

## GYMNOMITRANE-TYPE SESQUITERPENOIDS FROM THE LIVERWORT *PLAGIOCHILA TRABECULATA*\*

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**Key Word Index**—*Plagiochila trabeculata*; Plagiochilaceae; Hepaticae; 9 $\alpha$ -hydroxygymnomitryl acetate; 9 $\alpha$ -hydroxygymnomitryl cinnamate; 9-oxogymnomitryl acetate; gymnomitrol; gymnomitrene; sesquiterpenoids; chemosystematics.

**Abstract**—Three novel gymnomitrane-type sesquiterpenoids, 9 $\alpha$ -hydroxygymnomitryl acetate, 9-oxogymnomitryl acetate and 9 $\alpha$ -hydroxygymnomitryl cinnamate were isolated from the liverwort *Plagiochila trabeculata* along with the known gymnomitrene and gymnomitrol and their structures determined by NMR and chemical transformations. The present species is classified to the fifth chemotype in *Plagiochila*.

### INTRODUCTION

*Plagiochila* species are rich sources of 2,3-secoaromadendrane-type sesquiterpenoids [1-3], bibenzyls [3, 4] and cyclic bis (bibenzyl) derivatives [5]. They are classified as pungent and non-pungent species. The former produce plagiophilane A, a secoaromadendrane-type sesquiterpene which shows intense pungency, antifeedant, piscicidal, and cytotoxic activity (KB cell) [6-8]. The latter produce mainly aromatic compounds or different types of sesquiterpenoids and diterpenoids [9]. *Plagiochila trabeculata* belongs to the non-pungent group. It grows on rocks or trunks of deciduous trees and contains four to ten oil bodies per cell. In this paper, we wish to report the isolation and characterization of three new gymnomitrane-type sesquiterpenoids from *P. trabeculata* and discuss its chemosystematics.

### RESULTS AND DISCUSSION

TLC, GC and GC/MS showed the presence of limonene, gymnomitrol (6), gymnomitrene (7), bicyclogermacrene (9), 3 $\alpha$ -acetoxybicyclogermacrene (10), spathulenol (11) and anastreptene (12), and squalene in the dichloromethane extract of the dried liverwort. A combination of silica gel and Sephadex LH-20 column chromatography of the extract resulted in the isolation of three new gymnomitrane-type sesquiterpenoids (1, 2, 5), together with gymnomitrol (6), gymnomitrene (7), 3 $\alpha$ -acetoxy-*ent*-bicyclogermacrene (10) and spathulenol (11).

#### 9 $\alpha$ -Hydroxygymnomitryl acetate (1)

The IR spectrum of 1 indicated the presence of a hydroxyl group (3500 cm<sup>-1</sup>) and an acetoxy group

(1745, 1240 cm<sup>-1</sup>). The <sup>1</sup>H NMR spectrum (Table 1) contained the signals of three tertiary methyls, a secondary acetoxy group, an exomethylene group and an allylic secondary hydroxyl group. The mass spectrum contained the fragment peak *m/z* 218 ([M]<sup>+</sup> - AcOH) and thereby the molecular formula of 1 was estimated as C<sub>17</sub>H<sub>26</sub>O<sub>3</sub> after taking into account the <sup>13</sup>C NMR data (Table 2), which showed the presence of 17 carbon atoms: five methyls, four methylenes, one methine, two carbons bearing oxygen, an ester carbonyl, three quaternary carbons and an exomethylene. The spectral data suggested 1 to be a tricyclic sesquiterpene. The <sup>1</sup>H-<sup>1</sup>H correlated 2D-COSY NMR spectrum indicated the presence of a ■-(CH<sub>2</sub>)<sub>3</sub>-■ group, thus establishing that 1 was a gymnomitrane (= barbatane)-type sesquiterpene [10, 11] with C-(9)OH and C-(11)OAc groups. This was confirmed as follows. Oxidation of 1 with pyridinium chlorochromate (PCC) gave a conjugated ketone (5), C<sub>17</sub>H<sub>24</sub>O<sub>3</sub> ([M]<sup>+</sup> 276), whose <sup>1</sup>H NMR spectrum showed the presence of AB doublets (*J* = 19.5 Hz, H-8). Acetylation of 1 with acetic anhydride-pyridine afforded a diacetate (3), C<sub>19</sub>H<sub>28</sub>O<sub>4</sub> [δ<sub>H</sub> 2.08, 2.09 (each 3H, s)], which on reduction with lithium aluminium hydride gave a diol (4), C<sub>15</sub>H<sub>24</sub>O<sub>2</sub> (δ<sub>H</sub> 4.00, s, H-11; 4.36, *m*, H-9). Compound 3 was treated with boron trifluoride etherate to give an acetoxy migration product, whose spectral data were identical to those of 8-acetoxymethyl-1,2,6-trimethyl-tricyclo-[5.3.1.0<sup>2,6</sup>]undec-8-en-11-yl acetate (8) [11]. On the basis of the above spectral and chemical data and difference NOE spectral examinations (see Fig. 1), the structure of 1 was established to be 9 $\alpha$ -hydroxygymnomitryl acetate.

