Phytochemistry of three selected liverworts: *Conocephalum conicum*, *Plagiochila barteri* and *P. terebrans*

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Abstract

2α-Cinnamoyloxy-6β-acetoxybornane (**1**) has been isolated from the liverwort *Conocephalum conicum* (Conocephalaceae), while *Plagiochila barteri* and *P. terebrans* (Plagiochilaceae) furnished *ent*-spathulenol, marchantins C and H, 1(10),14-halimadien-13ξ-ol, and trifarienol B. Although the bisbibenzyl-type isoplagiochins have been reported to occur in some *Plagiochila* species, the presence of marchantins in the genus is very rare. Furthermore, the halimane diterpene usually found in *Jungermannia* species was isolated from *Plagiochila* sp. for the first time, and the relationship between the two genera *Jungermannia* and *Plagiochila* belonging to the Jungermanniales order (suborder: Jungermaniineae) is confirmed. All of the isolated compounds were tested for α-glucosidase inhibitory activity but only marchantin C showed moderate activity.

Keywords: Liverworts, *Conocephalum conicum*, *Plagiochila barteri*, *P. terebrans*, Chemical constituents, α-glucosidase inhibitor

Introduction

Mosses, liverworts, and hornworts are the three groups of bryophytes, which represent a grade or structural level in plant evolution. Their classification is often difficult morphologically since they have very small gametophytes. However, the lipophilic terpenoids and aromatic metabolites in their oil bodies can be a value in taxonomic investigation. The liverworts group (about 6.000 species recognized) is well-adapted to moist habitat. Their phytochemical studies have shown that they contain a wide variety of structurally interesting compounds, which are antibacterial, antifungal, anticancer, and diuretic.[1] In the continuation of our ongoing study aiming to find

novel and biologically active compounds from the moisture-loving plants, we wish to report the isolation and the structure determination of the chemical constituents of three liverworts: *Conocephalum conicum*, *Plagiochila barteri*, *P. terebrans*. The α-glucosidase inhibitory effect of the isolated compounds was evaluated.

Results and Discussion

A combination of Sephadex and silica gel column chromatography of *Conocephalum conicum* ether extract led to the isolation of a new compound (**1**) having a molecular formula $C_{21}H_{26}O_4$ as determined by HREIMS analysis. Its ^{1}H-NMR spectral data exhibited signals of a cinnamoyl moiety [(3H, δ 7.39, m; 2H, δ 7.55, m; 1H, δ 6.45, d, *J*= 15.9 Hz; 1H, δ 7.66, d, *J*= 15.9 Hz)], together with resonances of one acetoxy methyl (δ 2.01, s), two oxygen-bearing methines (δ 4.97; ddd, *J*= 9.6, 3.3, 1.9 Hz and δ 4.71, dd, *J*= 8.2, 5.0 Hz), and signals ascribable to oxygenated borneol. The ^{13}C-NMR spectrum showed 21 signals attributable to a cinnamoyl group, an acetyl group and 10 signals for the monoterpene moiety (Table 1). In order to establish the full structure of **1**, HSQC, COSY, HMBC, and NOESY experiments were carried out. Interpretation of the COSY spectral data allowed the allocation of the two oxygen-bearing to be at C-2 and C-6, which was substantiated by the partial structure -OCH-CH_2-CH-CH_2-CHO-. The attachment of the acetoxyl group to be at C-6 and the cinnamoyl group at C-2 was confirmed by careful analysis of the HMBC spectrum, which showed a cross peak from H-2 to the cinnamoyl carbonyl and from H-6 to the acetyl carbonyl. Moreover, the presence of the long-range coupling (1.9 Hz) of H-2 to H-4 and the NOE cross-peaks between H-2 and the C-8 and C-10 methyl protons (Figure 1) supported the pseudo equatorial orientation in the boat ring of the proton H-2. To ascertain the orientation of H-6, we have taken one of the C-5 protons (H-5a, δ 2.56, dd, *J*= 13.9, 8.2 Hz), which did not show any coupling with H-4 due to the dihedral angle between the two protons, as reference. The pseudo axial orientation of H-6 was thus deduced by presence of the NOE correlation between H-5a and H-6 as shown (Figure 1). The absolute configuration could not be determined due to the small amount of sample available. From the above data, the structure of **1** was deduced to be 2α-cinnamoyloxy-6β-acetoxybornane.

Each ether extract of *P. barteri* and *P. terebrans* was repeatedly chromatographed on sephadex LH-20 and silica-gel to give spathulenol (**2**),[1] marchantin C (**3**) and marchantin H (**4**),[2] together with a halimane diterpene: 1(10),14-halimadien-13ξ-ol (**5**) from *P. barteri* and trifarienol B (**6**)[3] from *P. terebrans*.

