*Phytochemistry*, Vol. 30, No. 9, pp. 3037–3040, 1991
Printed in Great Britain.

0031 9422/91 $3.00+0.00
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# SESQUITERPENOIDS FROM THE LIVERWORT *BAZZANIA JAPONICA**

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(*Received* 12 *February* 1991)

**Key Word Index**—*Bazzania japonica*; Jungermanniales; Hepaticae; cyclomyltaylan-3-ol; cyclomyltaylyl-3-caffeate; 2-hydroxycuparene; albicanol; albicanyl acetate; albicanyl caffeate; isobicyclogermacrenal; sesquiterpenoids; superoxide release inhibitory activity; chemosystematics.

**Abstract**—Two new tetracyclic sesquiterpenoids, cyclomyltaylan-3-ol and its caffeate, have been isolated from the stem-leafy liverwort *Bazzania japonica* together with the previously known five sesquiterpenoids and their absolute stereostructures established by a combination of the $^1H$ and $^{13}C$ NMR spectral data as well as X-ray crystallographic analysis and CD spectrum. *Bazzania japonica* is divided into two chemo-types, cyclomyltaylane-type and cuparene-drimane-type. Cyclomyltaylyl-3-caffeate inhibited release of superoxide anions at $IC_{50}$ 7.5 $\mu g\,ml^{-1}$.

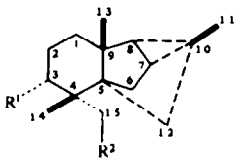
## INTRODUCTION

Recent chemical studies of the Hepaticae have shown them to be rich sources of terpenoids and aromatic compounds [2, 3], some of which possess interesting biological activity [4]. Previously we studied the chemical constituents of five *Bazzania* species, including *B. japonica*, and isolated several new sesquiterpenoids and characterized their structures [5–7]. Further fractionation of the methanol extract of *B. japonica* resulted in the isolation of two new sesquiterpenoids, cyclomyltaylan-3-ol (**1**) and cyclomyltaylyl-3-caffeate (**2**), along with the five previously known sesquiterpenoids (**8**–**12**). We now report their absolute stereostructures and discuss the chemosystematics and biological activity of the new compound.