#### 9 $\alpha$ -Hydroxygymnomitryl cinnamate (2)

The spectral data of 2, C<sub>24</sub>H<sub>30</sub>O<sub>3</sub> ([M]<sup>+</sup> 366), resembled those of 1, except for the replacement of the acetoxy group by a cinnamate group [*m/z* 131(base); <sup>13</sup>C NMR (INEPT) (Table 2)]. That compound 2 was 9 $\alpha$ -hydroxygymnomitryl cinnamate and not 9 $\alpha$ -cinnamoxygymnomitrol was confirmed by extensive spin decoupling and the

\*Part 25 in the series of 'Chemosystematics of Bryophytes'. For Part 24 see [17].

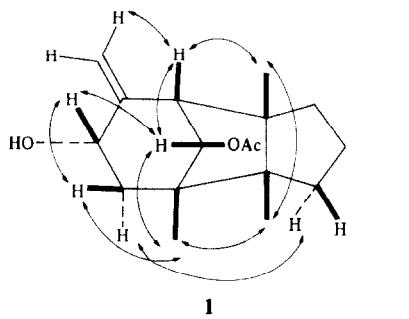
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Table 1.  $^1\text{H}$  NMR data of gymnomitrenes **1**, **2**, **5** and **6** (400 MHz,  $\text{CDCl}_3$ , TMS as internal standard)\*

H	<b>1</b>	<b>2</b>	<b>5</b>	<b>6</b>	<b>6</b> [11]
1	2.46 <i>s</i>	2.55 <i>s</i>	2.73 <i>s</i>	2.33 <i>s</i>	2.32 <i>s</i>
3	0.92–1.37 <i>m</i>	0.84–1.38 <i>m</i>	1.04–1.29 <i>m</i>		
4	1.39–1.44 <i>m</i>	1.41–1.50 <i>m</i>	1.51 <i>m</i>		
5	1.85 <i>m</i>		1.73 <i>m</i>		
8a	1.83 <i>dd</i> (14.7, 8.4)†	1.87 <i>dd</i> (15.0, 8.1)	1.81 <i>m</i>		
8b	1.96 <i>dd</i> (14.7, 9.9)	2.01 <i>dd</i> (15.0, 10.3)	2.23 <i>d</i> (19.5)		
9	4.47 <i>m</i>	4.52 <i>m</i>	2.80 <i>d</i> (19.5)		
11	5.03 <i>s</i>	5.17 <i>s</i>	5.04 <i>s</i>	3.72 <i>s</i>	3.70 <i>s</i>
12	1.15 <i>s</i>	1.21 <i>s</i>	1.20 <i>s</i>	1.24 <i>s</i>	1.21 <i>s</i>
13	1.03 <i>s</i>	1.11 <i>s</i>	1.11 <i>s</i>	1.09 <i>s</i>	1.07 <i>s</i>
14	0.94 <i>s</i>	1.00 <i>s</i>	0.96 <i>s</i>	0.96 <i>s</i>	0.97 <i>s</i>
15a	4.90 <i>d</i> (2.7)	4.92 <i>d</i> (2.6)	0.96 <i>s</i>	4.64 <i>s</i>	4.63 <i>s</i>
15b	5.20 <i>d</i> (2.2)	5.23 <i>d</i> (2.2)	5.20 <i>d</i> (2.0)	4.65 <i>s</i>	4.65 <i>s</i>
AcO–	2.07 <i>s</i>		6.07 <i>d</i> (2.0)		
Ph–CH=CH–		7.69 <i>d</i> (15.7)	2.13 <i>s</i>		
Ph–CH=CH–		6.42 <i>d</i> (15.7)			
		7.39 <i>m</i>			
$\text{C}_6\text{H}_5\text{-}$		7.54 <i>m</i>			

