Isocalamenenes from the Liverwort *Bazzania tridens*

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Four new sesquiterpenoids, 1,4-*trans*-6-hydroxyisocalamenene; 1,4-*trans*-6-methoxyisocalamenene; (+)-aristol-9-en-12β-ol, and eudesm-4(15)-en-6β-acetoxy-7β-ol, were isolated from *Bazzania tridens*, a liverwort species. Their structures were identified by means of spectroscopic methods. The two isocalamenenes are derived from a methyl rearrangement of the calamenene skeleton.

INTRODUCTION

The liverwort species *Bazzania tridens* is very rich and complex in sesquiterpenoids. In our previous findings, several chemotypes may be classified for this species on the basis of major oxygenated sesquiterpenes biosynthesized by every specimen collected at different localities.[1,2] In a continuous investigation of the same species collected from Fu-Shan, Taiwan, (+)-aristol-9-en-12β-al (**1**) and (+)-aristol-9-en-12β-oic acid (**2**)[3] were isolated as the major components. These two compounds have been observed in several *B. tridens* species collected from Taiwan and China since their first identification.[1] In addition, three new sesquiterpenes were also obtained along with other known compounds, such as tridensone (**3**),[3] spathulenol, and the triterpene friedelin.[4,5] Among the new sesquiterpenes, isocalamenenes **4** and **5** are noteworthy owing to possessing a rearranged calamenene skeleton.

RESULTS AND DISCUSSION

Compound **4** was obtained from the benzene eluate of the *n*-hexane-extract. Its GC-MS spectrum revealed the molecular ion peak at *m/z* 232 ($C_{15}H_{20}O_2$) and the base peak at *m/z* 189 ([M^+]-43). All other fragments appeared in fairly low intensities (< 15%). Hence the MS information indicated a relatively stable skeleton with the facility to lose an isopropyl group. ^{1}H-, ^{13}C- and DEPT spectra of **4** clearly supported the calamenene skeleton with a methoxy group (δ_H 3.81, s, δ_C 55.4) on the aromatic ring (δ_H 6.67, 6.98, all s, δ_C 109.7(CH), 129.2(CH), 123.6, 134.8, 138.4, 155.5, all quaternary) (Tables 1 & 2).

Since the compound was very likely to be one of the two known 7-methoxycalamenenes (**6** and **7**), careful comparisons of ^{1}H- and ^{13}C-NMR data among the three sesquiterpenes were carried out.[6,7] Their ^{1}H NMR data indicated that compound **4** appeared nearly identical with the *trans*-isomer of 7-methoxycalamenene (**6**) rather than its *cis*-isomer (**7**) as indicated by the chemical shifts at H-2, H-3, and H-4.[6,7] However, a comparison of their ^{13}C NMR data disclosed considerable difference for C-9 and C-10 as shown in Table 2. The subsequent NOESY and NOE difference experiments (Fig. 1) clarified that the aromatic proton at δ_H 6.98 correlated with the two methyls at δ_H 2.19 and 1.25, and that the other aromatic proton at δ_H 6.67 correlated with the methoxy methyl (δ_H 3.81), as well as the two methine protons at δ_H 2.21 and 2.68. Therefore, compound **4** must possess a rearranged calamenene skeleton with the methoxy and the methyl groups on the aromatic ring interchanged from that shown in **6**. Thus the new compound **4** is assigned as 6-methoxyisocalamenene.

Compound **5** was slightly more polar than compound **4**, and possessed a molecular formula of $C_{15}H_{22}O$ as indicated by its GC-MS showing [M^+] 218 and a base peak at *m/z* 175. Again, the mass spectrum of compound **5** showed the same characteristic as that of 7-hydroxycalamenene. The ^{1}H NMR data of **5** (Table 1) were indistinguishable from those of *trans*-7-hydroxycalamenene (**8**),[6] but their ^{13}C NMR chemical shifts (Table 2), again, differed significantly at C-9 and C-10. A better resolved proton spectrum, particularly at regions around δ_H 2.20 (H-11 and H-15) and 2.68 (H-1 and H-4) was obtained when a small amount of the shift reagent, Eu(fod)$_3$, was added to the solution of compound **5** (Fig. 1). The improved resolution permitted similar correlations as those observed in the NOE study for com-

