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SESQUITERPENES FROM JAPANESE LIVERWORTS

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Key Word Index—*Bazzania japonica*; *B. pompeana*; *B. tricrenata*; *B. tridens*; *B. trilobata*; Jungermanniales; Hepaticae; albicanyl 3,4-dihydroxycinnamate; albicanyl 2,4-dihydroxycinnamate; drimane-type sesquiterpene esters; tridensenone; aromadendrane-type sesquiterpene ketone; barbatane-, bazzanane-, cuparane-, amorphane- and chamigrane-type sesquiterpenes; chemosystematics.

Abstract—Five Japanese liverworts (*Bazzania* sp.) were examined for sesquiterpenes. *B. japonica* and *B. pompeana* contained two new drimane-type sesquiterpene esters, albicanyl 3,4-dihydroxycinnamate and albicanyl 2,4-dihydroxycinnamate. Tridensenone, a new aromadendrane-type sesquiterpene ketone was isolated from *B. tridens*. The stereostructures of these new sesquiterpenes were elucidated mainly by spectrometry. Barbatane-, bazzanane- and cuparane-type sesquiterpenes were found in all of the five species investigated. These sesquiterpenes, along with the new drimane- and aromadendrane-type sesquiterpenes are useful chemosystematic markers.

INTRODUCTION

As part of a systematic study of biologically active substances of bryophytes, we are continuing to investigate their chemical constituents. Most of the liverworts contain oil bodies which are mainly composed of mono-, sesqui- and di-terpenoids and/or lipophilic aromatic compounds. These substances are often obtained as the major components and thus we can apply them to chemosystematic investigations of Hepaticae [1–7].

Bazzania species (Lepidoziaceae, Jungermanniales) are a rich source of sesquiterpenes. In this paper, we wish to report the distribution of sesquiterpenoids in five Japanese *Bazzania* species, and the isolation and characterization of two new drimane-type sesquiterpene esters from *B. japonica* and *B. pompeana* and of a new aromadendrane-type sesquiterpene ketone from *B. tridens*.

RESULTS AND DISCUSSION

Fresh *Bazzania* species were air-dried, ground and extracted with ether. The crude extracts were directly analysed by GC/MS linked to a computer. The mass spectra obtained were identified by direct comparison with those of authentic samples and published information. The main constituents were isolated by a combination of column chromatography, preparative TLC and GLC, and their structures were confirmed by spectral data and chemical degradation. Table 3 lists the species studied and sesquiterpenoids isolated or detected.

Albicanyl 3,4-*dihydroxycinnamate* (1)

Compound 1 [$C_{24}H_{32}O_4$ (M^+ 384), mp 179–180°] was isolated from *B. japonica* and *B. pompeana* as the major component. The IR and UV spectra showed the presence of a hydroxyl group (3480, 3350 cm^{-1}), a benzene ring (1605, 1535 cm^{-1}; λ_{max} 246, 300, 331 nm) and a conjugated aromatic ester (1695, 1180 cm^{-1}). The ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2) of 1 indicated the presence of three tertiary methyl groups, a non-conjugated methylene group, a methylene group bearing an ester oxygen, five methylene groups, two sp^3 quaternary carbons, two sp^3 methines and a 3,4-disubstituted *trans*-cinnamate group. Methylation of 1 with MeI gave a dimethyl ether (3) [$C_{26}H_{36}O_4$ (M^+ 412)] which on hydrolysis gave a sesquiterpene alcohol (5) [$C_{15}H_{26}O$ (M^+ 222); 3550 cm^{-1}; mp 64–65°] and 3,4-dimethoxycinnamic acid. Oxidation of 5 with pyridium-chlorochromate gave a carboxylic acid (6) [$C_{15}H_{24}O_2$ (M^+ 236); 1715 cm^{-1}], together with an unidentified seven-membered aldehyde lacking exomethylene and vinyl methyl groups. The above chemical and spectral evidence suggested that the original compound (1) might be an ester of 3,4-dihydroxycinnamic acid and a drimane-type sesquiterpene alcohol. Ohta *et al.* [8] reported the isolation of albicanol (5), the double-bond isomer of drimenol (7), from the European liverwort *Diplophyllum albicans*. The ^1H NMR and IR spectra of the sesquiterpene alcohol (5) derived from 1 were superimposable on those of albicanol (5). While Ohta *et al.* [8] confirmed the absolute configuration of albicanol from the LIS values observed in the ^1H NMR spectrum on addition of Eu(fod)$_3$ and chemical correlation with drimenol (7), the absolute configuration of 5 obtained from the ester found in *B. japonica* and *B. pompeana* remained questionable because most of the liverworts produce sesquiterpenoids enantiomeric to those found in higher plants, although there are several exceptions as shown by the sesquiterpenoids of *Porella*, *Frullania*, *Wiesnerella* and *Conocephalum* species [9–15]. The absolute configuration of 5 from *Bazzania* species was confirmed as follows. Ozonolysis of 5 gave a saturated six-membered ketone (8), [$C_{14}H_{24}O_2$ (M^+ 224); 1695 cm^{-1} (hydrogen-bonded C=O)], whose CD spectrum showed a negative Cotton effect (307 nm, $\Delta\varepsilon$-1.20). Thus, the stereostructure of albicanol was shown to be 5α,10β,11β-