\* All assignments were carried out by spin decoupling, 2D-COSY and NOE difference experiments.

† Figures in parentheses are coupling constants in Hz.



NOEs (↔) observed by NOE difference spectra

Fig. 1.

2D-COSY NMR spectra. The stereochemistry of **2** was determined by NOE difference spectra.

#### 9-Oxogymnomitryl acetate (**5**)

The spectral data of the third compound were completely identical to those of **5** prepared from **1** by PCC oxidation as described above. In addition to the above new sesquiterpenoids, two gymnomitrene-type sesquiterpenoids, gymnomitrol (**6**) [10,11] and gymnomitrene ( $=\beta$ -barbatene) (**7**) [1,10,11] have been isolated, together with *ent*-spathulenol (**11**) and  $3\alpha$ -acetoxy-*ent*-bicyclogermacrene (**10**) [12] from *P. trabeculata*.

Gymnomitene (**7**) is widespread not only in the Jungermanniales but also in the thalloid liverworts Metzgeriales and Marchantiales [1]. However, the distribution of oxygenated gymnomitrenes is very restricted in a few genera; *Bazzania tricrenata* [13] and *Gymnomitrium obtusum* [10,11]. It is known that there are at least 2000 species of *Plagiochila* in the world. We have investigated two European [14], one New Zealand [4], 12 Japanese [14], 13 Chilean [2] and 30 Peruvian *Plagiochila* species

[3] and have classified them into eight chemotypes: 2,3-secoaromadendrane-type (**I**), bibenzyl-type (**II**), cuparene-isocuparene-type (**III**), bibenzyl-cuparene-isocuparene-type (**IV**), gymnomitrene ( $=\beta$ -barbatene)-bicyclogermacrene-type (**V**), bicyclogermacrene-spathulenol-type (**VI**), pinguisane-type (**VII**) and sesquiterpene lactone-type (**VIII**) [2, 3].

The present species produces gymnomitrene- and *ent*-bicyclogermacrene-types sesquiterpenoids like Chilean *P. engelii*, *P. neesiana* and *P. parvidens* [2]. Thus *P. trabeculata* is classified to chemotype **V**.

#### EXPERIMENTAL

The solvents used for spectral determination were TMS- $\text{CDCl}_3$  [ $^1\text{H}$  NMR (400 MHz);  $^{13}\text{C}$  NMR (100 MHz)];  $\text{CHCl}_3$  (IR).  $\text{CHCl}_3$ -MeOH (1:1) was used for CC on Sephadex LH-20. TLC, GC and GC/MS were carried out as previously reported [15, 16].

*Plant material.* *Plagiochila trabeculata* Steph. was collected in Yakushima, in Dec. 1983 and identified by Dr H. Inoue. The voucher specimen was deposited at the Institute of Pharmacognosy, Tokushima Bunri University.

