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Sesquiterpene constituents from the liverwort *Bazzania japonica*

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Abstract

The hydrodistillation products of the liverwort *Bazzania japonica* were separated by preparative gas chromatography (GC) and investigated by spectroscopic methods. Seven unknown compounds were isolated and identified by GC–MS and NMR. Four of them, the norsesquiterpene hydrocarbons 4-*epi*-11-*nor*-aristola-1(10),11-diene (**1**), 4-*epi*-11-*nor*-aristola-1,9,11-triene (**2**), 4-*epi*-11-*nor*-aristola-9,11-diene (**3**), and one oxygenated sesquiterpene, (−)-aristol-1(10)-en-12-ol (**5**) are new natural compounds, and one, (+)-himachala-2,4-diene (**7**), has for the first time been isolated from liverworts. The absolute configurations of **5** and **7** were derived by chemical correlation reactions and/or enantioselective GC using cyclodextrin phases. **1**, **2** and **3** have identical absolute configuration.
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Keywords: *Bazzania japonica*; Liverwort; Norsesquiterpene hydrocarbons; Oxygenated sesquiterpenes

1. Introduction

Bazzania japonica is a stem-leafy liverwort and rich in sesquiterpenes. The plant was collected at Aioi-cho, Nakagun, near Tokushima, Japan. In earlier work on this liverwort the isolation of albicanol, albicanyl-3,4-dihydroxycinnamate, albicanyl-2,4-dihydroxycinnamate, bicyclogermacrene, α-barbatene, β-barbatene, bazzanene, calamenene, cuparene, 2-hydroxycuparene, (−)-isobicyclogermacrenal, albicanyl caffeate, albicanyl acetate, cyclomyltaylane-3-ol, cyclomyltaylyl-3-caffeate, and friedelin from the ether and methanol extracts was reported (Toyota et al., 1981; Asakawa et al., 1991). In this article we describe the isolation and characterization of seven compounds, including three new *nor*-aristolane-type sesquiterpene hydrocarbons and a new oxygenated sesquiterpene.

2. Results and discussion

The hydrodistillation product of *Bazzania japonica* was examined by GC and GC–MS which revealed the presence of cyclomyltaylane (0.9%) (Wu and Chang, 1992), anastreptene (0.3%), *E*-β-caryophyllene (8.0%), calarene (4.4%), β-barbatene (7.2%), myltayl-8(12)-ene (0.9%) (Adio et al., 2002), β-acoradiene (0.5%), β-chamigrene (0.4%), α-muurolene (1.2%), β-himachalene (0.4%), *trans*-calamenene (0.4%), α-calacorene (0.7%), δ-cuprenene (1.7%), 4-dehydroviridiflorol (0.4%), viridiflorol (4.3%), rosifoliol (0.4%), cubenol (0.6%), cadalene (0.8%), isophyllocladene (2.3%), and traces of α-pinene, γ-terpinene, α-chamigrene, maaliol, caryolan-1-ol, 1-*epi*-cubenol and manooloxide. These compounds were identified by comparison of their mass spectra and retention indices with published data and a spectral library established under identical experimental conditions (Joulain and König, 1998; Hochmuth et al., 2002). The GC–MS also revealed the presence of some unknown compounds: three norsesquiterpene hydrocarbons as well as other sesquiterpene hydrocarbons

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and oxygenated sesquiterpenes which were isolated by preparative GC by using a thick-film capillary column with improved separation capability as compared with previously employed packed GC columns and investigated by 1D and 2D NMR.