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Table 1. ^1H NMR data of the new drimane- and aromadendrane-type sesquiterpenes and their related compounds* (90 MHz, TMS as internal standard)

	1 (acetone-d_6)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**6** (CCl_4)	**8** ($CDCl_3$)	**10** ($CDCl_3$)	Δ†
H-3						5.95 *m*	−0.47
H-5						3.17, *d* (*br*), *J* = 9	−0.29
H-6						0.85 *t* (*br*), *J* = 9	−0.13
H-7						0.67 *m*	−0.13
H-8						1.82 *m*	−0.08
H-9						2.48 *m*	−0.22
H-11	4.35 *d*, *J* = 9	4.36 *d*, *J* = 9	4.29 *d*, *J* = 9	2.75 *bs*	3.60 *dd*, *J* = 13.5, 3		
	4.42 *d*, *J* = 5	4.43 *d*, *J* = 5	4.33 *d*, *J* = 5		3.96 *dd*, *J* = 13.5, 9		
H-12	4.60 *s* (*br*)	4.60 *s* (*br*)	4.52 *s* (*br*)	4.76 *bs*		1.05 *s*	−0.07
	4.85 *s* (*br*)	4.86 *s* (*br*)	4.82 *s* (*br*)	4.82 *bs*			
H-13	0.90 *s*	0.90 *s*	0.87 *s*	1.03 *s*	0.96 *s*	1.20 *s*	−0.09
H-14	0.85 *s*	0.83 *s*	0.82 *s*	0.89 *s*	0.86 *s*	2.02 *d*, *J* = 2	−0.13
H-15	0.82 *s*	0.81 *s*	0.76 *s*	0.86 *s*	0.80 *s*	2.22 *s* (*br*)	−0.48
H-17	6.20 *d*, *J* = 15	6.25 *d*, *J* = 15	5.76 *d*, *J* = 15				
H-18	7.50 *d*, *J* = 15	7.59 *d*, *J* = 15	6.76 *d*, *J* = 15				
H-2′	7.13 *d*, *J* = 2	7.03 *d*, *J* = 2					
H-3′			7.60 *d*, *J* = 2				
H-5′	6.83 *d*, *J* = 8	6.38 *d*, *J* = 8	7.16 *dd*, *J* = 8, 2				
H-6′	7.02 *dd*, *J* = 8, 2	7.07 *dd*, *J* = 8, 2	6.79 *d*, *J* = 8				
OH	8.22 *s* (*br*)				3.02 *s* (*br*)		
OMe		3.90 *s*	3.88 *s*				
			3.90 *s*				

* All assignments were confirmed by the double resonance experiments.

† $\Delta Eu = \delta_{CDCl_3} - \delta_{Eu(fod)_3}$, tridensenone (10 mg) containing 10 mg of $Eu(fod)_3$.

Table 2. ^{13}C NMR data of the new drimane- and aromadendrane-type sesquiterpenes (22.6 MHz, TMS as int. standard)

	1 (acetone-d_6)	**5** ($CDCl_3$)	**10** ($CDCl_3$)
C-1	38.28 *t*	37.94 *t*	132.75 *s*
C-2	19.83 *t*	19.26 *t*	198.63 *s*
C-3	42.64 *t*	42.06 *t*	132.75 *d*
C-4	33.93 *s*	33.51 *s*	172.16 *s*
C-5	55.74 *d*	55.28 *d*	44.10 *d*
C-6	24.65 *t*	24.26 *t*	31.16 *d*
C-7	39.71 *t*	39.10 *t*	25.03 *d*
C-8	148.74 *s*	147.93 *s*	21.99 *t*
C-9	55.74 *d*	59.24 *d*	39.17 *t*
C-10	39.71 *s*	39.10 *s*	149.97 *s*
C-11	61.59 *t*	58.78 *t*	18.91 *s*
C-12	107.71 t	106.40 t	15.94 *q*
C-13	15.48 *q*	15.32 *q*	28.27 *q*
C-14	33.93 *q*	33.66 *q*	16.56 *q*
C-15	22.11 *q*	21.76 *q*	20.49 *q*
C-16	167.58 *s*		
C-17	122.50 *d*		
C-18	145.62 *d*		
C-1′	127.67 *s*		
C-2′	115.22**d*		
C-3′	146.31 *s*		
C-4′	147.89 *s*		
C-5′	116.38 *d*		
C-6′	115.88**d*		

* Signals may be interchangeable.

drim-8(12)-en-11-ol (**5**) which was identical to that isolated from *D. albicans* [8]. On the basis of the above evidence, the new sesquiterpene ester was assigned structure **1**.