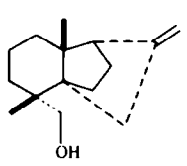
## RESULTS AND DISCUSSION

*Bazzania japonica* was collected at two places, Kainan-cho, Tokushima (KT) and Kainan-cho, Asakawa, Tokushima (KAT). The sample collected in the former place was extracted with methanol and the one in the latter place with ether. A combination of silica gel and Sephadex LH-20 column chromatography of the crude extract from the former sample resulted in the isolation of two new tetracyclic sesquiterpenoids named $Bj_1$ (**1**) and $Bj_2$ (**2**) and their absolute stereostructures established [8]. However, the same skeletal compound (**4**) was isolated later from the liverwort *Mylia taylorii* together with the related compound (**5**) and named cyclomyltaylanol [9, 10]. To avoid confusion, we use the name of cyclomyltaylane for our new compounds. Thus, $Bj_1$ and $Bj_2$ are named cyclomyltaylan-3-ol (**1**) and cyclomyltaylyl-3-caffeate (**2**). The ether extract from the latter sample was treated in the manner described above to give 2-hydroxycuparene (**8**), albicanol (**9**), albicanyl acetate (**10**), albicanyl caffeate (**11**), isobicyclogermacrenal (**12**) and friedelin. The molecular formula, $C_{15}H_{24}O$ ($[M]^+$ at $m/z$ 220.1833), of **1** was determined by high resolution mass spectrometry. The IR and $^{13}C$ NMR spectra showed the presence of a secondary hydroxyl group (3450 $cm^{-1}$; $\delta$76.6, *d*) which was further confirmed by oxidation with pyridinium chlorochromate (PCC) to afford a mono ketone (**6**) ($[M]^+$ 218; 1695 $cm^{-1}$; $\delta$217.1, *s*). The $^1H$ NMR spectrum (Table 1) of **1** contained the signals of four tertiary methyls, two protons on the cyclopropane ring and one proton on the carbon bearing hydroxyl group. The $^{13}C$ NMR spectrum (Table 2) of **1** further indicated the presence of four methyls, four methylenes, two methines and four quaternary carbons. The above results coupled with the molecular formula indicated that **1** is a tetracyclic sesquiterpene alcohol including a cyclopropane ring. In order to clarify the framework of **1**, the $^1H$–$^1H$ (Table 3), $^{13}C$–$^1H$ and long range $^{13}C$–$^1H$ 2D COSY NMR spectra (Table 4) were measured and spin decoupling experiments carried out. These led to the conclusion that compound **1** might be represented as **1** or **7**. As further evidence for determination of two alternative structures was not obtained by the above spectral data, we performed an X-ray crystallographic analysis of the *p*-bromobenzoate (**3**) of **1**, which was prepared from **1** by benzoylation with *p*-bromobenzoyl chloride. Recrystallization from methanol afforded **3** as thin needles. The ORTEP drawing of **3** is shown in Fig. 1. The absolute configuration of **1** was determined by the negative Cotton effect at 297 nm of **6**. The spectral data of compound **2**, $C_{24}H_{30}O_4$ ($[M]^+$ at $m/z$ 382.2110), showed the presence of a hydroxyl group (3550 $cm^{-1}$), an aromatic ring (1607, 1273, 1170 $cm^{-1}$), an unsaturated ester carbonyl group (1685 $cm^{-1}$; $\delta$168.0, *s*) and a *trans*-ethylenic double bond ($\delta$6.28 and 7.58, each *d*, $J$ = 15.8 Hz). The $^1H$ and $^{13}C$ NMR (Tables 1 and 2) of **2** resembled those of **1**, except for the presence of the signals of the aromatic ring, indicating that **2** possessed the same skeleton as that of **1**. This was further confirmed by the $^1H$–$^1H$ and $^{13}C$–$^1H$ 2D-COSY NMR spectra. The

*Part 46, in the series 'Chemosystematics of Bryophytes'. For Part 45, see ref. [1].



**1** $R^1$ = OH, $R^2$ = H
**2** $R^1$ = $OCOCH{=}CH{-}C_6H_3(OH)_2$, $R^2$ = H
**3** $R^1$ = $OCOC_6H_4Br(p)$, $R^2$ = H
**4** $R^1$ = H, $R^2$ = OH



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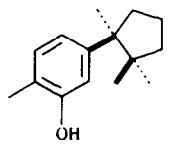

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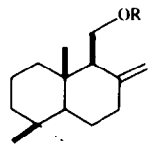
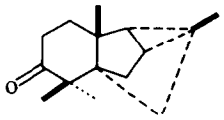


**9** R = H
**10** R = Ac
**11** R = $COCH{=}CH{-}C_6H_3(OH)_2$



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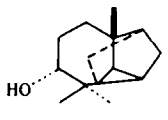

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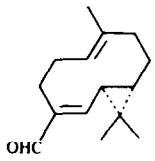

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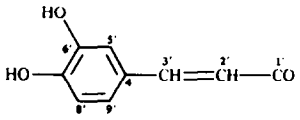

