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SESQUITERPENOIDS FROM THE LIVERWORT *BAZZANIA FAURIANA**

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Key Word Index—*Bazzania fauriana*; Jungermanniales; Hepaticae; sesquiterpenoids; bazzanane; drimane; valencane; eudesmane; gymnomitrane; cuparane; bazzanenyl caffeate; drimenyl caffeate; 7α-hydroxyvalenc-1(10)-ene; 6β-hydroxyeudesm-3-ene; chemosystematics.

Abstract—Four new sesquiterpenoids, bazzanenyl caffeate, drimenyl caffeate, 6β-hydroxyeudesm-3-ene and 7α-valenc-1(10)-ene have been isolated from the liverwort *Bazzania fauriana* and their structures determined by chemical transformation and extensive NMR spectral experiments. The present species belongs to the new chemotype of *Bazzania* species.

INTRODUCTION

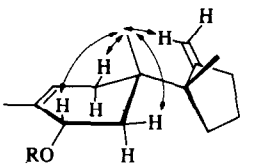
Bazzania species are rich sources of sesquiterpenoids [1]. In previous papers [2, 3] we reported the distribution of sesquiterpenoids of five Japanese *Bazzania* species and discussed their chemosystematics. *Bazzania fauriana* grows on rock or humus soil in southern Japan and its stem shoot may be up to 8 cm long. The morphology of this species is quite different from the other *Bazzania* species so far examined. The distinctive trinor sesquiterpene hydrocarbon, *trans*-8,10-dimethyl-1(9)-octalin (**21**) has been found in the Taiwanese *B. fauriana* [4]. As part of a chemosystematic study of the Lepidoziaceae, we have examined the lipophilic components of the Japanese *B. fauriana* and have isolated four new sesquiterpenoids. In this paper, we wish to report the chemical structures of these new compounds and to discuss the chemosystematics of *B. fauriana*.

RESULTS AND DISCUSSION

The methanol extract of dried *B. fauriana* was examined by TLC, GC and GC/MS to reveal bazzanene (**4**), drimenol (**7**), β-gymnomitrene (=β-barbatene) (**15**), α-gymnomitrene (=α-barbatene) (**16**), cuparene (**18**), 2-hydroxycuparene (**19**) and 2,3-dihydroxycuparene (**20**) [1, 2]. A combination of column chromatography on silica gel, silica gel impregnated with silver nitrate and Shephadex LH-20 resulted in the isolation of four new sesquiterpenoids (**1**, **5**, **8**, **9**), along with the previously known sesquiterpenoids, β-bazzanene (**4**), eudesm-3,7(11)-diene-8-one (**12**), albicanyl caffeate (**13**) and gymnomitrol (**14**) [1].

Bazzanenyl caffeate (**1**)

The IR spectrum of **1** [$C_{24}H_{30}O_4$ (M^+ 382)] indicated the presence of a hydroxyl group (3550, 3300 cm^{-1}), a conjugated carbonyl group (1690 cm^{-1}) and a benzene ring (1635, 1605 cm^{-1}). The ^1H NMR spectrum (Table 1) contained the signals of two tertiary methyls, an exomethylene, a vinylic methyl which was long range coupled (^4J) with a vinylic proton, and a 3,4-disubstituted *trans*-cinnamate group. Methylation of **1** with methyl iodide gave the dimethyl ether (**2**) [$C_{26}H_{34}O_4$ (M^+ at *m/z* 410)], indicating the presence of two phenolic hydroxyl groups on the benzene ring. Reduction of **2** with lithium aluminium hydride ($LiAlH_4$) gave 3,4-dimethoxycinnamyl alcohol and a sesquiterpene alcohol [$C_{15}H_{24}O$ (M^+ at *m/z* 220), δ5.41 (1H, *br s*)], whose spectral data were identical to bazzanenol (**3**). The structures of bazzanene and bazzanenol were reported by Hayashi *et al.* [5, 6], but the structure of the former compound was recently revised as depicted in **4** [7]. The structure of the latter compound has been revised to be β-hydroxy bazzanene without physical and spectral data [8]. The stereochemistry at C-2 of **3** was still ambiguous. The extensive spin decoupling, ^1H–^1H and ^1H–^{13}C correlated 2D NMR spectral data of **1** and **2** confirmed the structure of the new sesquiterpene ester and the α-configuration of the caffeate at C-2 of **1** and the hydroxyl group at C-2 of **3** was further established by difference NOE experiments of **1** and **3**, as shown in Fig. 1. On the basis of