Albicanyl 2,4-*dihydroxycinnamate* (**2**)

Compound **2** could not be isolated as such, because of the presence of a small amount of an unknown sesquiterpene ester. After the absence of any methoxyl groups in the crude material was confirmed by the ^1H NMR spectrum, the mixture was methylated with MeI and then chromatographed on Si gel to give the dimethyl ether **4** [$C_{26}H_{36}O_4$ (M^+ 412)], whose MS spectrum was identical with that of the dimethyl ether (**3**) derived from **1**. The other spectral data of **4** were quite similar to those of **3**, indicating that **4** might be an isomer **3**. Hydrolysis of **4** gave albicanol (**5**) and a dimethoxycinnamic acid whose spectral data and chromatographic behavior were identical with those of the authentic 2,4-dimethoxycinnamic acid. Thus, the structure of the original ester was established as **2**.

Tridensenone (**10**)

Compound **10** [$C_{15}H_{20}O$ (M^+ 216)] was isolated from *B. tridens* and its structure was deduced from the spectrophotometric data. The ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2) showed the presence of two vinyl methyls, two tertiary methyls, one vinylic proton, a proton on a cyclopropane ring, two methylene groups, one of which was located in an allylic position, three sp^3 methines, one quaternary sp^3 carbon, a tetrasubstituted double bond and a carbonyl group. The presence of a cross-conjugated cyclopentenone group was suggested by

Table 3. Sesquiterpenoids found in *Bazzania* species

	*B. japonica**	*B. pompeana**		*B. tricrenata†*		*B. tridens**	*B. trilobata†*	
Albicanyl 3,4-dihydroxycinnamate (**1**)	+ + + + + + ‡§	+ + + + + + ‡						
Abicanyl 2,4-dihydroxycinnamate (**2**)	+	+						[36]
Drimenol (**7**)							+ + +	+
Tridensenone (**10**)						+		
Bicyclogermacrene (**11**)	+	+	[28–30]			+		[25]
α-Barbatene (**12**)¶	+	+	+		[25]	+	+	+
Gymnomitrol (**13**)					+			
β-Barbatene (**14**)¶	+ +	+ +	+	+ +	+	+ +	+ + +	+
Bazzanene (**15**)	+ +	+ +	+	+	+	+ +	+ + +	+
Calamenene (**16**)	+ +			+	+	+	+	+
5-Hydroxycalamenene (**17**)				+	+		+ + +	
7-Hydroxycalamenene (**18**)				+			+	
Cuparene (**19**)	+		+	+	+	+	+	
2-Hydroxycuparene (**20**)	+ + + + + +§	+	+					
β-Chamigrene (**21**)				+				

* Collected in Sept. 1980, Kito-son, Nakagun, Tokushima, Japan.

† Collected in Sept. 1980, Yatsugatake, Japan.

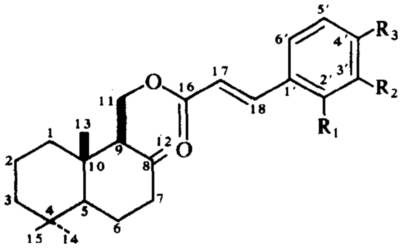
‡ Except for compounds **1** and **2** which were estimated by the relative sizes of their TLC spots, +, + +, + + + etc are the relative concentrations estimated by GC/MS.

§ **1** and **20** made up 25% of the total extract and their ratio was estimated to be 1:1 (TLC spots and weight).

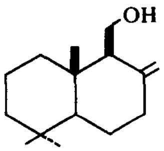
¶ The names of α-pompene and β-pompene have been used for these compounds by Matsuo *et al.* [28, 31].

intense absorption bands at λ_{max} 258 nm (log ε, 4.13) and 1680 cm^{-1}. The above spectral data, coupled with the molecular formula, showed that tridensenone (**10**) was a tricyclic sesquiterpene ketone having a cyclopropane ring and two double bonds. The chemical shifts of the two vinyl methyls in the ^{1}H NMR spectrum, the strong absorption band at 1620 cm^{-1} assignable to a *cis*oid carbonyl group and the UV absorption maximum observed in tridensenone were quite similar to those of the natural sesquiterpene lactones matricarin, desacetoxymatricarin [16, 17], lactucin [18, 19] and the achillins [20], indicating that **10** might possess partial structure **9**. The remaining partial structure of **10** was confirmed by spin decoupling experiments. Irradiation of the doublet signal at δ 2.02 (H-14) collapsed the multiplet at 5.95 (H-3) to a sharp doublet (J = 2 Hz). Reverse irradiation at δ 5.95 caused the doublet at 2.02 and the broad doublet at 3.17 (H-5) to collapse to a sharp singlet and a sharp doublet, respectively. Irradiation at the triplet at δ 0.85 (H-6) caused the doublet at 3.17 to collapse to a broad singlet. Reverse irradiation at δ 3.17 collapsed the triplet at 0.85 to a doublet. Irradiation at the centre of the multiplet at δ 1.82 (H-8) collapsed the triplet-like signal at 2.48 (H-9) and the complex multiplet at 0.67 (H-7) to a broad singlet and a broad doublet, respectively. The above decoupling experiments along with the well-separated IR absorption bands at 1360 and 1375 cm^{-1}, attributable to a gem-dimethyl group, the loss of a C_3H_7 group (m/z 173, 92 %), and the co-existence of (−)-bicyclogermacrene (**11**) [10, 21–23], which may be an important precursor for tridensenone (**10**), led to structure **10** for tridensenone. The absolute configuration of C-5 was further determined by the coupling constant (J = 9) between H-5 and H-6.