Caffeoyl group

Table 1. $^1$H NMR spectral data of compounds **1**, **2** and **6** (400 MHz, TMS–$CDCl_3$)*

| H | 1 | 2 | 6 |
|---|---|---|---|
| 1α | 1.77 *ddd* (13.7, 13.7, 3.9) | 1.65–1.75 *m* | 1.87 *ddd* (13.8, 13.8, 4.9) |
| 1β | 1.19 *m* | 1.22–1.29 *m* | 1.63 *dddd* (13.4, 6.8, 2.0, 0.7) |
| 2α | 1.61 *ddd* (14.2, 6.4, 2.9) | 1.65–1.75 *m* | 2.19 *ddd* (15.4, 4.9, 2.1) |
| 2β | 1.94 *dddd* (14.2, 14.2, 4.4, 4.4) | 1.93 *m* | 2.70 *ddd* (15.3, 13.8, 7.0) |
| 3 | 3.48 *dd* (2.9, 2.9) | 4.85 *br s* | |
| 6α | 2.17 *dd* (10.3, 1.5) | 2.17 *d* (10.3) | 1.69 *d* (10.5) |
| 6β | 1.19 *m* | 1.22–1.29 *m* | 1.29 *d* (10.5) |
| 7 | 0.76 *br d* (5.4) | 0.83 *d* (5.4) | 0.95 *br d* (5.3) |
| 8 | 0.66 *d* (5.4) | 0.72 *d* (5.4) | 0.79 *d* (5.2) |
| 11 | 1.14 *s* | 1.20 *s* | 1.15 *s* |
| 12 *endo* | 1.29 *dd* (9.8, 1.0) | 1.32 *d* (9.8) | 1.26 *d* (10.9) |
| 12 *exo* | 1.45 *br d* (9.8) | | 1.39 *dd* (10.9, 1.5) |
| 13 | 0.94 *s* | 0.97 *s* | 1.19 *s* |
| 14 | 0.90 *s* | 0.90 *s* | 1.00 *s* |
| 15 | 0.98 *s* | 1.00 *s* | 1.15 *s* |
| 2′ | | 6.28 *d* (15.8) | |
| 3′ | | 7.58 *d* (15.8) | |
| 5′ | | 7.14 *br s* | |
| 8′ | | 6.90 *d* (8.0) | |
| 9′ | | 7.10 *d* (8.0) | |

*Coupling constants (*J* in Hz) are given in parentheses.

aromatic part of **1** was confirmed as caffeic acid because the $^{13}$C NMR signal pattern in the aromatic region was almost identical with that of caffeic acid (Table 2). Thus, **2** is cyclomyltaylyl-3-caffeate.

Takaoka *et al.* [9, 10] isolated myltaylanol (**5**) and cyclomyltaylanol (**4**) from the liverwort *Mylia taylorii* and proposed the relative structure (**4**) by spectral data and on consideration of the co-existence of **5**. The present data support the structure **4**. It is noteworthy that *Bazzania* (Lepidoziaceae) and *Mylia* (Jungermanniaceae) produce the same biogenetically unique cyclomyltaylane-type sesquiterpenoids, although the two families are morphologically quite distinct.

Cyclomyltaylane-type sesquiterpenoids have not been detected in the ether extract of *B. japonica* collected in KAT and 2-hydroxycuparene (**8**) obtained as the major

Table 2. $^{13}C$ NMR spectral data for compounds **1**, **2** and **6** (100 MHz, TMS–$CDCl_3$)*

| C | 1 | 2 | 6 |
|---|---|---|---|
| 1 | 26.6* | 27.2 | 31.6 |
| 2 | 26.7* | 24.2 | 34.2 |
| 3 | 76.6 | 78.7 | 217.1 |
| 4 | 49.4 | 49.7 | 54.1 |
| 5 | 36.9 | 36.7 | 47.6 |
| 6 | 43.0 | 42.7 | 31.1 |
| 7 | 17.9 | 18.2 | 20.6 |
| 8 | 33.0 | 33.0 | 32.9 |
| 9 | 44.6 | 44.6 | 44.5 |
| 10 | 19.6 | 19.5 | 19.5 |
| 11 | 16.3 | 16.7 | 16.1 |
| 12 | 31.7 | 31.6 | 42.6 |
| 13 | 21.2 | 21.1 | 20.9 |
| 14 | 24.2 | 24.2 | 22.1 |
| 15 | 25.0 | 24.7 | 23.8 |
| 1′ | | 168.0 (167.1)† | |
| 2′ | | 115.9 (115.2) | |
| 3′ | | 145.2 (145.6) | |
| 4′ | | 127.2 (127.1) | |
| 5′ | | 114.5 (114.4) | |
| 6′ | | 144.1 (143.3) | |
| 7′ | | 146.7 (147.0) | |
| 8′ | | 115.5 (115.6) | |
| 9′ | | 122.4 (122.5) | |