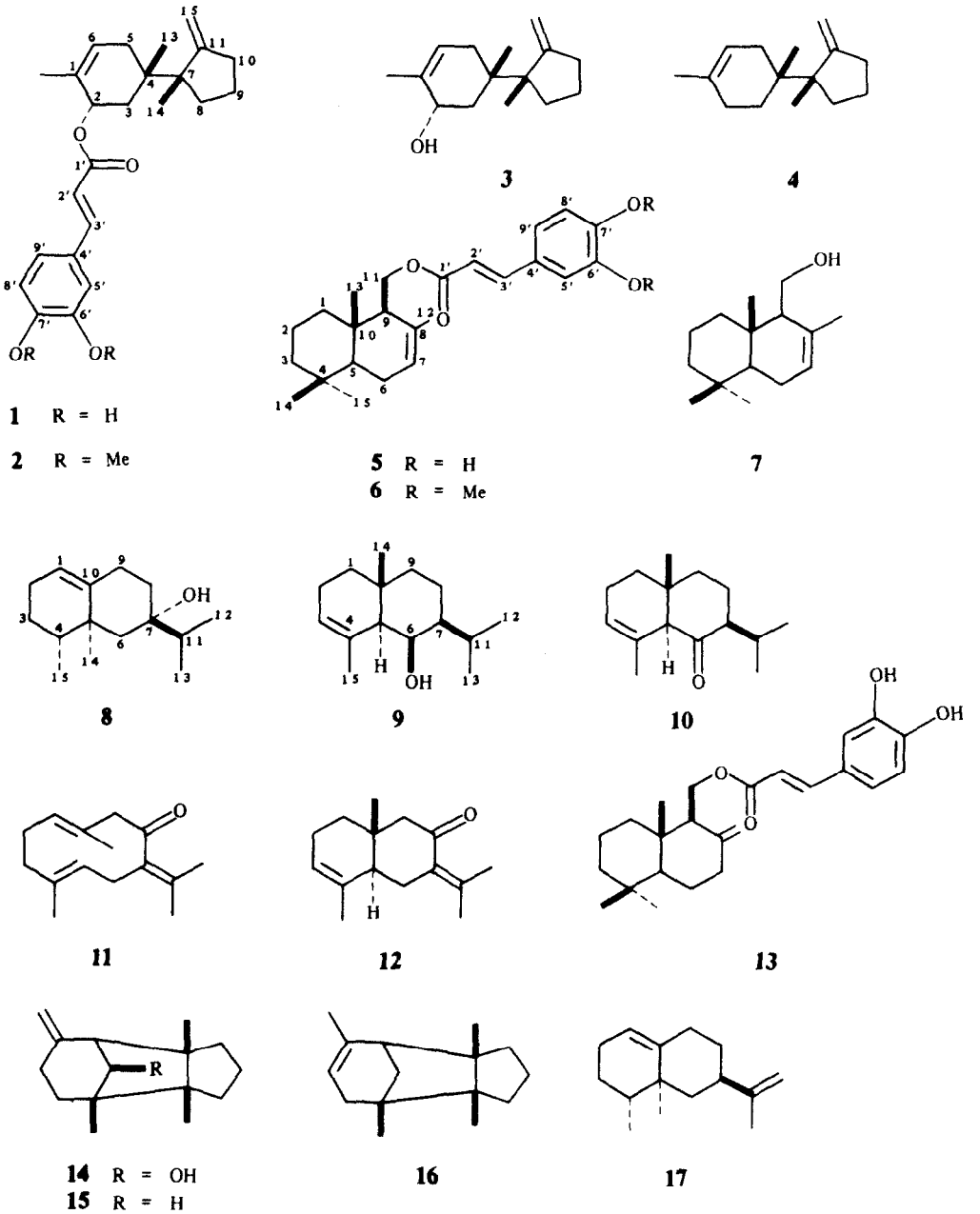


1 R = Caffeyl
2 R = H

NOEs ( ) observed by NOE difference spectra

Fig. 1.