The structure of compound **5** was tentatively determined to be the same as 1(10),14-halimadien-13ξ-ol by comparison of its ^{1}H- and ^{13}C-NMR spectral with those of (**7**), previously isolated from *Jungermannia infusca*.[4] The orientation of the C-8 and C-9 methyl groups was determined as depicted by interpretation of the NOESY spectral data (Figure 2). Interestingly, compounds **5** and **7** were differentiated by their optical rotation values ($[\alpha]^{18}_{D,}$ = 28.2° and −60.2°, respectively), suggesting that they are *enantiomers*. However, the big difference in

optical rotation could be also interpreted as due to the configuration difference of C-13. Attempt was made to determine the stereochemistry but the amount of the sample as well as the quaternary nature of the C-13 hydroxyl, could not allow us to make derivative products suitable for stereochemical determination.

The structure of compounds **2**– **4** and **6**, were established by comparison of their physical and spectroscopical data with the reported data in the literature.[1-3]

Table 1. ^{1}H- and ^{13}C-NMR spectral data for compound **1** (600 and 150 MHz, in $CDCl_3$)

	1	
Position	H	C
1		47.7
2	4.97; ddd (9.6, 3.3, 1.9)	77.9
3a	2.49; ddd (14.7, 9.6, 5.0)	34.0
3b	1.05; dd (14.7, 3.2)	
4	1.95; d (5.0)	49.8
5a	2.56; dd (13.9, 8.2)	37.2
5b	1.58; d, m (14.5)	
6	4.71; dd (8.2, 5.0)	77.1
7		49.8
8	0.95; s	19.5
9	1.06; s	20.4
10	0.93; s	12.8
1’		134.3
2’	7.39; m	128.8
3’	7.55; m	128.1
4’	7.39; m	130.2
5’	7.55; m	128.1
6’	7.39; m	128.8
7’	6.45; d (15.9)	118.2
8’	7.66; d (15.9)	144.6
C=O		167.1
$\underline{CH_3}C=O$	2.01, s	21.3
$CH_3\underline{C=O}$		170.6

Assignments based on COSY, HSQC, and HMBC experiments

Chemosystematic significance

Toyota[5] has reported the presence of three chemotypes in *C. conicum* (chemotype I: sabinene, chemotype II: (+)-bornyl acetate, chemotype III: methyl cinnamate) by analyzing 400 samples of *C. conicum* from Tokushima prefecture (Japan). GC/MS analysis of the present sample (collected from Bizan, Tokushima city) showed the high content of bornyl acetate confirming its

classification in chemotype II as previously mentioned. The isolation of compound **1** in the yield of 1.441% suggested that the present sample might be the link between chemotypes II and III. Noteworthy, sabinene was detected in the sample but in trace.

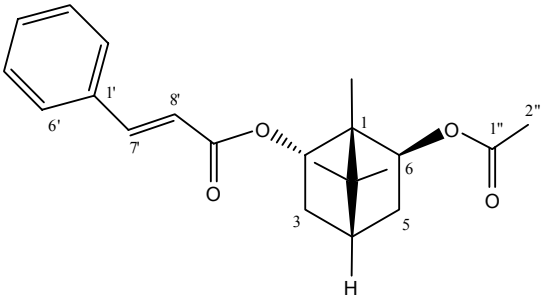
The genus *Plagiochila* (family Plagiochilaceae, suborder Jungermanniineae, order Jungermanniales) is one of the largest neotropical and tropical liverworts genera. Although the presence of bibenzyls and bisbibenzyls as isoplagiochins has been reported, marchantins C and H have been only isolated from *Plagiochila sciophila* (=*P. acanthopylla* subsp. *japonica*).[6] The Malagasy *P. barteri* is thus the second species of *Plagiochila*, which has been known to contain marchantin type bisbibenzyls.

The genera *Jungermannia* (Jungermannioideae) and *Plagiochila* (Plagiochilaceae) belong to the suborder Jungermaniineae. Although, halimane diterpenes are known to be present in the *Jungermannia* species,[4] their occurrence in *Plagiochila* species is very rare. One of the important points of the present results is that liverwort chemical constituents can prove the relationship between the two species belonging to the same suborder.