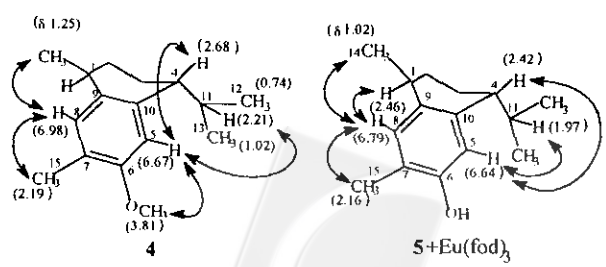


Fig. 1. Key NOE's observed for compounds **4** and **5**.

Table 1. ^{1}H NMR Spectral Data of Compounds **4**, **5**, **12** and **16**

H	4	5	12	16
1	2.70*	2.68*[2.46]	2.16 (md, J = 13.8 Hz)	1.33*
			1.96 (mt, J = 13.8 Hz)	1.39*
2	1.93 (m)	1.94 (m)	1.67 (m)	1.54 (m)
	1.36 (m)	1.33 (m)	1.40*	1.54 ?
3	1.56 (m)	1.54 (m)	1.47*	2.04*
	1.82 (m)	1.82 (m)	1.60*	2.29 (br d, J = 13.1 Hz)
4	2.68*	2.68*[2.42]	1.58*	-
5	6.67 (br. s)	6.63 (br. s)[6.64]	-	2.53 (br. s)
6	-	-	0.72 (d, J = 9.6 Hz)	5.22 (br. s)
7	-	-	0.89 (br d, J = 9.6 Hz)	-
8	6.98 (br. s)	6.96 (br. s)[6.79]	2.33 (dqnt, J = 18.9, 2.9 Hz)	1.57*
				1.63*
			2.05 (ddd, J = 18.9, 4.5, 1.9 Hz)	
9	-	-	5.10 (br. s)	1.37*
				1.61 (m)
11	2.21	2.20*[1.97]	-	1.49 (sept, J = 6.8)
	(br sept, J = 6.8)			
12	0.74 (d, J = 6.8)	0.72 (d, J = 6.7)	3.32 (s)	0.86 (d, J = 6.8)
13	1.02 (d, J = 6.8)	1.01 (d, J = 6.8)	1.18 (s)	0.94 (d, J = 6.8)
14	1.25 (d, J = 6.8)	1.25 (d, J = 6.8)	1.05 (s)	0.92 (s)
		[1.02]		
15	2.19 (s)	2.21 (s)[2.16]	0.92 (d, J = 6.9)	4.51 (d, J = 1.4)
				4.71 (d, J = 1.4)
OMe	3.81 (s)	-	-	-
OH	-	4.42 (br. s)	-	-
OAc	-	-	-	2.01 (s)

* Overlapped or obscured by contaminant peaks.

[δ_H] Observed chemical shifts upon addition of Eu(fod)$_3$.