In addition to the albicanyl cinnamates (**1** and **2**), *B. japonica* produces a large amount of (*S*)-2-hydroxycuparene (δ-cuparenol) (**20**). Japanese *B. tricrenata* and *B.*



1 R_1 = H, R_2 = R_3 = OH
2 R_1 = R_3 = OH, R_2 = H
3 R_1 = H, R_2 = R_3 = OMe
4 R_1 = R_3 = OMe, R_2 = H



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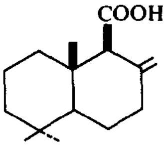

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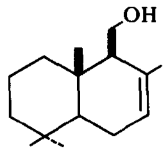

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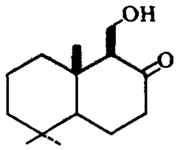

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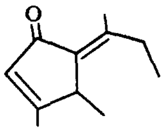

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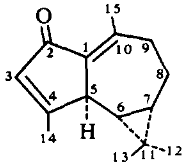

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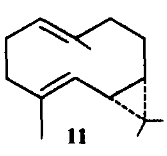

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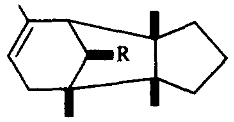

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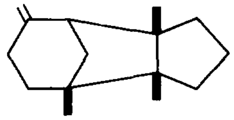
13 R = OH



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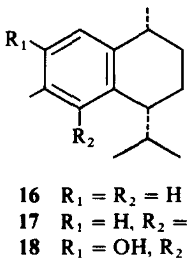
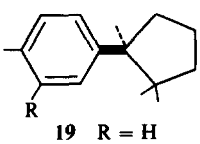
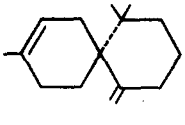


16 R_1 = R_2 = H
17 R_1 = H, R_2 = OH
18 R_1 = OH, R_2 = H



19 R = H
20 R = OH



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trilobata (Table 3) have also been investigated chemically. The present five *Bazzania* species contained barbatane- (**12–14**), bazanane- (**15**) and cuparane-type sesquiterpenes (**19–20**) whose chemical structures have been fully elucidated by Connolly, Andersen and Matsuo *et al.* [24–31]. Calamenene (**16**) was detected in four (*B. japonica*, *B. tricentata*, *B. tridens* and *B. trilobata*) out of the five species. 5-Hydroxycalamenene (**17**) and 7-hydroxycalamenene (**18**) were detected in *B. tricrenata* and *B. trilobata*. Free drimenol (**7**), the double bond isomer of albicanol (**5**), was isolated from *B. trilobata*. As seen in Table 3, *B. japonica* and *B. pompeana* are different from the other three species, since the former two species produce albicanyl dihydroxycinnamates (**1** and **2**). *B. tridens* is slightly different from the other four species since it elaborates a unique aromadendrane-type sesquiterpene ketone (**10**). Thus the barbatane-, bazzanane, cuparane-, drimane-, aromadendrane-type sesquiterpenes, and calamenene and its hydroxy derivatives are significant chemosystematic markers of *Bazzania* species.

It is suggested that the sesquiterpenoids found in *Bazzania* species may be formed by cyclization of *cis,trans*- and *trans,trans*-farnesyl pyrophosphate. Four possible biogenetic pathways for the formations of each sesquiterpene isolated or detected in *Bazzania* species are represented in Fig. 1.

EXPERIMENTAL

The solvents used for spectral determination were: CCl_4, $CDCl_3$ or Me_2CO-d_6 [^1H NMR (90 MHz), ^{13}C NMR (22.6 MHz)]; $CHCl_3$ or MeOH ($[\alpha]_D$ and CD)]; 95% EtOH (UV). IR spectra were run in $CHCl_3$, CCl_4 or liquid film. TLC and prep. TLC: precoated Si gel (0.25 mesh) F_{254}, *n*-hexane–EtOAc (4:1), C_6H_6–EtOAc (4:1) and $CHCl_3$–MeOH (1:1). Compounds were detected by I_2 vapour, H_2SO_4 and UV light. Prep. GLC: SE-30 2% and 5%, glass column (3 m × 2 mm), oven temp. 100–220°, He 30 ml/min. MS: EIMS (DI method), 70 eV; GC/MS: 70 eV, SE-30 1%, glass column (3 m × 2 mm), temp. programme 50–270° at 5°/min, inject. temp. 260°, He 30 ml/min.