*Extraction and isolation.* *P. trabeculata* was air-dried and ground mechanically to give a powder (1.20 kg) which was extracted with  $\text{CH}_2\text{Cl}_2$  for 1 month. A small amount of the green oil, after removal of the solvent, was analysed by TLC, GC, and GC/MS and the presence of limonene, gymnomitrol (**6**), gymnomitrene ( $=\beta$ -barbatene) (**7**), bicyclogermacrene (**9**),  $3\alpha$ -acetoxy-*ent*-bicyclogermacrene (**10**), spathulenol (**11**) and anastreptene (**12**), and squalene were confirmed by comparison of the MS data with those of authentic samples. The remaining crude extract (28.50 g) was chromatographed on silica gel using a *n*-hexane-EtOAc gradient to give 18 fractions. Rechromatography of Fr. 9 (*n*-hexane-EtOAc 9:1) (1.00 g) on Sephadex LH-20 and silica gel impregnated with  $\text{AgNO}_3$  (10%) gave *ent*-spathulenol (**11**) (13 mg) [1]. Fr. 7 and 8 (*n*-hexane-EtOAc 9:1) contained a viscous oil which was dissolved in MeOH. The MeOH-

Table 2.  $^{13}\text{C}$  NMR data of gymnomitrenes **1**, **2** and **5** (100 MHz,  $\text{CDCl}_3$ , TMS as internal standard)\*

C	<b>1</b>	<b>2</b>	<b>5</b>
1	58.2	58.3	58.2
2	54.9 <sup>a</sup>	55.0 <sup>a</sup>	55.8 <sup>a</sup>
3	39.5 <sup>b</sup>	39.5 <sup>b</sup>	38.9 <sup>b</sup>
4	24.7	24.7	25.5
5	40.0 <sup>b</sup>	40.0 <sup>b</sup>	40.3 <sup>b</sup>
6	54.3 <sup>a</sup>	54.4 <sup>a</sup>	55.0 <sup>a</sup>
7	48.6 <sup>a</sup>	48.8 <sup>a</sup>	48.4 <sup>a</sup>
8	42.5	42.6	51.5
9	65.0	65.1	198.8
10	150.4	150.5	145.3
11	84.3	83.6	87.0
12	28.0	28.1	28.0
13	24.6	27.4	24.2
14	21.7	21.8	19.9
15	111.7	111.8	123.1
Me—COO	21.4	21.3	
COO	170.4	170.1	
Cinnamoyl			
Ph-CH=	145.0		
Ph-CH=CH	130.4		
$\text{C}_6\text{H}_5-$	118.3		
	128.1 ( $\times 2$ )		
	128.9 ( $\times 2$ )		
	134.3		
CO	166.4		

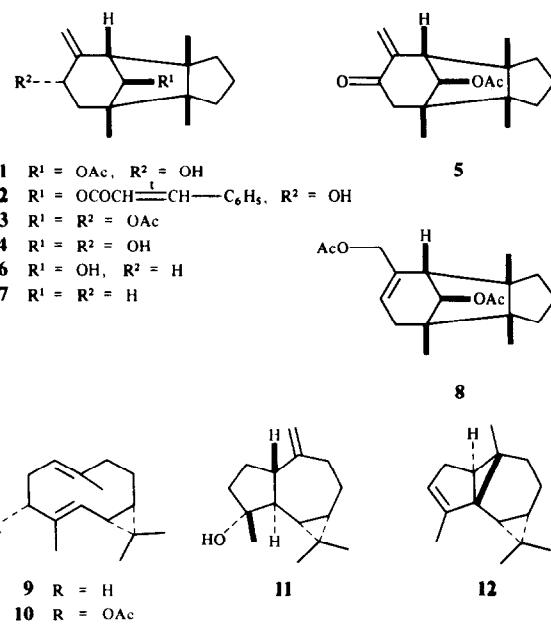
\*All assignments were carried out by LSPD and selective decoupling.

<sup>a, b</sup>Values in any vertical column may be interchanged.