* Part 26 in the series of 'Chemosystematics of Bryophytes'. For part 25 see ref. [15].



the above evidence, the structure of the new sesquiterpene ester was determined to be bazzanenyl caffeate (**1**).

Drimenyl caffeate (**5**)

The ^{1}H NMR spectral data (Table 1) of **5** showed the presence of a 3,4-disubstituted *trans*-cinnamate group, three tertiary methyl groups, a vinylic methyl, a vinylic proton and two protons bearing ester oxygen. Methylation of **5** with methyl iodide gave a dimethyl ether **6**, [$C_{26}H_{36}O_4$ (M^+ at *m/z* 412)] which on reduction with $LiAlH_4$ afforded 3,4-dimethoxycinnamyl alcohol and a sesquiterpene alcohol whose spectral data and physical constants were identical to those of drimenol (**7**) [1]. Thus the structure of the original ester was established to be drimenyl caffeate (**5**).

7α-Hydroxyvalenc-1(10)-ene (**8**)

Compound **8** ($C_{15}H_{26}O$, M^+ at *m/z* 222) contained a tertiary hydroxyl group [3500 cm^{-1}, δ_c 74.4 (*s*)]. The ^{1}H NMR spectrum (Table 1) had signals for an isopropyl group, one secondary methyl group and one tertiary methyl group. This spectrum was very similar to that of authentic valencene (**17**), except for the absence of the signals corresponding to the isopropenyl group, suggesting that **8** was valencene with a tertiary hydroxyl group. The above assumption and the presence of a 7α-hydroxyl group were confirmed by ^{1}H–^{1}H and ^{1}H–^{13}C 2D COSY, spin decoupling, LIS and difference NOE experiments. On the above spectral evidence, the structure of **8** was established to be 7α-hydroxyvalenc-1(10)-ene.

Table 1. ^{1}H NMR spectral data of compounds **1**, **5**, **8** and **9** (400 MHz, $CDCl_3$, TMS)*

H	1	5	8	9
1			5.35 *br s*	1.40 *m*
				2.05 *m*
2	5.48 *br s*		2.00 *m*	2.05 *m*
			1.93 *m*	1.40 *m*
3	1.53 *t* (11)†		1.35 *m*	5.36 *br s*
	2.03 *m*		1.40 *m*	
4			1.35 *m*	
5	2.27 *m*			2.07 *br d* (3)
	1.65 *m*			
6	5.53 *m*		1.12 *m*	3.96 *dd* (4, 3)
			1.84 *m*	
7		5.53 *br s*		1.60 *m*
8	1.38 *m*		1.36 *m*	1.40 *m*
	1.82 *m*		1.67 *m*	1.60 *m*
9	1.38 *m*		1.93 *m*	1.50 *m*
	1.65 *m*		2.61 *m*	1.30 *m*
10	2.17 *m*			
	2.31 *m*			
11		4.23 *dd* (11, 5)	1.50 *m*	1.78 *m*
		4.40 *dd* (11, 3)		
12	1.65 *s*	1.68 *s*	0.89 *d* (6.8)‡	0.95 *d* (6.8)‡
13	0.94 *s*	0.85 *s*‡	0.91 *d* (6.8)‡	1.03 *d* (6.8)‡
14	1.01 *s*	0.87 *s*‡	1.11 *s*	0.96 *s*
15	4.79 *br s*	0.89 *s*‡	0.87 *s*	1.87 *br s*
	4.96 *br s*			
2′	6.20 *d* (16)	6.18 *d* (16)		
3′	7.53 *d* (16)	7.50 *d* (16)		
5′	7.08 *br s*	7.06 *br s*		
8′	6.85 *br d* (8)	6.84 *br d* (8)		
9′	6.84 *br d* (8)	6.83 *br d* (8)		

* All assignments were confirmed by spin decoupling, ^{1}H–^{1}H and ^{1}H–^{13}C 2D NMR and difference NOE spectral experiments.