Plant materials. *Bazzania* species were identified by Drs. S. Hattori, H. Inoue and M. Mizutani and are deposited in the Herbarium, Instit. of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. *Bazzania japonica* (Lac.) Lindb., *B. pompeana* (Lac.) Mitt., *B. tricrenata* (Wahl.) Trev., *B. tridens* (Reinw. *et al.*) Trev. and *B. trilobata* (L.) S. Gray were collected in Tokushima and Yatsugatake (Table 3) and air-dried for 5 days. The dried material (1 g) was ground and then extrd with Et_2O for 1 week. The crude extract was filtered through a short column packed with Si gel (230–400 mesh) and the solvent was evapd *in vacuo*. Each green oil was monitored on TLC for the presence of

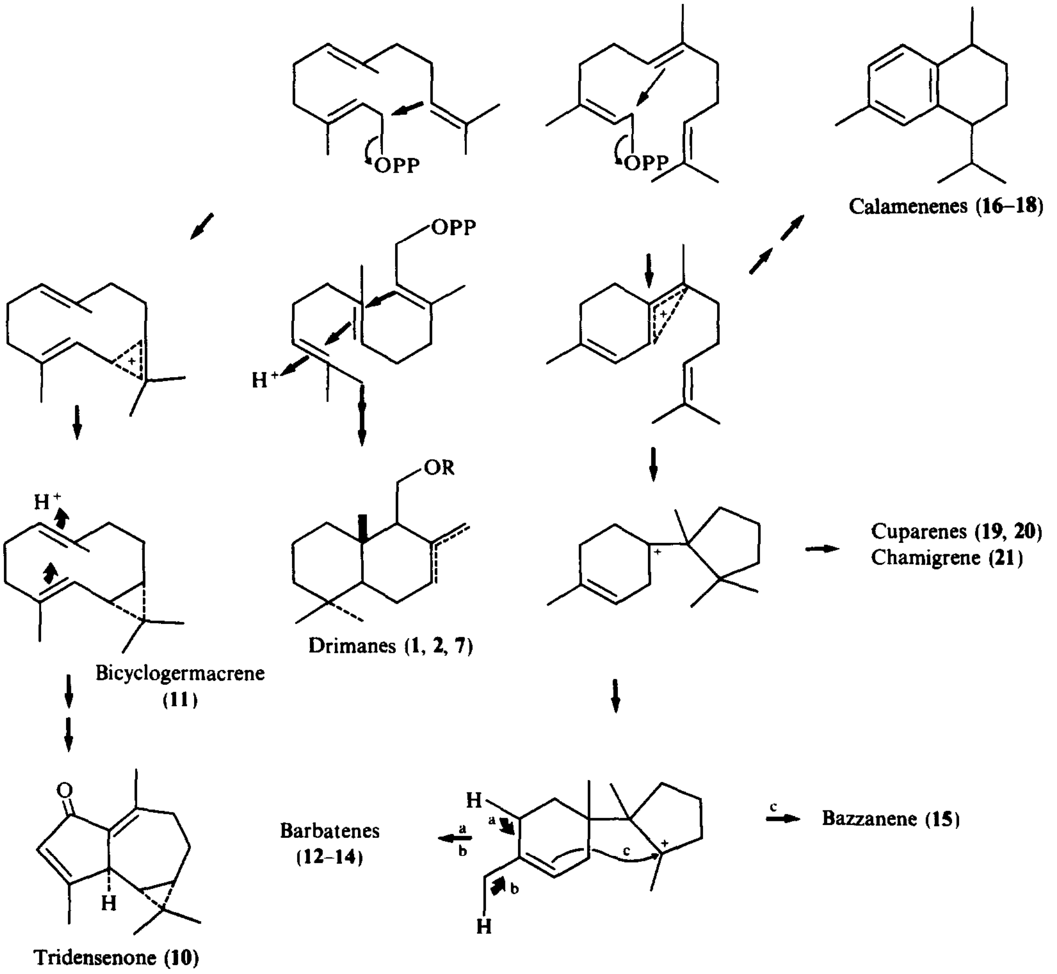

Fig. 1. Possible biogenetic pathways for the formation of the sesquiterpenes found in *Bazzania* species.

major components. To obtain the first indication of the chemical constituents of each species, the green oils were analysed by computerized GC/MS. The remaining ground materials (250 g of *B. japonica*, 140 g of *B. pompeana*, 55 g of *B. tricrenata*, 30 g of *B. tridens* and 530 g of *B. trilobata*) were also extrd with Et_2O for 2 weeks. The sesquiterpenoids were then isolated from the extracts by the combination of CC, prep. TLC and GLC.