*All assignments were confirmed by INEPT, $^{13}C$–$^{1}H$ and long range $^{13}C$–$^{1}H$ 2D COSY NMR spectral data.

†Chemical shifts of caffeic acid are given in parentheses.

*Assignments may be interchangeable.

Table 3. $^{1}H$–$^{1}H$ 2D COSY NMR spectral data for compound **1** (400 MHz, TMS–$CDCl_3$)

| H | Correlated H |
|---|---|
| 1 | H-1, H-13* |
| 2 | H-1, H-2 |
| 3 | H-2 |
| 6 | H-6, H-12* |
| 7 | H-6, H-8 |
| 8 | H-7 |
| 12 | H-6*, H-11*, H-12 |

*Long range coupling.

Table 4. Long range $^{13}C$–$^{1}H$ 2D COSY NMR spectral data for compound **1** [$^{13}C$ NMR (100 MHz), $^{1}H$ NMR (400 MHz), TMS-$CDCl_3$]

| C | Correlated H |
|---|---|
| 1 | H-13 |
| 4 | H-14, H-15 |
| 5 | H-14, H-15 |
| 7 | H-6(α), H-11 |
| 8 | H-1, H-11, H-12(*endo*), H-13 |
| 9 | H-1, H-12(*endo*), H-13 |
| 10 | H-11, H-12(*exo*) |

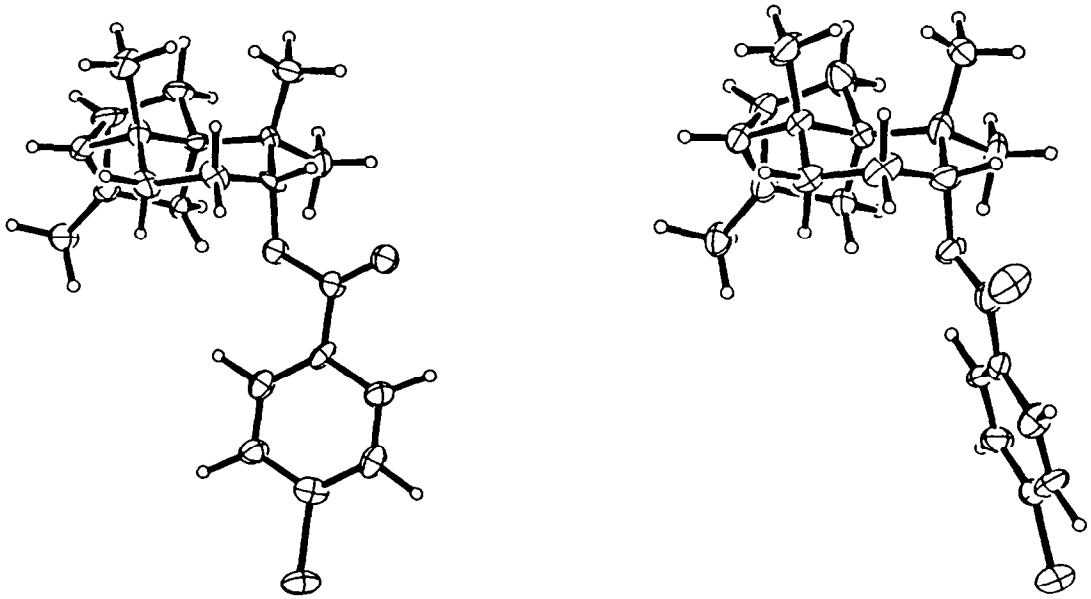

Fig. 1. ORTEP drawing of the molecular structure of compound **3**.

