Chemical Constituents of Malagasy Liverworts, Part III: Sesquiterpenoids from *Bazzania decrescens* and *Bazzania madagassa*

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Received December 20, 2004; accepted January 26, 2005

In the continuation of our investigation of the phytochemical constituents of Malagasy liverworts, a new cuparane-type sesquiterpenoid together with five known compounds was isolated from *Bazzania decresens*. *Bazzania madagassa* furnished a new cyclomyltaylane-type sesquiterpenoid and a new acoradienol. The structures of the isolated compounds were determined based on a combination of physical and spectroscopic evidence. The chemosystematics of the genus *Bazzania* as well as the biogenesis of cyclomyltaylane sesquiterpenoids in liverworts is discussed.

Key words liverworts; *Bazzania decrescens*; *Bazzania madagassa*; cuparane; cyclomyltaylane; chemosystematics

The genus *Bazzania* is one of the most phytochemically studied among the liverworts and has been shown to produce a wide range of sesquiterpenoids and aromatic compounds. The sesquiterpenoids that have been detected are of the aromadendrane, bicyclogermacrene, bazzanene, barbatene, calamenane, cuparane, chamigrene, drimane, pinguisane, myltaylane, and cyclomyltaylane types.[1,2] Lignans and bisbibenzyls were also isolated from *Bazzania trilobata*.[3,4] Interestingly, sesquiterpene caffeates, which have been shown to have useful activities such as cytotoxic, antimicrobial, antifungal, and superoxide anion release inhibitory activity have only been found in *Bazzania* species.[1,2,5] In the course of our ongoing phytochemical studies of the Malagasy liverworts,[6,7] six compounds including a new cuparane-type sesquiterpene (**1**) were isolated from *Bazzania decrescens*, while *Bazzania madagassa* produced a new cyclomyltaylane-type sesquiterpenoid (**2**) and a new acoradienol: 1*S**,4*S**,5*S**, acora-8(15),9-dien-7*R**-ol (**3**). The biogenesis of the cyclomyltaylane-type sesquiterpenoid is discussed.

Results and Discussion

Bazzania Decrescens The ether extract of *B. decrescens* was analyzed with GC/MS and shown to contain α-barbatene (1.7%), cuparene (6%), and bazzanene (41.6%). Silica gel column chromatography of the remaining extract afforded five fractions. Purification on Sephadex LH-20, silica gel, and preparative HPLC of each fraction yielded isobicyclogermacrenal,[8] drimenol and a mixture of drimenyl *cis* and *trans* caffeate,[9] which was converted to the *trans* form on standing at room temperature overnight, 2-methoxy-5-hydroxycuparene (HM-1),[10] and friedelin together with a new cuparane-type sesquiterpenoid (**1**) (Fig. 1). The structures of the known compounds were determined by comparison of their spectral data with those of authentic samples and reported in the literature.

Compound **1** exhibited the molecular formula of $C_{16}H_{24}O_2$ as determined using HR-EI-MS. Its IR spectrum showed absorption bands for an hydroxyl group (3328 cm^{-1}) and aromatic methine strech (3086 cm^{-1}), while the UV spectrum displayed an aromatic ring (206, 272 nm). The ^{1}H-NMR spectral data (Table 1) showed the presence of four quaternary methyl proton signals (δ 0.59, 1.07, 1.22 each singlet, including an aromatic methyl proton signal at δ 2.08), two *meta*-coupled aromatic proton signals (δ 6.46, 6.48, each doublet, J=1.6 Hz), a signal of a methoxyl group (δ 3.81, s), and three methylene signals (H-8ab, H-9ab, H-10ab), which showed further COSY coupling from H-8a/b (δ 2.43, dt, J=12.6, 9.0 Hz and δ 1.66, m) to H-10a/b (δ 1.57, m, δ 1.70, m). Inspection of the ^{13}C-NMR spectral data revealed the presence of 16 carbon signals attributable to a cuparene-type sesquiterpene[1] with a methoxyl group (δ 55.6), two oxygenated aromatic carbons (δ 153.4, δ 157.7), and an upfield methyl signal at δ 7.7. The positions of the hydroxyl group at C-2, methyl group at C-3, and methoxyl group at C-4 were determined by careful interpretation of the HMBC spectrum (Fig. 2). Thus the structure of **1** was determined to be 2-hydroxy-4-methoxy-cuparene.