The crude extract (7.30 g) of *B. japonica* was chromatographed on Si gel using a *n*-hexane–EtOAc gradient. The first fraction eluted with *n*-hexane contained the mixture of sesquiterpene hydrocarbons (200 mg) in which the presence of bicyclogermacrene (**11**), α-barbatene (**12**), β-barbatene, bazzanene, calamenene and cuparene (**19**) were shown by GC/MS. The hydrocarbon mixtures were rechromatographed on 5% $AgNO_3$–Si gel using *n*-hexane to afford (−)-β-barbatene (**14**) (70 mg), $[\alpha]_D$ −18° (*c*, 0.8) [25], (+)-bazzanene (**15**) (84 mg), $[\alpha]_D$ +37° (*c* 1.8) [25] and (+)-calamenene (**16**) (62 mg), $[\alpha]_D$ +51° (*c*, 0.50) [32,33]. The second fraction eluted with *n*-hexane–EtOAc (19:1) gave a mixture of carotenoids and triglycerides (1.20 g). The third fraction (9:1) gave an oil (900 mg) whose spectral and physical data were identical to those of (*S*)-(−)-2-hydroxycuparene (**20**), $[\alpha]_D$ −62° (*c*, 2.5) [29,34]. The fourth fraction (4:1) gave a sterol mixture (campesterol, stigmasterol, sitosterol, 1:2:3 by GC/MS) (80 mg). The fifth fraction (1:1) gave a brown oil (2.40 g) which was rechromatographed on Sephadex LH-20 using $CHCl_3$–MeOH (1:1) to afford albicanyl 3,4-dihydroxycinnamte (**1**) (910 mg) and an additional sesquiterpene ester (160 mg) which was methylated with MeI and chromatographed on Si gel using a *n*-hexane–EtOAc gradient to afford albicanyl 2,4-dimethoxycinnamate (**4**) (100 mg).

Albicanyl 3,4-dihydroxycinnamate (**1**). Mp 179–180°; $[\alpha]_D$ −18.3° (*c*, 3.2 in MeOH); IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 3480, 3350 (OH), 1695, 1180 (–C=C–COO), 1640 (C=C), 1605, 1535, 1445 (aromatic), 1385, 1365 (gem-dimethyl), 1300, 1275, 1240, 1110, 980, 890, 845, 810; UV $\lambda_{\max}$ nm (log ε): 218 (4.12), 246 (3.95), 300 (4.08), 331 (4.23); MS *m/z* (rel. int.) 384 (M^+, $C_{24}H_{32}O_4$, 5), 236 (21), 205 (10), 204 (14), 189 (13), 181 (13), 180 [$(HO)_2$–C_6H_3CH=CH–$COOH^+$, 100], 163 (55), 137 (16), 136 (14), 135 (17), 134 (13), 123 (16), 121 (10), 107 (12), 95 (14), 93 (16), 89 (11), 81 (19), 79 (12), 69 (20), 55 (16), 41 (16).

Albicanyl 2,4-dimethoxycinnamate (**4**). IR $\nu_{\max}^{\mathrm{liq}}$ cm^{-1}: 1718, 1170 (–C=C–COO), 1630 (C=C), 1605, 1590, 1520, 1466 (aromatic), 1390, 1370 (gem-dimethyl), 1425, 1345, 1280, 1260, 1146, 1030, 890, 825, 760, 735, 665; UV $\lambda_{\max}$ nm (log ε): 298 (3.92), 321 (3.98); MS *m/z* (rel. int.): 412 (M^+, $C_{26}H_{36}O_4$, 12), 208 [$(MeO)_2C_6H_3CH$=CH–$COOH^+$, 100], 191 (28), 138 (10), 69 (11).

The crude extract (8.10 g) of *B. pompeana* was directly chromatographed on Bio-Beads S-X2 (200–400 mesh) using C_6H_6 (three fractions collected). The first fraction gave a brown oil which was rechromatographed on Sephadex LH-20 using $CHCl_3$–MeOH (1:1) to afford albicanyl 3,4-dihydroxycinnamate (1.70 g) and a mixture of sesquiterpene esters (50 mg) which was methylated with MeI, followed by chromatography on Si gel using *n*-hexane–EtOAc to afford the pure dimethyl ether **4** (20 mg). The second fraction contained sterols (campesterol, stigmasterol, sitosterol, 1:2:1 in GC/MS) (130 mg). The third fraction gave a mixture of carotenoids, triglycerides and sesquiterpene hydrocarbons (1.50 g) which was rechromatographed on Si gel using a *n*-hexane–EtOAc gradient. The first fraction (*n*-hexane) contained the sesquiterpene hydrocarbons (320 mg) of which bicyclogermacrene (**11**), α-barbatene (**12**), β-barbatene (**14**), bazzanene (**15**) and cuparene (**19**) were detected by GC/MS. The second fraction (*n*-hexane–EtOAc, 19:1) gave carotenoids (50 mg). The third fraction (9:1) (870 mg) was treated with MeOH and divided into a MeOH-soluble portion and a MeOH-insoluble portion. The solvent of the former was evapd and the residue was purified by prep. TLC to afford a sesquiterpene phenol (12 mg) whose spectral and physical data were in agreement with those of (*S*)-(−)-2-hydroxycuparene (**20**) isolated from *B. japonica*.