soluble part (800 mg) was rechromatographed on Sephadex LH-20 to give gymnomitrol (**6**) (14 mg) [10, 11] and  $3\alpha$ -acetoxy-*ent*-bicyclogermacrene (**10**) (13 mg) [12]. The insoluble part contained triglyceride (700 mg). Fr. 11 (9:1-4:1) (2.40 g) was further chromatographed on Sephadex LH-20 and collected as 3 fractions (11A-C). Fr. 11B (1.50 g) was purified by prep. TLC (*n*-hexane-EtOAc 4:1) to give 9-oxogymnomitryl acetate (**5**) (15 mg); IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 1735, 1234 (AcO), 1690 (C=C-CO), 1614 (C=C), 1380, 1365, 1355, 1010; EIMS  $m/z$  (rel. int.): 276 [ $\text{M}]^+$  (1), 234 (17), 216 (14), 201 (13), 138 (61), 123 (14), 122 (46), 121 (16), 113 (33), 110 (11), 96 (36), 95 (100), 43 (23). Fr. 13 (4:1-7:3) (2.05 g) was rechromatographed on Sephadex LH-20 and collected as 3 fractions (13 A-C). Fr. 13 C (370 mg) was further chromatographed on silica gel using MeOH-CHCl<sub>3</sub> (1:49), followed by Sephadex LH-20 to afford 9 $\alpha$ -hydroxygymnomitryl acetate (1) (100 mg) and 9 $\alpha$ -hydroxygymnomitryl cinnamate (2) (9 mg). 1: IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3500 (OH), 1745, 1240 (AcO), 1465, 1360, 1060, 1040, 1030; EIMS  $m/z$  (rel. int.): 218 [ $\text{M}]^+$ —AcOH (8), 140 (19), 124 (21), 123 (38), 122 (49), 107 (19), 106 (19), 96 (27), 95 (100), 94 (19), 91 (20), 81 (36), 79 (20), 55 (20), 43 (60). 2: IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3600 (OH), 1705 (COO), 1635, 1600 (benzene ring), 1160, 1035; EIMS  $m/z$  (rel. int.): 366 [ $\text{M}]^+$  (6), 218 (8), 131 (100), 102 (22), 95 (12).

**Oxidation of 1.** Compound **1** (20 mg) in  $\text{CH}_2\text{Cl}_2$  (2 ml) was mixed with pyridinium chlorochromate (PCC) (40 mg) and stirred at room temp. overnight. Work-up as usual gave **5** (15 mg), whose spectra were superimposable on those of the natural 9-oxogymnomitryl acetate (**5**).

**Acetylation of 1.** Compound **1** (45 mg) was acetylated with  $\text{Ac}_2\text{O}-\text{C}_5\text{H}_5\text{N}$  (each 0.5 ml, overnight). Work-up as usual gave a diacetate (**3**) (40 mg); IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 1745, 1230 (AcO), 1360, 1035;  $^1\text{H}$  NMR:  $\delta$  0.93, 1.04, 1.16 (each 3H, s), 2.08, 2.09 (each,



3H, s), 2.48 (1H, s), 4.91 (1H, d,  $J=2.4$  Hz, H-15a), 5.01 (1H, s, H-11), 5.04 (1H, d,  $J=2.4$  Hz, H-15b), 5.54 (1H, m, H-9); EIMS  $m/z$  (rel. int.): 218 [ $\text{M}-\text{AcOH}-42$ ] $^+$  (23), 123 (26), 122 (71), 106 (23), 96 (22), 95 (100), 81 (23), 43 (84).

**Reduction of 1.** To a suspension of  $\text{LiAlH}_4$  (15 mg) in  $\text{Et}_2\text{O}$  was added compound **1** (10 mg) in  $\text{Et}_2\text{O}$ . The mixture was stirred at 0° for 1 hr, after which work-up as usual gave a diol (**4**) (8.5 mg);  $^1\text{H}$  NMR:  $\delta$  1.02, 1.06, 1.24 (each 3H, s), 2.43 (1H, s), 4.00 (1H, s, H-11), 4.36 (1H, m, H-9), 4.90, 5.16 (each, 1H, br s, H-15a and H-15b).

**Reaction of 3 with  $\text{BF}_3$ -etherate.** To compound **3** (30 mg) in  $\text{C}_6\text{H}_6$  (2 ml) was added  $\text{BF}_3$ -etherate (one drop). The mixture was stirred at room temp. for 5 min and, after removal of the solvent, purified by prep. TLC (*n*-hexane-EtOAc 4:1) to give a diacetate (**8**) (2.3 mg), whose spectral data were identical to those of 8-acetoxymethyl-1,2,6-trimethyl-tricyclo-[5.3.1.0<sup>2,6</sup>]undec-8-en-11yl acetate [11].

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