133.8 (C-1), 136.8 (C-2).

(3-Hydroxy-5-oxo-4-phenyl-5*H*-furan-2-ylidene)-phenylacetic Acid 6-Hydroxy-1,7(11)-humuladienyl-10-yl Ester (**3**): An oil; $[\alpha]_D$ −87.5° (c=0.4, $CHCl_3$); EI-MS *m/z* (rel. int.): 528 $(M)^+$(8), 308 (58), 290 (100), 145 (59), 89 (26); HR-EI-MS *m/z*: 528.2513: $(M)^+$ (Calcd for $C_{33}H_{36}O_6$: 528.2512); IR ($CHCl_3$) cm^{-1}: 3526, 1774, 1440; UV λ_{max} (EtOH) nm (log ε): 377 (3.93), 291 (4.44).

Crystallographic Analysis of 1 and 4 Yellow single crystals of **1** and **4** grown from *n*-hexane–ethyl acetate were used for the X-ray crystal analysis and data were collected by a MacScience DIP-2020 diffractmeter with Mo$K\alpha$ radiation (λ=0.71073 Å), respectively. The crystal data of **1** and **4** were as follows: **1**; $C_{33}H_{36}O_5$, M_r=512.646, Orthorhombic, space group $P2_12_12_1$, a=17.5800(5) Å, b=18.6080(5) Å, c=35.2940(12) Å, Z=16, V=11545.7(6) Å^3, D_x=1.180 Mg m^{-3}, R_1=0.0592, R_w=0.1446, **4**; $C_{19}H_{14}O_5$, M_r=322.316, Monoclinic, space group $P2_1/n$, a=7.8460(6) Å, b=7.8420(5) Å, c=26.032(3) Å, β=94.306(4)°, Z=4, V=1597.2(2) Å^3, D_x=1.340 Mg m^{-3}, R_1=0.0607, R_w=0.1583, All diagrams and calculations were performed using maXus (Bruker Nonius, Delft & MacScience, Japan).

Alkaline Hydrolysis of 1 The mixture of **1** (10 mg) and 1% KOH (3 ml) in EtOH was refluxed for 16 h. After removal of the solvent in vacuum, H_2O (1 ml) was added to the mixture. The reaction mixture was extracted with ether, which was then evaporated to give a residue. The mixture was purified by HPLC [column: 5SL-II SiO_2, i.d. 6.0×250 mm; mobile phase, EtOAc–*n*-hexane; flow rate, 2.0 ml/min] to give sesquiterpene **2** (1 mg) and a polar fraction (10 mg) which was still a complicated mixture.

Esterification of 1,6-Humuladien 10-Ol (2) Isolated from *M. emarginata* subsp. *tosana* by (*R*)-(+)- and (*S*)-(−)-α-Methoxy-α-trifluoromethylphenylacetic Acid (MTPA) To a solution of **2** (10 mg) in dry CH_2Cl_2 (0.7 ml), *R*-(+)-MTPA (64 mg), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (82 mg) and 4-dimethylaminopyridine (DMAP) (20 mg) were added and the reaction mixture was stirred for 6 d. To the mix, H_2O was added and the solution was washed with 1 N HCl, saturated $NaHCO_3$ and NaCl. After evaporation, the CH_2Cl_2 layer was purified by preparative HPLC to give a mono *R*-(+)-MTPA ester (1.4 mg). *S*-(−)-MTPA ester (1.1 mg) of **2** was obtained by *S*-(−)-MTPA with in the same manner as above.

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