The crude extract (1.45 g) from *B. tridens* was chromatographed on Si gel using the same solvent system as was used for the *B. japonica* extract. The first fraction (*n*-hexane) contained sesquiterpene hydrocarbons (120 mg) of which bicyclogermacrene (**11**), α-barbatene (**12**) and β-barbatene (**14**), bazzanene (**15**), calemenene (**16**) and cuparene (**19**) were detected by GC/MS. This mixture was rechromatographed on Si gel eluted with *n*-hexane to afford (−)-bicyclogermacrene (**11**) (12 mg), $[\alpha]_D$ −63° (*c*, 0.2) [11,21–23] and (+)-bazzanene (**15**) (15 mg) in the pure state. The second fraction eluted with *n*-hexane–EtOAc (19:1) contained a mixture of carotenoids and triglycerides (50 mg). The third fraction (9:1) gave tridensenone (**10**) (60 mg, pure).

Tridensenone (**10**). Mp 89–90°; $[\alpha]_D$ −55.5° (*c*, 0.5); $C_{15}H_{20}O$; IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1680 (–C=C–CO), 1635 (*trans*oid C=C), 1620 (*cis*oid C=C), 1375, 1360 (gem-dimethyl), 1460, 1430, 1415, 1315, 1275, 1240, 1220, 1190, 1145, 1070, 1040, 985, 950, 880, 860, 640, 600; UV $\lambda_{\max}$ nm (log ε): 258 (4.13); $\Delta\varepsilon_{358\,\mathrm{nm}}^{\mathrm{CHCl_3}}$: −1.50; MS *m/z* (rel. int.): 216 (M^+, 100), 201 (19), 174 (24), 173 (M^+, $-C_3H_7$, 93), 159 (27), 158 (20), 148 (22), 145 (46), 134 (23), 133 (20), 129 (13), 115 (18), 105 (27), 91 (45), 77 (25), 69 (36), 41 (44), 39 (22).

The crude extract (18.80 g) from *B. trilobata* was chromatographed on Si gel using the solvent system just described. The first fraction (*n*-hexane) gave a mixture of sesquiterpene hydrocarbons (2.75 g) in which α-barbatene (**12**) and β-barbatene (**14**), and bazzanene (**15**) were detected by GC/MS. Prep. GLC gave (+)-α-barbatene (25 mg), $[\alpha]_D$ +42° (*c*, 0.8) [25], (−)-β-barbatene (67 mg) and (+)-bazzanene (80 mg). The second fraction (*n*-hexane) contained two sesquiterpene hydrocarbons (160 mg) which were purified by prep. GLC to afford (+)-calamenene (**16**) (32 mg) and (−)-cuparene (**19**) (26 mg), $[\alpha]_D$ −57° (*c*, 0.4) [29]. The third fraction (*n*-hexane–EtOAc, 19:1) contained a mixture of carotenoids and triglycerides (5.30 g). The fourth fraction (9:1) gave a yellow viscous oil (2.65 g) which was rechromatographed on Si gel using a C_6H_6–EtOAc gradient to give a pure sesquiterpene phenol [250 mg, $C_{15}H_{22}O$, MS *m/z* (rel. int.) 218 (M^+, 15), 175 (M^+ $-C_3H_7$, 100)], whose ^1H NMR spectral data were identical to that of 5-hydroxycalamenene (**17**) [25] and a small amount of an additional sesquiterpene phenol [6 mg, $C_{15}H_{20}O$, MS *m/z* (rel. int.) 218 (M^+, 18), 175 (M^+ $-C_3H_7$, 100)] whose structure was tentatively assigned to be 7-hydroxycalamenene (**18**) [33,35] by comparison of its MS spectrum to that of 5-hydroxycalamenene (**17**). The fifth fraction (4:1) contained a mixture of sterols (campesterol, stigmasterol, sitosterol, 2:3:2 by GC/MS). The sixth fraction (7:3) (152 mg) was not identified. The seventh fraction (1:1) gave a viscous oil (560 mg) which was rechromatographed on Si gel using a C_6H_6–EtOAc gradient to afford (−)-drimenol (145 mg) $[\alpha]_D$ −22° (*c*, 0.7) [36] and fatty acids (360 mg). The eighth fraction (1:2) contained flavonoid-like compounds (140 mg).

The crude extract (1.40 g) from *B. tricrenata* was chromatographed on Si gel using a *n*-hexane–EtOAc gradient. The first fraction (*n*-hexane) contained the sesquiterpene hydrocarbons (125 mg) of which β-barbatene (**12**), bazzanene (**15**), calamenene (**16**), cuparene (**19**) and β-chamigrene (**21**) were detected by GC/MS. The second fraction (*n*-hexane–EtOAc, 19:1) contained carotenoids and triglycerides (650 mg). The third fraction (9:1) gave sesquiterpene phenols (65 mg) of which 5-hydroxycalamenene (**17**) and 7-hydroxycalamenene (**18**) were detected by GC/MS. The fourth fraction (4:1) gave a green mass which was washed with *n*-hexane to afford sterols (campesterol, stigmasterol, sitosterol, 1:2:1 by GC/MS). The last fraction (1:1) gave fatty acids (35 mg).