Compound HM-1 (**4**) was previously isolated from the phytopathogenic fungus *Helicobasidium mompa*,[10] but has not been found in liverworts of the *Bazzania* genus even though they are known to contain cuparane-type sesquiterpenes as chemical markers.[1]

Bazzania Madagassa GC/MS analysis of the ether extract of *B. madagassa* revealed β-barbatene (5.8%), isobazzanene (9.2%), chamigrene (6.8%), δ-cuparene (16.2%), and acora-3,5 diene (1.3%). A combination of column chromatography on Sephadex LH-20, silica gel, and ODS RP-18 of the remaining extract yielded two new compounds (**2**, **3**).

Compound **2** exhibited the molecular formula $C_{19}H_{26}O_5$ as determined using HR-EI-MS. Its IR spectrum showed ab-

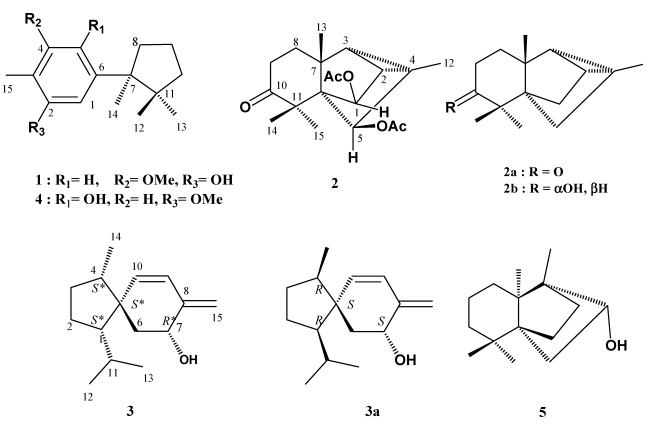

Fig. 1

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1**, **3**, and **3a** (600, 150 MHz, Respectively)

	1		**3**		**3a**
	H	C	H	C	
1	6.46 d (1.6)	107.1	1.40 m	57.3	57.9
2		153.4	1.47 m	26.3	27.3
3a		109	1.25 m	31.5	29.3
3b			1.78 m		
4		157.7	1.89 m	47.4	47.3
5	6.48 d (1.6)	102.5		51.0	50.9
6a		146.6	1.76 dd (12.8, 2.1)	45.6	31.5
6b			1.92 t (12.8)		
7			4.50 dt (12.8, 2.1)	67.7	67.8
8	2.43 dt (12.6, 9.0)	50.5		147.0	146.8
	1.66 m	36.7			
9	1.76 m	19.5	6.19 d (10.1)	127.7	127.4
10a	1.57 m	38.7	5.54 d (10.1)	131.9	140.1
10b	1.70 m				
11		44.1	1.63 dd (13.7, 6.7)	29.5	30.4
12	1.07 s	24.2	0.88 d (6.7)	23.4	22.9
13	0.59 s	26.5	0.85 d (6.7)	21.6	23.4
14	1.22 s	24.4	0.88 d (6.7)	17.9	13.7
15	2.08 s	7.7	4.95 br m	108.2	107.7
			5.20 br m		
OCH_3	3.81 s	55.6			