product, together with drimane- (**9**–**11**) and isobicyclogermacrenal (**12**). This species is chemically identical to that previously reported [5]. On the other hand, neither cuparene- nor drimane-type sesquiterpenoids have been detected in the methanol extract of the same species collected in KT. The first collection point KAT is 100 m away from the second point (KT). Thus, it is suggested that there are two chemo-types in *Bazzania japonica*: cyclomyltaylane-type and cuparene-drimane-type [5].

Free albicanol (**9**) has been found in the liverwort *Diplophyllum albicans* (Scapaniaceae) [11] and its acetate (**10**) in the marine organism [12]. (−)-Isobicyclogermacrenal (**12**) has been isolated from the liverwort *Lepidozia vitrea* [13] which belongs to the same family as that of *Bazzania* species. These three sesquiterpenoids are the first example of the isolation from the *Bazzania* genus.

Cyclomyltaylyl-3-caffeate (**2**) inhibited release of the superoxide anion from guinea-pig peritoneal macrophage induced by $O_2^-$ stimulant FMLP (formyl-methionyl-leucyl-phenylalanine; $10^{-7}$ M) at $ID_{50}$ 7.5 $\mu g\,ml^{-1}$.

## EXPERIMENTAL

The solvent used for spectral measurements were TMS–$CDCl_3$ [$^1$H NMR (400 MHz); $^{13}$C NMR (100 MHz)]; $CHCl_3$ (IR, CD and $[\alpha]_D$) unless otherwise stated. MeOH–$CHCl_3$ (1:1) was used for CC on Sephadex LH-20.

*Plant material. Bazzania japonica* (Lac.) Lindb. was collected in Kainan-cho, Tokushima (KT) and Kainan-cho, Asakawa, Tokushima (KAT) in August 1986 and identified by Dr M. Mizutani. The voucher specimens are deposited at the Herbarium in the Institute of Pharmacognosy, Tokushima Bunri University.

*Extraction and isolation.* Dried and ground *B. japonica* (120 g) collected in KT was extracted with MeOH for 4 months. The crude extract (2.0 g) was chromatographed on silica gel using a *n*-hexane–EtOAc gradient to give 6 frs: Fr. 1 (100% *n*-hexane); Fr. 2 (5% EtOAc), Fr. 3 (10% EtOAc), Fr. 4 (20% EtOAc), Fr. 5 (50% EtOAc) and Fr. 6 (100% EtOAc). Fr. 2 (93 mg) was rechromatographed on Sephadex LH-20 to afford cyclomyltaylan-3-ol (**1**) (28 mg). Fr. 3 (166 mg) was purified by CC on Sephadex LH-20 to give cyclomyltaylyl-3-caffeate (**2**) (52 mg).

*Compound* **1**. Oil, $[\alpha]_D$ −19.1° (*c* 0.52), IR $\nu_{max}^{neat}$ $cm^{-1}$: 3450, 1450, 1377, 990, $^1$H and $^{13}$C NMR (Tables 1 and 2), HRMS: found: $[M]^+$ at *m/z* 220.1833 $C_{15}H_{24}O$ requires 220.1827, EIMS: *m/z* (rel. int.): 220 $[M]^+$ (25), 202 (34), 187 (47), 176 (17), 161 (42), 145 (50), 132 (100), 119 (85), 105 (73), 93 (63), 77 (38), 69 (33), 55 (46).