The unique sesquiterpene trifarienol B was only isolated from the Malaysian liverwort belonging to the family Lejeuneaceae (Subfamily Lejeuneoidieae), *Cheilolejeunia trifaria*. *Plagiochila terebrans* is the second liverwort containing compound **6**. Its biosynthesis in *Plagiochila* species can be the same as reported by Tazaki and co-workers,[7] since the genus is known to contain pinguisane sesquiterpenes.[1]

Alpha-glucosidase inhibitory activity

Apart from the reported biological activities,[1] there is increasing evidence that liverworts secondary metabolites may cause other beneficial effects. Diabetes mellitus is one of the most common chronic diseases and a major contributor to the development of cardiovascular diseases. It is due to a deficiency or a failure of normal action of insulin, which is responsible of the use of the sugar from the diet. The number of cases of non-insulin dependent diabetes mellitus has increased dramatically due to the changes in lifestyle, increasing prevalence of obesity, and aging of populations.[8] Inhibition of α-glucosidase, which catalyzes the final step in the digestive process of carbohydrates can give contribution to the worldwide battle against the growing increase of non-insulin dependent diabetes mellitus patients. The uses of plants and herbal remedies are very common in Asia, where traditional Chinese medicine demonstrates a good practice and shows a bright future in the therapy of diabetes and its complications.[9] Therefore, the inhibitory activity toward α-glucosidase by compounds **1**, **3**– **6**, and trifarienol A (**8**), previously isolated from the Malaysian *Cheilolejeunia trifaria* was evaluated,[3] and only marchantin C (**3**) showed 52.2% inhibition of the enzyme activity at 1 mM. It is interesting to note that the insolubility of marchantin H in the medium could effect on the activity. Although, the present activity is lower than that of the 1-deoxynojirimycin (100% at 0.4 mM), used as reference, the present results can give a hope for the research on finding new important molecular chromophores for inhibition of α-glucosidase activity. As far as we are aware, this is the first report on the α-glucosidase inhibitory activity of marchantins.



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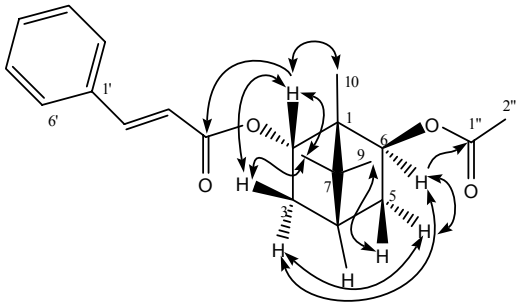


Figure 1. Important HMBC (arrow) and NOESY (double arrow) correlations observed in **1.**

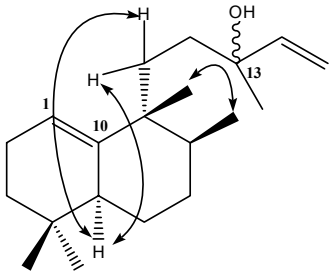
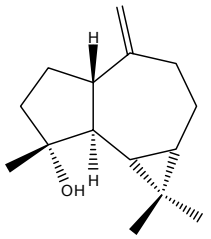


Figure 2. Important NOE correlations observed in **5**



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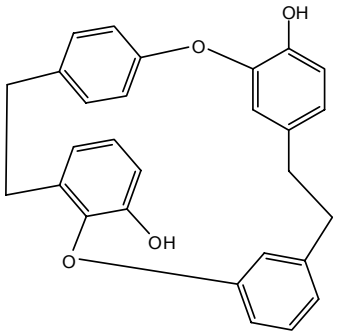

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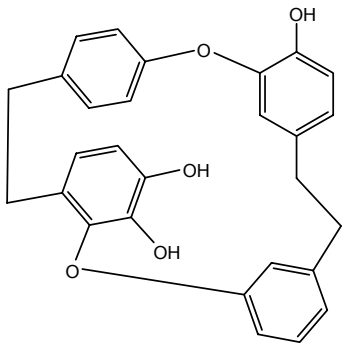

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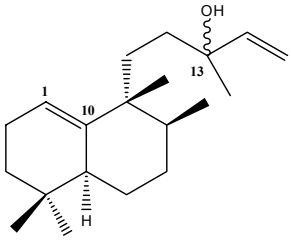

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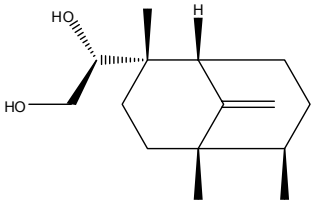

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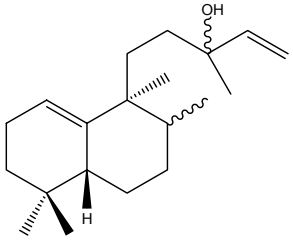

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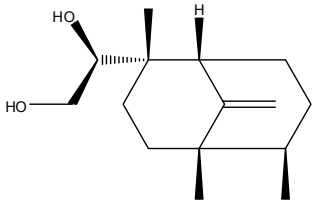