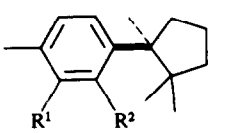
† Figures in parentheses are coupling constants in Hz.

‡ The values in any vertical column may be interchanged.

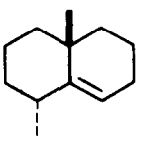
6β-Hydroxyeudesm-3-ene (**9**)

Compounds **9** and **12** were isolated as oils. The spectral data of the latter compound was identical to those of α-cyclogermacrone prepared from germacrone (**11**) by a cyclization reaction with acidic methanol and it has also been isolated from higher plant [9]. The ^{1}H–^{1}H, ^{1}H–^{13}C COSY NMR spectral and spin decoupling experiments suggested that **9** was an eudesmane type sesquiterpene with an isopropyl group, a vinylic methyl coupled with a vinylic proton (^4J) and a secondary alcohol, which was confirmed by pyridinium chlorochromate (PCC) oxidation to afford a six membered ketone (M^+ at *m/z* 220, 1710 cm^{-1}) whose spectral data were identical to isoacoramone (**10**) [10]. The above NMR experiments and the presence of the singlet signal (δ3.28, H-5) of **10** and co-occurrence of **12** established the structure **9** as 6β-hydroxyeudesm-3-ene.

The isolation of trinorsesquiterpene hydrocarbon (**21**) from Taiwanese *B. fauriana* has been reported [4]. However, even a trace amount of this compound was not detected by GC and GC/MS in the same species from Japan. Bazzanene (**4**)- and gymnomitrane (= barbatane)-types (e.g. **15**, **16**) sesquiterpenoids are widely distributed, not only in *Bazzania* species, but also in other liverworts [1, 11–13]. *Bazzania japonica* and *B. pompeana* (chemotype 1) elaborate albicanyl caffeate (**13**) as a major component but no bazzanenyl and drimenyl caffeate have been detected [1, 2]. The above two species also produce cuparene type sesquiterpenoids (**18**, **19**). There are the other different types (chemotype 2) of *Bazzania* species which produce calamenene-type sesquiterpenoids [2]. The present species biosynthesize bazzanenyl, drim-



18 $R^1 = R^2 = H$
19 $R^1 = OH$, $R^2 = H$
20 $R^1 = R^2 = OH$



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enyl and albicanyl caffeate, together with eudesmane- and valencane-type sesquiterpenoids not detected in any other *Bazzania* species so far examined. Thus, *B. fauriana* is chemically different from the above two chemotypes and we propose chemotype 3 for *B. fauriana*. The present chemical results are correlated to the morphological difference between *B. fauriana* and the other *Bazzania* species.

EXPERIMENTAL

The solvents used for spectral determination were: TMS-$CDCl_3$[^{1}H NMR (400 MHz); ^{13}C NMR (100 MHz)]; $CHCl_3$ (IR and $[\alpha]_D$). $CHCl_3$–MeOH (1:1) were used for Sephadex LH-20 CC. GC/MS was carried out at 70 eV using OV-17 (2%) column, temp. programmed from 50° to 250° at 5°/min, inject. temp. 250°; He 40 ml/min. TLC and GC were carried out as previously reported [14].