*Compound* **2**. Oil, IR $\nu_{max}$ $cm^{-1}$: 3550, 1685, 1607, 1273, 1170, $^1$H and $^{13}$C NMR (Tables 1 and 2), HRMS: found: $[M]^+$ at *m/z* 382.2110 $C_{24}H_{30}O_4$ requires 382.2144, EIMS: *m/z* (rel. int.): 382 $[M]^+$ (21), 202 (27), 163 (100), 145 (16), 119 (13), 95 (8), 69 (7), 41 (9). The remaining fractions were treated in the same manner as described above, however, a small amount of phytosterol, triglyceride and fatty acid mixtures were obtained and no other sesquiterpenoids isolated.

Dried *B. japonica* (2.75 kg) collected in KAT was extracted with $Et_2O$ for 20 days to give a crude extract (44.9 g) which was chromatographed on silica gel using a *n*-hexane–EtOAc gradient to give 4 frs: Fr. 1 (0–5% EtOAc: 8.2 g) was chromatographed on Sephadex LH-20 to afford 2-hydroxycuparene (**8**) (6.4 g) [2, 3]. From Fr. 2 (10% EtOAc: 2.2 g), friedelin (433 mg) [3] was obtained. Fr. 3 (20% EtOAc: 3.6 g) was further purified by CC on Sephadex LH-20 to afford albicanol (**9**) (71 mg) [11]. Fr. 4 (EtOAc 30% 6.4 g) was rechromatographed on Sephadex LH-20 and silica gel using a $C_6H_6$–EtOAc gradient to furnish albicanyl caffeate (**11**) (347 mg) [5], (−)-isobicyclogermacrenal (**12**) (38 mg) [13] and the sesquiterpene mixture containing an acetate which was further chromatographed on Sephadex LH-20 to give albicanyl acetate (**10**) (52 mg) [12].

*Benzoylation of* **1**. To compound **1** (10 mg) in pyridine (1 ml) was added *p*-bromobenzoyl chloride (50 mg) and the mixture was allowed to stand overnight. $H_2O$ (3 ml) was added to the reaction mixture and extracted with $Et_2O$, washed with $H_2O$, dried over $Na_2SO_4$ and the solvent was evapd to afford the *p*-bromobenzoate (**3**) (15 mg) which was recrystallized from MeOH to furnish a single crystal, mp 56.5–57.5°. Crystal data: monoclinic, $a = 13.341$ (4), $b = 7.462$ (2), $c = 20.245$ (4) Å, $\beta = 95.04$ (2)°, space group, $P2_1$, $z = 4$. EIMS: *m/z* (rel. int.): 404 $[M+2]^+$ (13), $[M+1]^+$ (4), 402 $[M]^+$ (13), 202 (100), 183 (85), 173 (13), 159 (60), 145 (74), 132 (91), 119 (53), 105 (49), 95 (43), 81 (29), 69 (23), 55 (26), 41 (36).

*Oxidation of compound* **1**. To a soln of **1** (17 mg) in dry $CH_2Cl_2$ (2 ml) was added pyridinium chlorochromate (50 mg) and the resulting mixture stirred at room temp. for 12 hr. The reaction mixture was diluted with $Et_2O$, filtered through a short column packed with Florisil, the eluate dried over $Na_2SO_4$, filtered and the solvent evapd to give a monoketone (**6**) (17 mg). IR $\nu_{max}$ $cm^{-1}$: 1695, 1455, 1370, 1093; $^1$H and $^{13}$C NMR (Tables 1 and 2), EIMS: *m/z* (rel. int.): 218 $[M]^+$ (56), 203 (12), 185 (5), 175 (24), 161 (41), 147 (29), 132 (100), 119 (54), 107 (49), 97 (9), 91 (45), 77 (26), 69 (15), 55 (22), 41 (38). CD: $\Delta\varepsilon_{297\,nm}$ −1.67.

*Acknowledgements*– We thank Dr M. Mizutani (The Hattori Botanical Laboratory, Nichinan, Japan) for the identification of the liverworts and Otsuka Pharmaceutical Co. Ltd for carrying out biological screening. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare.

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