Methylation of **1**. To the solution of **1** (100 mg) in dry Me_2CO (3 ml) was added MeI (1 ml) in the presence of K_2CO_3 (3 g). The mixture was refluxed for 1 hr. Usual work-up gave a dimethyl ether (**3**) (90 mg): mp 119–120°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705, 1180 (–C=C–COO), 1640 (C=C), 1605, 1590, 1520, 1470 (aromatic), 1390, 1373 (gem-dimethyl), 1430, 1345, 1310, 1365, 1165, 1145, 1030, 985, 900, 855, 815; UV λ_{max} nm (log ε): 295 (4.14), 322.5 (4.24); MS *m/z* (rel. int.): 412 (M^+, $C_{26}H_{36}O_4$, 14), 208 [$(MeO)_2C_6H_3CH{=}CH{-}COOH^+$, 100], 191 (32), 69 (10).

Hydrolysis of **3**. **3** (90 mg) was dissolved in 3% KOH–MeOH (5 ml) and the mixture was refluxed for 30 min. The reaction mixture was worked up to give an alcohol (**5**) (50 mg): mp 64–65°; $[\alpha]_D$ +6° (*c*, 2.6); MS *m/z* (rel. int.): 222 (M^+, $C_{15}H_{26}O$, 10), 137 (100), 136 (30), 123 (31), 121 (21), 109 (26), 107 (25), 95 (43), 93 (22), 91 (21), 81 (41), 69 (34), 55 (30), 41 (30); ^1H NMR and IR spectra superimposable on those of albicanol (**5**) [8]. The K salt was acidified by 5% HCl and then extrd with Et_2O. The solvent was evapd to give a carboxylic acid which was then converted into the methyl ester (30 mg). The spectral data of the acid and its methyl ester were identical to those of 3,4-dimethoxycinnamic acid and its methyl ester, respectively.

Oxidation of albicanol (**5**). To a stirred (magnetically) suspension of pyridium chlorochromate (160 mg) in anhydrous CH_2Cl_2 (2 ml) was added **5** (70 mg). After 1 hr, dry Et_2O (50 ml) was added and decanted from the black gum. The organic layer was evapd, *in vacuo* and the residue was purified by prep. TLC to afford albicanic acid (**6**) (30 mg) and an unidentified ring expanded α,β-unsaturated aldehyde (20 mg).

Albicanic acid (**6**): IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3020, 1715 (COOH), 1650 (C=C), 1385, 1365 (gem-dimethyl), 1455, 1440, 1420, 1265, 1225, 1210, 1105, 1040, 935, 895, 850, 695, 640; MS *m/z* (rel. int.): 236 (M^+, $C_{15}H_{24}O_2$, 22), 137 (100), 123 (43), 121 (26), 109 (31), 107 (26), 95 (44), 93 (24), 91 (28), 81 (40), 79 (26), 69 (57), 67 (24), 55 (37), 41 (58).

α,β-Unsaturated aldehyde. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1695 (–C=C–CHO), 1645 (C=C), 1390, 1375 (gem-dimethyl); ^1H NMR: δ 0.88, 0.93, 1.05 (each 3 H, *s*), 6.23 (1 H, *m*), 9.26 (1 H, *s*); MS *m/z* (rel. int.): 222 (M^+, $C_{15}H_{24}O$, 1), 191 ($M^+ - CHO$, 21), 41 (100). The structure of the above aldehyde is now under investigation.

Ozonolysis of **5**. A stream of ozonized-O_2 was passed through an EtOAc (5 ml) soln of **5** (60 mg) at −70° for 3 hr. To the reaction mixture was added 30% H_2O_2 (1 ml), AcOH (2 ml), H_2O (0.5 ml) and 1 drop of conc. HCl. The mixture was then stirred for 20 hr. Work-up as usual gave a saturated ketol (**8**) (12 mg): $[\alpha]_D$ −29° (*c*, 0.6); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3660 (free OH), 3570 (intermolecular OH), 1695 (hydrogen-bonded C=O), 1390, 1370 (gem-dimethyl), 1330, 1305, 1180, 1120, 1070, 1025, 965; $\Delta\varepsilon_{307nm}^{CHCl_3}$: −1.20; MS *m/z* (rel. int.): 224 (M^+, $C_{14}H_{24}O_2$ 17), 137 (39), 123 (34), 109 (32), 99 (52), 95 (44), 86 (100), 81 (53), 71 (33), 69 (63), 67 (32), 55 (59), 41 (61).

Hydrolysis of **4**. **4** (70 mg) was hydrolysed by 3% KOH–MeOH (3 ml) and worked up as usual to afford albicanol (**5**) (27 mg) and an unsaturated carboxylic acid (15 mg) whose spectral data were identical to those of authentic 2,4-dimethoxycinnamic acid.

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