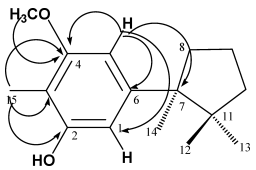

Fig. 2. Important HMBC Correlations Observed for **1**

sorption bands for a ketone (1709 cm^{-1}) and ester carbonyl groups (1739 cm^{-1}). The NMR spectra of **2** (Table 2) displayed four quaternary methyl proton signals (δ_H 0.78, 1.03, 1.07, 1.62, each singlet), five quaternary carbon resonances including a signal of a ketone (δ_C 214.7), two methylene carbon signals (δ_C 32.5, 33.3), two oxygen-bearing methines (δ_H 5.20, s, and 5.55, d, J=1.3 Hz), and two acetyl groups. The above data coupled with the molecular formula indicated that **2** is a tetracyclic sesquiterpene ketone. Compound **2** consists of two partial structures (–CH_2–CH_2– and –CHO–CH–CH–) as deduced from coupling patterns and the ^{1}H–^{1}H COSY spectrum. To clarify the complete structure, comprehensive HMQC, HMBC, and NOESY studies were carried out. The important long-range correlations observed between H9a/b (δ_H 2.72, 2.34) and C=O (δ 214.7), C-8 (δ 32.5), C-7 (δ 46.2); the methyl protons at δ_H 1.62 and C-6 (δ 56.4), C-7 (δ 46.2), C-3 (δ 34.8), and C-8 (δ 32.5); the methyl protons at δ_H1.03 and C-5 (δ 82.5), C-3 (δ 34.8), C-2 (δ 26.0), C-4 (δ 23.8); the downfield methine protons H-5 and H-1, (δ_H 5.20, 5.55, respectively), and the acetyl carbonyls (δ 170.4, 170.5, respectively), together with those shown in Fig. 3 enabled us to deduce that the structure of **2** is 1,5-diacetoxy cyclomyltaylan-10-one. The orientation of the acetoxyl group at C-5 as depicted was substantiated by the observation of NOE cross peaks between H-5 (δ_H 5.20) and H-8ax, while one acetyl methyl proton showed NOE correlations with H_3-13 and H_3-15. The second acetoxyl group at C-1 was thus pseudoequatorially oriented in the boat ring (Fig. 3). The absolute configuration was determined by comparison of the CD spectrum of a monoketone-derived product (**2a**) of cyclomyltaylyl-10-ol (**2b**) by oxidation with pyridinium chlorochromate (PCC).[11] Both ketones showed a negative Cotton effect at 300 and 297 nm, indicating that they have the same absolute configuration. Thus the structure of **2** was determined to be 1*R*,5*R*-diacetoxy-cyclomyltaylane-10-one.

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data for **2** (600, 150 MHz, Respectively)

	2		
	H	C	DEPT
1	5.55 d (1.3)	77.5	(d)
2	1.57 dd (4.9, 1.3)	26.0	(d)
3	1.38 d (4.9)	34.8	(d)
4		23.8	(s)
5	5.20 s	82.5	(d)
6		56.4	(s)
7		46.2	(s)
8a	1.77 ddd (14.2, 7.6, 1.6)	32.5	(t)
8b	2.04 m		
9a	2.72 ddd (15.6, 13.1, 7.6)	33.3	(t)
9b	2.34 m		
10		214.7	(s)
11		47.7	(s)
12	1.03 s	13.0	(q)
13	1.62 s	24.2	(q)
14	1.07 s	22.4	(q)
15	0.78 s	24.6	(q)
1-$\underline{CH_3}$C=O	2.07 s	21.4	(q)
5-$\underline{CH_3}$C=O	2.10 s	21.7	(q)
1-$CH_3\underline{C}$=O		170.5	(s)
5-$CH_3\underline{C}$=O		170.4	(s)

The molecular formula of compound **3** was determined to be $C_{15}H_{24}O$, as indicated by HR-EI-MS. The three secondary methyl groups at δ 0.85 (d, J=6.7 Hz, 3H), and 0.88 (d, J=6.7 Hz, 6H), the two methylene signals at δ 1.25 and 1.78