Plant material. Bazzania fauriana (Steph.) Hatt. was collected in Yakushima, Japan in Dec. 1983 and identified by Dr M. Mizutani. The voucher specimen was deposited at the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. B. fauriana was air-dried for 3-days and the dried material (800 g) was extracted with MeOH to obtain a green oil (5.26 g). A small amount of the crude extract was checked by TLC, GC and GC/MS. The presence of bazzanene (**4**), drimenol (**7**), α- and β-gymnomitrenes (**15**, **16**), cuparene (**18**), 2-hydroxycuparene (**19**) and 2,3-dihydroxycuparene (**20**) were identified by direct comparison of mass spectra with those of authentic samples. The GC/MS analysis also showed the presence of three unidentified sesquiterpenoids: *m/z* 204 $[M]^+$, 119 (100%), 204 $[M]^+$, 119 (100%) and 218 $[M]^+$, 175 (100%). The remaining material (5.2 g) was chromatographed on silica gel using a *n*-hexane–EtOAc gradient to provide 6 fractions. Fraction A (*n*-hexane 100%) was rechromatographed on silica gel impregnated with $AgNO_3$ (10%) using the same solvent system described above to give bazzanene (**4**) [7] (120 mg). Fraction B (*n*-hexane–EtOAc, 9:1) was further chromatographed on Sephadex LH-20 to give three fractions. Fraction B-1 was purified by column chromatography on silica gel–$AgNO_3$ (10%) using C_6H_6–EtOAc (19:1) to afford eudesm-3,7(11)-diene-8-one (**12**) (62 mg) [9]. Fraction B-2 was rechromatographed on silica gel (C_6H_6–EtOAc gradient) to give 6β-hydroxy eudesm-3-ene (**9**) (19 mg): $[\alpha]_D$ −35° (*c* 1.1); IR ν_{max}cm^{-1}: 3500, 1465, 1380, 1210, 1130, 1040, 970; ^{1}H NMR (Table 1); ^{13}C NMR: δ21.5, 21.6, 22.5, 22.7 (Me) 20.3, 23.0, 37.5, 39.8 (CH_2) 27.3, 45.7, 50.4 (CH) 31.7 (C) 70.1 (CH−O) 122.6 (=CH) 134.9 (=C); EIMS *m/z* (rel. int.): 207 $[M-Me]^+$ (18), 204 $[M-H_2O]^+$ (24), 161 (69), 109 (100), 107 (63), 105 (36), 93 (44), 81 (27). Fraction B-3 was rechromatographed on silica gel–$AgNO_3$ (10%) (*n*-hexane–EtOAc gradient) to furnish 7α-hydroxyvalenc-1(10)-ene (**8**) (27 mg) and gymnomitrol (**14**) (29 mg): **8**: $[\alpha]_D$ +75° (*c* 0.36); IR ν_{max}cm^{-1}: 3500, 1465, 1380, 1205, 1055, 975, 945; ^{13}C NMR: δ15.6, 16.9 (×2), 20.6 (Me) 25.8, 26.7, 28.4, 35.7, 47.1 (CH_2), 39.6, 41.9 (CH), 35.3 (C), 74.4 (C–O), 119.7 (=CH) 143.6 (=C); EIMS *m/z* (rel. int.): 179 $[M-C_3H_7]^+$ (100), 161 (76), 121 (60), 119 (25), 105 (22), 93 (24).

Fraction F (*n*-hexane–EtOAc 4:1) was also successively chromatographed on Sephadex LH-20, silica gel (EtOAc–C_6H_6 1:9) and Sephadex LH-20 to afford bazzanenyl caffeate (**1**) (85 mg), drimenyl caffeate (**5**) (8 mg) and albicanyl caffeate (**13**) [1, 2] (11 mg), respectively. **1**: IR ν_{max}cm^{-1}: 3550, 3300, 1690, 1635, 1605, 1515, 1445, 1270, 1180, 1110, 980; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2) **5**; IR ν_{max}cm^{-1}: 3480, 3350, 1695, 1605, 1180; ^{1}H NMR (Table 1) EIMS *m/z* (rel. int.): 180 (100), 163 (80).