8

Experimental Section

General Procedures. Optical rotations were measured on a JASCO DIP-1000 polarimeter with $CHCl_3$ as solvent. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on a Varian Unity 600 and/or 300 NMR spectrometer (600 or 300 MHz for ^1H and 150 or 75 MHz for ^{13}C), using $CDCl_3$ as solvent. Chemical shifts are given relative to tetramethylsilane (TMS, δ 0.00) as internal standard (^1H) and δ77.02 from $CDCl_3$ as standards (^{13}C). Mass spectra were recorded on a JEOL JMS AX-500 spectrometer. Column chromatography was carried out on Sephadex LH-20 (Amersham Pharmacia Biotech, CH_2Cl_2-MeOH, 1:1) and silica gel (Kieselgel 60: 0.040-0.063 mm, Merck). The preparative HPLC experiment was performed using a Cosmosil reversed phase column, a JASCO 880-PU pump, JASCO 875-UV UV detector and ERC-7512 ERMA CR INC RI detector.

Plant material. *C. conicum* was collected from Bizan (Tokushima city, Japan) in April 2004, while *P. barteri* and *P. terebrans* were collected in Moramanga (near Andasibe), Madagascar in March 2004. After being purified, each sample was air-dried at room temperature.

Extraction and isolation procedure. Dried liverworts of *C. conicum*, *P. barteri* and *P. terebrans* were mechanically powdered and extracted with ether at room temperature for one month. The extracts was filtered and concentrated *in vacuo* to give 69.4 mg extract for *C. conicum*, 327 mg for *P. barteri*, and 43.9 mg for *P. terebrans*. *Conocephalum conicum* ether extract was divided into three fractions by size exclusion chromatography on sephadex LH-20. The third fraction was rechromatographed on silica gel (hexane: EtOAc; 85:15) to give a fraction rich in compound **1**. Preparative TLC on silica gel (hexane: EtOAc; 4:1) resulted the isolation of compound **1** in pure state (1 mg). The ether extract of *P. barteri* was subjected to a sephadex LH-20 column chromatography to give six fractions. Compound **5** (12.6 mg) was isolated from fraction 3 by a combination of silica gel and ODS flash column chromatography (hexane: EtOAc gradient and 90% MeOH, respectively), while compounds **2** (5 mg) and **3** (0.4 mg) were obtained from fraction 4 by silica gel column chromatography (hexane: EtOAc gradient) and preparative TLC on ODS (80% MeOH), respectively. Silica gel column chromatography (hexane: EtOAc; 6.5:3.5) of the fifth fraction afforded compound **4** (9.2 mg). Size exclusion chromatography of the ether extract of *P. terebrans* gave five fractions. The fourth fraction was applied to a silica gel column chromatography (hexane- EtOAc, 7:3) to afford compound **6** (1.7 mg).

2α-Cinnamoyloxy-6β-acetoxybornane. Oil ($[\alpha]^{18}_{D}$ +18.3^{o} (*c* 0.2, $CHCl_3$); IR (KBr) cm^{-1}: 1731, 1712, 1450; Positive HREIMS: *m/z* 342.1831 $[M]^+$ ($C_{21}H_{26}O_4$, requires 342.4287); ^{1}H- and ^{13}C-NMR: see Table 1.

Enzyme inhibition assay. α-Glucosidase inhibitory activity was performed according to the method described by Oki and co-workers[10] with slight modifications. α-Glucosidase was purchased from TOYOBO Co. Ltd, 2-8 Dojima Hama, 2-Chome, Kitaku, Osaka, Japan. Pure Chemical Industries Ltd. The enzyme solution (ES) was prepared by dissolving 0.6 U/ml of α-glucosidase in 100 mM phosphate buffer (pH 7) containing 2 g/l bovine serum albumin and 0.2 g/l NaN_3. *p*-Nitrophenyl-α-D-glucopyranoside (5 mM) in the same buffer solution (pH 7) was used as a substrate solution (SS). The ES (50 µl) and the test compounds (10 µl) dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mM were mixed in each well of the microliter 96-well culture plates and measured spectrophotometrically (Abs 415 nm) at zero time by using a microplate reader (BIO-RAD model 550 Microplate reader). The mixture was preincubated for 5 min at room temperature before SS addition (50 µM) followed by 5 min incubation at room temperature. The increase in absorbance from zero time was measured. The inhibitory activity was expressed as 10 minus relative absorbance difference (%) of test compounds to absorbance change of the control where test solution was replaced by DMSO. Experiences were performed in triplicate and the averages are presented. 1-Deoxynojirimycin (Wako Pure Chemical Industries, Ltd.) was used as positive control.

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