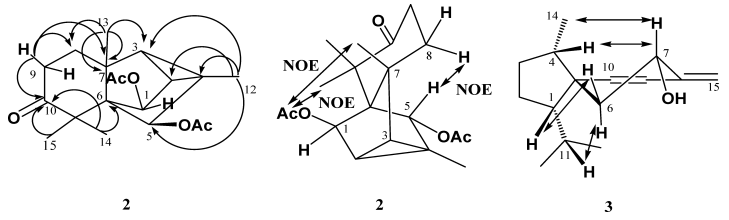

Fig. 3. Important HMBC and NOE Correlations Observed for **2** and **3**

(each multiplet, H-3a/b, respectively), and δ 1.47 (m, H-2ab) as shown in the ^{1}H-NMR spectrum, and the quaternary carbon signal at δ_C 51.0 were attributable to an isopropylmethylcyclopentane. The remaining signals were those of an exomethylene (δ_H 4.95, 5.20, each brm, δ_C 108.2, 147.0), two *cis*-oriented olefinic protons (δ_H 5.54, 6.19, each d, J=10.1 Hz), an oxygen atom-bearing methine (δ_H 4.50, dt, J=12.8, 2.1 Hz), and a methylene group (δ_H 1.76, dd, J=12.8, 2.1 Hz and δ_H 1.92, t, J=12.8 Hz). These data, in addition to the observed 15 carbon signals, which appeared as three quartets, four triplets, six doublets, and two singlets in the DEPT spectrum, suggested the structure of acoradienol.[1)] Since the optical rotation, and UV, IR, and CD spectral data of **3** were very similar to those of shizuka-acoradienol (**3a**) previously isolated from the higher plant *Chloranthus japonicus*,[12)] a similar planar structure could be deduced. The NMR spectral data, however, indicated an upfield shift of the olefinic carbon at C-10 of **3** (δ 131.9 instead of 140.1 in **3a**) and a downfield shift of C-6 (δ 45.6 instead of 31.5 in **3a**). To determine the exact structure, careful interpretation of the two-dimensional NMR spectral data was carried out. The *cis* configuration of the C-1 isopropyl and C-4 methyl group was substantiated from the observation of NOE correlations between: H-1 (δ 1.40, m) and H-4 (δ 1.89, m); H-10 (δ 5.54) and H-11 (δ 1.63, dd, J=13.7, 6.7 Hz) together with methyl protons at CH_3-14 (δ 0.88, d, J=6.7 Hz) and H-10. Moreover, the NOE interaction clearly observed between CH_3-14 and H-7 and H-1 and H-6eq together with the coupling constant of H-7 (axial oriented, dd, J=12.8, 2.1 Hz) and H-6ax (t, J=12.8 Hz) indicated that **3** has the 5*S**,7*R** configuration (Fig. 3). The differences between **3a** and **3** were the presence of the γ-effect (substituent chemical shift) of the C-4 methyl group to C-10 as well as the orientation of the methyl groups at C-1 and the configuration of C-7. From the above spectral data, the structure of **3** was determined to be 1*S**,4*S**,5*S**, acora-8(15),9-dien-7*R**-ol.