Table 2. ^{13}C NMR Spectral Data of Compounds **4-6**, **8**, **12** and **16**

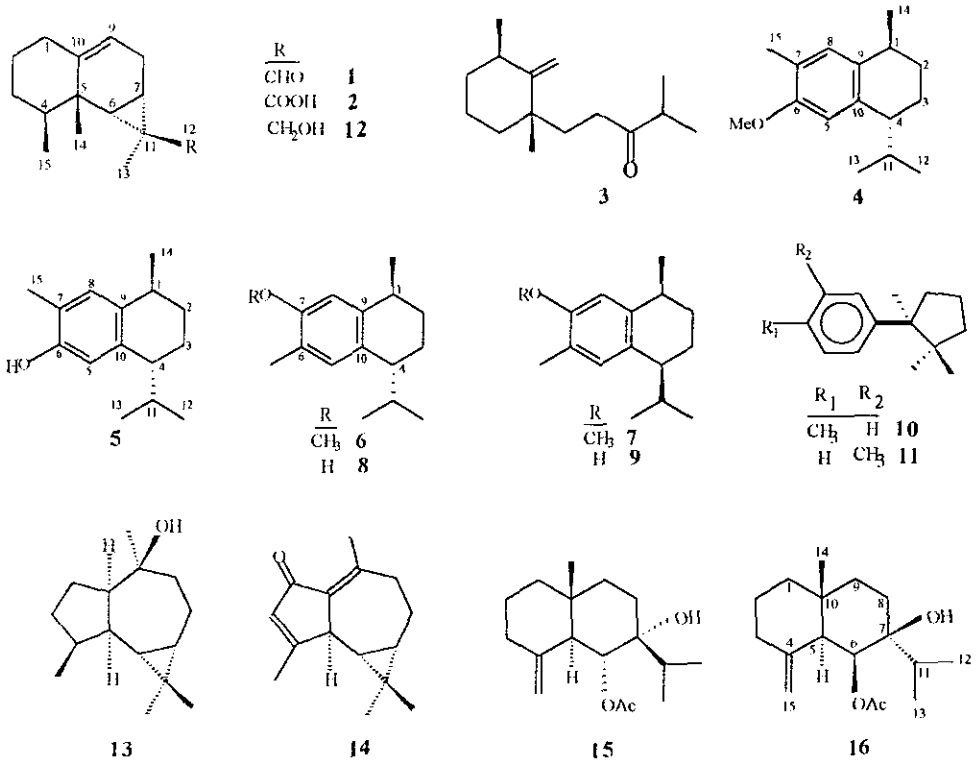
C	4	5	6*	8*	12	16
1	32.10	32.2	32.9	32.6	33.0	44.3
2	29.7	31.1	30.7	30.8	27.0	22.7
3	21.6	21.5	21.4	21.6	31.4	37.7
4	44.0	43.6	43.0	43.1	37.5	147.4
5	109.7	114.3	130.3	130.5	36.2	47.2
6	155.5	151.4	123.4	120.5	29.2	72.7
7	123.6	120.6	155.4	151.3	16.1	74.6
8	129.2	129.3	108.4	113.0	21.3	27.1
9	134.8	135.4	141.3	142.1	118.2	36.0
10	138.4	139.0	131.6	132.2	141.7	35.2
11	32.05	31.7	31.8	31.8	24.7	31.8
12	17.4	17.2	17.4	17.2	74.5	16.3
13	21.3	21.2	21.3	21.2	11.1	16.3
14	22.5	22.4	22.5	22.2	21.3	19.8
15	15.9	15.4	16.0	15.5	15.9	106.9
OMe	55.4		55.2			
OAc						21.4
						170.0

* Data from ref. [6].

pound **4** as shown in Fig. 1. Thus, the structure of 6-hydroxyisocalamenene was assigned to compound **5**.

The calamenene skeleton occurs commonly among liverwort constituents. Both (-)-(1*S*,4*S*)-*cis*-7-hydroxycalamenene (**9**)[8] and (+)-(1*S*,4*R*)-*trans*-7-hydroxycalamenene (**8**),[6] as well as (+)-(1*S*,4*R*)-*trans*-methoxycalamenene (**6**)[6] have been found in liverworts. Since the substituents on the phenyl ring of the calamenene skeleton should not affect much of the optical chirality,[9] the dextrorotatory property of compound **4** ($[\alpha]_D^{25}$ = +33) supported either a (1*S*,4*R*)-*trans* relationship of the two substituents or a (1*R*,4*R*)-*cis* relationship as compared with the optical data of the known calamenenes.[6,8,9] The assignment of a *trans*-configuration was then judged on the ^{1}H NMR shifts[6-8] as stated above. Therefore, the absolute configurations of the two new isocalamenenes, **4** and **5**, depicted here are tentatively assumed to be 1*S*,4*R*.

A methyl migration on the benzene ring has been observed on the cuparene skeleton (**10**) that has resulted in a series of herbertene-type (**11**) compounds in liverwort con-



stituents. Herbertene (**11**) shares the same fragmentation pattern as that of cuparene (**10**), and generally elutes from GC column (DBWAX) slightly earlier. These two compounds, **10** and **11**, cannot be easily distinguished on GC, if there is no authentic sample for comparison. Due to similar fragmentation patterns and retention times, the hydrocarbon isocalamenene may have been identified as calamenene by GC-MS. We suspect that many isocalamenene-type compounds identified by GC-MS may also have been misinterpreted as calamenene derivatives.