Table 2. ^{13}C NMR spectral data of compounds **1**, **2**, **3** and **4** (100 MHz, $CDCl_3$, TMS)*

C	1[a,b,c]	2[a,c]	3[c]	4[a,b,c]
1	131.3	131.7	134.7	132.5
2	73.0	72.2	69.5	27.8
3	34.6	34.8	38.9	28.1
4	39.5	39.5	39.6	36.7
5	32.5	32.5	32.7	32.4
6	125.8	125.6	123.6	120.5
7	50.5	50.1	50.0	50.2
8	36.8	36.9	36.8	37.0
9	23.3	23.4	23.4	23.5
10	38.8	38.8	38.9	39.0
11	158.9	159.0	159.2	159.8
12	18.8	18.8	18.8	23.3
13	18.4	18.5	18.4	17.7
14	23.4	23.5	23.4	23.7
15	107.0	107.0	106.8	106.3
1′	168.5	167.1		
2′	115.2	111.0		
3′	145.6	144.5		
4′	127.1	127.5		
5′	114.4	109.6		
6′	144.3	149.3		
7′	147.0	151.1		
8′	115.6	116.3		
9′	122.5	122.6		
6′-OMe		55.9†		
7′-OMe		56.0†		

* All assignments were confirmed by [a] ^{1}H–^{13}C 2D COSY; [b] long range ^{1}H–^{13}C 2D COSY and [c] INEPT spectral experiments.

† The values may be interchanged.

Methylation of bazzanenyl caffeate (**1**). Caffeate **1** (39 mg) in Me_2CO was methylated with MeI (2 ml) in the presence of K_2CO_3 (500 mg) for 3 hr. Work-up as usual gave the dimethyl ether (**2**) (25 mg).; IR ν_{max}cm^{-1}: 1690, 1645, 1510, 1270; ^{1}H NMR: δ0.98, 1.04, 1.68, 3.91, 3.92 (each 3H, *s*) 4.81, 4.98, 5.52, 5.54 (each 1H, *br s*) 6.33, 7.64 (each 1H, *d*, *J* = 16 Hz) 6.86 (1H, *d*, *J* = 8.3 Hz) 7.06 (1H *d*, *J* = 1.9 Hz) 7.11 (1H *dd*, *J* = 8.3, 1.9 Hz); EIMS *m/z* (rel. int.): 410 $[M]^+$ (2), 208 (49), 191 (100), 107 (35).

Reduction of **2** *with* $LiAlH_4$. To suspension of $LiAlH_4$ (50 mg) in dry Et_2O was added **2** (29 mg) in dry Et_2O and stirred at 0° for 1 hr. Work-up as usual gave bazzanenol (**3**) (10 mg) [8] and 3,4-dimethoxycinnamyl alcohol (8 mg).

Methylation of drimenyl caffeate (**5**). Caffeate **5** (10 mg) in Me_2CO was methylated with MeI (1 ml) in the presence of K_2CO_3 (200 mg) for 2 hr. Work-up as usual gave a dimethyl ether **6** (8 mg).; IR ν_{max}cm^{-1}: 1720, 1630, 1603, 1520; ^{1}H NMR: δ0.86, 0.87, 0.90, 1.70, 3.90, 3.91 (each 3H, *s*) 4.23 (1H, *dd*, *J* = 11, 5 Hz) 4.40 (1H, *dd*, *J* = 11, 3 Hz) 6.23, 7.59 (each 1H, *d*, *J* = 16 Hz) 6.83 (1H, *d*, *J* = 8 Hz) 7.03 (1H, *d*, *J* = 2 Hz) 7.06 (1H, *dd*, *J* = 8,2 Hz); EIMS *m/z* (rel. int.): 208 (100), 191 (30).

Reduction of dimethylcaffeate **6**. Compound **6** (8 mg) was reduced by $LiAlH_4$ in the same manner as described above to afford drimenol **7** (3 mg) [1] and 3,4-dimethoxycinnamyl alcohol.

Oxidation of 6β-hydroxyeudesm-3-ene (**9**). To a soln of **9** (10 mg) in dry CH_2Cl_2 was added pyridinium chlorochromate (PCC) (50 mg) and the mixture stirred. After 3 hr, Et_2O (20 ml)

was added and the resulting mixture was filtered through a short pad of Celite to give the ketone (**10**) (5 mg) [10].

Cyclization of germacrone (**11**). A soln of **11** (10 mg) in MeOH (5 ml) containing one drop of concd HCl was allowed to stand for 1 hr, then the reaction mixture was poured into H_2O and extracted with Et_2O, followed by prep. TLC to afford α-bicyclogermacrone (**12**) (4 mg) [9].

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