Although this is the first report on the phytochemical investigation of Malagasy *Bazzania*, many studies have been carried out on species from Japan, Taiwan, Europe, and South America. The isolated chemical constituents classify *Bazzania decrescens* in the albicanyl (drimenyl)-caffeate-cuparene chemotype of *Bazzania*. *B. decrescens* metabolites are very similar to those from *Bazzania japonica*,[11)] although the latter contains albicanol and albicanyl caffeate instead of drimenol and drimenyl caffeate. The only difference between the two species is the presence of cyclomyltaylane-type sesquiterpenoids in *B. japonica*. *B. madagassa* is a species morphologically close to *B. decrescens*. However, neither myltaylanes nor cylcomyltaylane have been detected in *B. decrescens*. This confirms that liverwort secondary metabolites can assist in the differentiation of the two species. Myltaylanes and cyclomyltaylanes are very rare sesquiterpenoids found only in liverworts from the genera *Mylia*, *Bazzania*, *Reboulia*, and *Mannia*. The myltaylane framework may be derived from C-3, C-7 cyclization of β-chamigrene, followed by migration of the methyl group to the vicinal proton.[13)] Interestingly, Barrero and coworkers[14)] reported the isolation of a similar sesquiterpene, junicedranol (**5**), which was also proposed to be biosynthetically derived from chamigrene by conversion of cuparene and/or thujopsane. The presence of cuparene in *Bazzania* and *Reboulia* species confirms this fact. However, neither cuparene- nor thujopsane-related compounds have been detected in *Mylia* species, which contain aromadendrane, bicyclogermacrane, and secoaromadendrane-type sesquiterpenoids. Taking into account the presence of chamigrene in *B. madagassa* as a precursor the biosynthesis of myltaylanes and cyclomyltaylanes in liverworts is suggested as shown in Fig. 4.

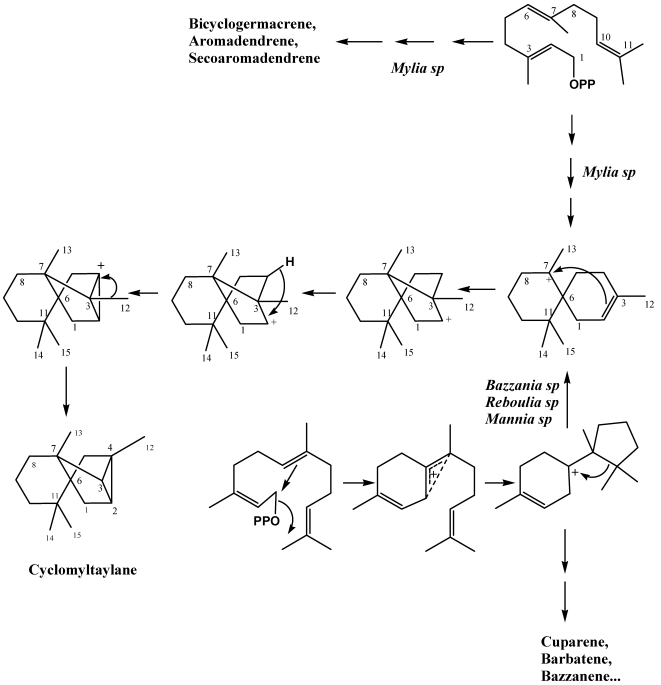

Fig. 4. Possible Biosynthesis of Cyclomyltaylane in *Bazzania*, *Reboulia*, *Mannia* and *Mylia* Species

Experimental

General Procedures Optical rotations were measured on a JASCO DIP-1000 polarimeter with MeOH as solvent. UV spectra were obtained on a Shimadzu UV-1650PC instrument in MeOH. IR spectra were measured on Perkin Elmer Spectrum One FT-IR Spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity 600 NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C), using $CDCl_3$ as a solvent. Chemical shifts are given relative to TMS (δ 0.00) as an internal standard (^{1}H) and δ 77.0 (ppm) from $CDCl_3$ as a standard (^{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 as the solvent system) and silica gel (Kieselgel 60: 0.040—0.063, Merck). The preparative HPLC experiment was performed using a Cosmosil reverse-phase column, JASCO 880-PU pump, JASCO 875-UV UV detector, and ERC-7512 Erma CR Inc, RI detector. RP-18 F_{254S} (20×20 cm) was used for preparative TLC. The temperature programming of the GC-MS analysis was performed from 50 °C, then 50—250 °C at 15 °C min^{-1}, and finally isothermal at 250 °C. A fused silica column coated with DB-17 (30 m×0.25 mm i.d., film thickness 0.25 mm) using He as carrier gas (1 ml min^{-1}). Mass spectra were measured at 70 eV.