From the same Fu Shan species of *B. tridens*, another minor component with a molecular ion at *m/z* 220 ($C_{15}H_{24}O$) was obtained. Since its mass spectrum showed the same base peak (*m/z* 105) as that of aristol-9-en-12β-al (**1**),[3] the major compound of the plant, structure **12** was suspected to be the corresponding alcohol. The ^{1}H- and ^{13}C-NMR spectra of **12** exhibited a sharp singlet at δ_H 3.32 (2H) and δ_C 74.5 for a primary alcohol, and a pair of protons with large coupling constant (J = 18.9 Hz) at δ_H 2.33 and 2.05 attributable to H-8β and H-8α, which confirmed the speculation. The detailed assignments of all protons and carbons (Tables 1 & 2) for the new compound aristol-9-en-12β-ol (**12**) were derived from ^{13}C-DEPT, HMBC, HMQC, ^{1}H-^{1}H COSY and NOESY data. Finally, alcohol **12** was confirmed as aristol-9-en-12β-ol by $NaBH_4$ reduction of **1** to **12**.

The examination of another specimen of *B. tridens* collected from Taiping Shan by GC-MS displayed a quite different profile from that shown by the same species from Fu Shan as described above. The former contained three major sesquiterpenoids, two of them were identified to be ledol (**13**) and tridensenone (**14**).[10,1] The ^{1}H- and ^{13}C-NMR of the third component indicated a secondary acetoxy group (δ_H 2.01, s, 5.22, s, δ_C 21.4, 170.0, 72.7), an exomethylene group (δ_H 4.51, 4.71, all br s, δ_C 106.9, 147.4), and a tertiary alcohol (δ_C 74.6), in addition to three methyl groups (δ_H 0.86, d, J = 6.8 Hz; 0.93, d, J = 6.8 Hz; 0.91, s, δ_C 16.3, 16.3, 19.8). The NMR features and mass fragments (base peak *m/z* 177) of the third compound were reminiscent of the data obtained for eudesm-3-en-6α-acetoxy-7α-ol (**15**), isolated from *Lepidozia vitrea* recently.[11] The eudesmane skeleton and the substitution pattern of this component were derived from the HSQC and HMBC experiments. The relative configuration was established from a NOESY study as shown in Fig. 2. Thus this component should be eudesm-4(15)-en-

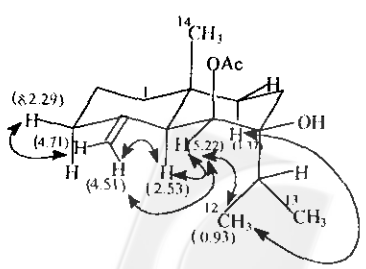


Fig. 2. Key NOE's observed for compound **16**.

6β-acetoxy-7β-ol (**16**).

As mentioned in the beginning, the species of *B. tridens* biosynthesizes a variety of oxygenated sesquiterpenes depending on the locality collected. Several chemotypes may be classified for *B. tridens* according to the major sesquiterpenoids produced.[1,2] Obviously, the present two Taiwanese specimens, one from Fu Shan, the other from Taiping Shan, belong to two different chemotypes as shown in Table 3.

Table 3. Comparison of Skeletal-types of Major Components of *B. tridens* from Fu Shan and Taiping Shan

Sesquiterpene skeleton	Location	
	Fu Shan	Taiping Shan
aristolane (e.g. **1**)	+++	
aromadendrane (e.g. **14**)		++
eudesmane (e.g. **16**)		++

EXPERIMENTAL SECTION

General

NMR spectra were measured in $CDCl_3$ on Bruker AM-300WB and DMX-500. All GC-MS(EI) spectra were taken at 70 eV. A DBWAX, 30 m × 0.25 mm (i.d.), fused silica capillary column was used for both GC and GC-MS. The column temperature was programmed from 50° to 220° C at 5°/min. IR spectra were measured in $CHCl_3$ or CH_2Cl_2 on NaCl pellets after the solvent was evaporated. UV was recorded in MeOH and optical rotation measured in $CHCl_3$.

Plant Materials

Bazzania tridens (Reinw. et al. Trev.) was collected at Fu Shan (800 m, alt.) in 1995 and Taiping Shan (2000 m) in 1994, respectively, both in Ilan Hsien, Taiwan. Specimens were identified by Dr. Kohsaku Yamada (Ise-shi, Japan) and deposited at the Dept. of Chemistry, Tamkang Univ.