Plant Materials *B. decrescens* and *B. madagassa* were collected in Moramanga (Madagascar) in June 2003 and identified by Prof. emeritus T. Pocs of the Hungarian Academy of Science. Voucher specimens (*B. decrescens*: 2003LIV4; *B. madagassa*: 2003LIV5) were deposited in the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and Isolation The powdered *B. decrescens* (33 g) and *B. madagassa* (7 g) were extracted with ether at room temperature for 5 d. Each extract was filtrated and concentrated *in vacuo* to yield 1.35 g of green oils for *B. decrescens* and 260 mg for *B. madagassa*. The former extract was divided into five fractions based on size-exclusion chromatography (Sephadex LH-20). Fraction 3 was applied to silica gel column chromatography using hexane and ethyl acetate (4 : 1 to 100% EtOAc) as solvent to afford three subfractions. Fraction 3-1 was rechromathographed on a asilica gel column to afford drimenyl caffeate (71 mg) and HM-1 (6 mg). Fraction 3-2 was purified using silica gel (hexane–EtOAc, 9 : 1) and ODS RP-18 (80% MeOH) column chromatography to yield drimenol (1.4 mg) and compound **1** (47.7 mg) with preparative HPLC (70% MeOH). Fraction 4 was purified on silica gel chromatography (solvent systems: hexane–EtOAc 9 : 1; 1 : 1; and 100% EtOAc) to give friedelin (0.8 mg) and isobicyclogermacrenal (1.2 mg).

The greenish oil extract (260 mg) from *B. madagassa* was fractionated on Sephadex LH-20 column chromatography to afford six fractions. Compound **3** (6.2 mg) was obtained from the fourth fraction after separation on silica gel column chromatography (hexane–EtOAc 7 : 3 as solvent system). Further purification of the fifth fraction on silica gel column produced compound **2** (3 mg).

2-Hydroxy-4-methoxycuparene (**1**): Oil, $[\alpha]_D^{20}$ −52.2° (c=1.8, MeOH). Positive HR-EI-MS: m/z 248.1776 $[M]^+$, $C_{16}H_{24}O_2$, requires 248.1775. UV λ_{max} (MeOH) nm (log ε): 206 (3.4), 272 (3.2). IR (KBr) cm^{-1}: 3328, 3086. ^{1}H- and ^{13}C-NMR, see Table 1.

1*R*,5*R*-Diacetoxycyclomyltaylan-10-one (**2**): Amorphous powder, $[\alpha]_D^{20}$ −23.2° (c=0.9, MeOH). Positive HR-EI-MS: m/z 334.1784 $[M]^+$, $C_{19}H_{26}O_5$, requires 334.1780. CD λ^{MeOH} nm ($\Delta\varepsilon$): 300 (−13). IR (KBr) cm^{-1}: 2917, 1739, 1709. ^{1}H- and ^{13}C-NMR, see Table 2.

1*S**,4*S**,5*S**, Acora-8(15),9-dien-7*R**-ol (**3**): Oil, $[\alpha]_D^{20}$ −165.0° (c=0.2, MeOH). Positive HR-EI-MS: m/z 220.1850 $[M]^+$, $C_{15}H_{24}O$, requires 220.1845. UV λ_{max} (MeOH) nm (log ε): 202 (4), 232 (4.2). IR (KBr) cm^{-1}: 2960, 2924, 1610. ^{1}H- and ^{13}C-NMR, see Table 1.

Acknowledgments We thank Prof. emeritus T. Pocs of the Hungarian Academy of Science for the identification of the liverworts; Drs. T. Hashimoto, M. Toyota, and F. Nagashima for the valuable discussions, Dr. T. Tanaka for the NMR measurement; and Ms. Y. Okamoto (TBU) for recording mass spectra. The present work was supported by a Grant-in-Aid for the Scientific Research (A) (No. 11309012) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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