Extraction and Isolation

The material (250 g) collected at Fu Shan was frozen-crushed and extracted with *n*-hexane. The crude oil (8.2 g, 3.3% of dry wt.) was chromatographed on silica gel and eluted with *n*-hexane, benzene, and EtOAc, respectively. The first benzene fraction afforded compound **4** (5 mg) after purification on prep. TLC. The second benzene fraction furnished compounds **1** (870 mg),[3] **5** (3 mg), **3** (8 mg),[3] and friedelin (3 mg)[4,5] upon successive chromatography on Sephadex LH-20 ($CHCl_3$/MeOH = 1:1) and $AgNO_3$-impregnated silica gel, as well as prep. TLC. The EtOAc-eluted fractions afforded spathulenol (100 mg), compounds **12** (3.4 mg) and **2** (58 mg)[3] after repeated chromatography on silica gel (*n*-hexane/EtOAc gradient).

A small amount of the crude oil was examined by GC-MS, and the following sesquiterpenes were identified in addition to the components isolated: aristol-9-ene, γ-cadinene, β-barbatene, β-chamigrene, δ-cuprenene, cyclocolorenone, and drimenol.

Frozen-crushed plant material (60 g) from Taiping Shan was extracted with EtOAc to furnish 1.2 g (2% of dry wt) of a greenish crude oil. Chromatography of the oil on silica gel (*n*-hexane/EtOAc gradient) afforded ledol (**13**) (13 mg)[10] and **16** (18 mg) in the 15% EtOAc/*n*-hexane eluate. In the GC-MS examination, β-barbatene, cuparene, δ-cuprenene, tridensone (**3**),[3] spathulenol, fusicoccadiene,[12] and tridensenone (**14**)[13] could be identified.

1,4-*trans*-6-Methoxyisocalamenene (**4**): $[\alpha]_D^{25}$ +33 (c 0.15, $CHCl_3$); ν_{max}/cm^{-1}: 3015, 1634, and 1213; λ_{max}/nm: 239.5, 280; GC Rt = 27.63 min.; TLC Rf = 0.40 (benzene/*n*-hexane = 1/9); GC-EIMS *m/z* (ref. int.): 232 ([M$^+$], 11), 190 (14), 189 (100).

1,4-*trans*-6-Hydroxyisocalamenene (**5**): GC Rt = 38.98 min.; TLC Rf = 0.39 (EtOAc/*n*-hexane = 1/9); GC-EIMS *m/z* (rel. int.): 218 (M$^+$], 10), 176 (13), 175 (100).

(+)-Aristol-9-en-12β-ol (**12**): $[\alpha]_D^{25}$ +31.5 (c 0.13, $CHCl_3$); ν_{max}/cm^{-1}: 3530; GC Rt = 31.60 min.; TLC Rf = 0.36 (EtOAc/*n*-hexane = 1/4); GC-EIMS *m/z* (rel. int.): 220 ([M$^+$], 11), 189 (59), 161 (29), 145 (25), 13 (31), 119 (29), 105 (100), 91 (65), 77 (22). To 20 mg of (+)-aristol-9-en-12β-al (**1**) in CH_3OH, 2 mg of $NaBH_4$ was added. The mixture was stirred at RT for 1.5 h. After work-up, compound **12** was obtained in 90% yield as checked by GC-MS.

Eudesm-4(15)-en-6β-acetoxy-7β-ol (**16**): $[\alpha]_D^{25}$ +22.2 (c 0.31, $CHCl_3$); ν_{max}/cm^{-1}: 3470, 1740, 1640, 1230; GC Rt = 37.19 min.; GC-EIMS *m/z* (rel. int.): 262 ([M$^+$] 18, 3), 177 (100), 149 (11), 121 (19), 107 (10), 93 (14).

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Key Words

Bazzania tridens; *Bazzania japonica*; Jungermanniaceae; Liverwort; 1,4-*trans*-6-Hydroxyisocalamenene; 1,4-*trans*-6-Methoxyisocalamenene; (+)-Aristol-9-en-12β-ol; Eudesm-4(15)-en-6β-acetoxy-7β-ol.

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