2.1. 4-epi-11-nor-Aristola-1(10),11-diene (1)

The mass spectrum of **1** exhibited a molecular ion peak at *m*/*z* 188 and the elemental composition of $C_{14}H_{20}$. An 4-*epi*-aristolane skeleton was derived from its MS, 1D and 2D NMR data. The ^{1}H NMR spectrum showed signals of one doublet and one singlet for methyl groups at δ 0.99 (3H, *d*, *J* = 6.6 Hz) and 1.10 (3 H, *s*), respectively. The olefinic carbon signals at δ 141.5 (*q*) and 102.0 (*s*) suggested presence of exomethylene double bond, which was confirmed by a signal in the ^{1}H NMR spectrum at δ 5.44 (2H, *br.d*). The olefinic carbon signals at δ 122.2 (*t*) and 142.0 (*q*) showed the presence of an olefinic double bond in this structure, indicated by a vinylic proton at δ 5.35 (1H, *br.s*). Additional information from ^{13}C NMR, (DEPT) as well as from ^{1}H–^{1}H COSY, HMQC and HMBC (Tables 1 and 2) led to structure **1** for this compound. Its relative configuration was derived from the NOESY spectrum (Table 2) and chemical correlations with compounds **2** and **3** with the same skeleton by catalytic hydrogenation and analysis by capillary GC using cyclodextrin derived chiral stationary phases (Fig. 1).

2.2. 4-epi-11-nor-Aristola-1,9,11-triene (2)

This new compound was also isolated by preparative GC. The mass spectrum exhibited a molecular ion peak at *m*/*z* 186 and the elemental composition $C_{14}H_{18}$ with six double bond equivalents. An 4-*epi*-aristolane skeleton was deduced from its MS and NMR data. Accordingly, this compound had three double bonds. The ^{1}H NMR spectrum of **2** showed two methyl groups at δ 0.94 (3H, *d*, 6.6 Hz) and 1.01 (3H, *s*). Three protons belonging to two conjugated *endo*cyclic double bonds gave signals at δ 5.94 (1H, *d*, 9.8 Hz), 5.52 (1H, *dd*, 9.8 and 4.1 Hz) and 5.14 (1H, *t*, 7.2 Hz). Two additional olefinic protons of an *exo*cyclic double bond were indicated by a signal at δ 5.47 (2H, *d*, 7.25 Hz). The ^{1}H–^{1}H COSY, HMQC and HMBC spectra (Tables 1 and 2) confirmed the structure of compound **2**. The relative configuration was determined from the NOESY spectrum (Table 2). Chemical correlation by comparing the hydrogenated products of **2** by enantioselective GC with compounds **1** and **3** showed that all have the same skeleton (Fig. 1).

2.3. 4-epi-11-nor-Aristola-9,11-diene (3)

The mass spectrum of compound **3** exhibited a molecular ion peak at *m*/*z* 188 and the elemental composition $C_{14}H_{20}$. The ^{1}H NMR spectrum revealed the presence of two methyl groups at δ 0.95 (3H, *d*, 6.6 Hz) and 1.06 (3H, *s*). One tri-substituted *endo*cyclic and one *exo*cyclic olefinic double bond were indicated by signals at δ 5.04 (1H, *m*) and 5.50 (2H, *br.d*, 5.4 Hz). The structure was derived from its MS data, ^{1}H NMR, H–H COSY, HMBC and NOESY spectra and chemical correlation with **1** and **2** (Fig. 1).

To confirm the structures of compounds **1**–**3**, they were submitted to catalytic hydrogenation. The products were compared by enantioselective GC on a modified cyclodextrin stationary phase. The hydrogenated products of **1**–**3** compounds had at least two major peaks in common (Fig. 1).

Table 1
^{1}H, ^{13}C NMR, and ^{1}H–^{1}H COSY data of compounds **1** and **2** (in C_6D_6)

Atom no.	**1**			**2**		
	^{13}C (ppm)	^{1}H (ppm)	^{1}H–^{1}H COSY	^{13}C (ppm)	^{1}H (ppm)	^{1}H–^{1}H COSY
1	122.2 (*t*)	5.35 (*br.s*)	H-2, H-9	130.3 (*t*)	5.94 (*d*, 9.8 Hz)	
2	25.7 (*s*)	1.99–1.83 (*m*)	H-3	125.4 (*t*)	5.52 (*d*, 9.8 Hz, 4.1 Hz)	
3	27.0 (*s*)	1.42–1.32 (*m*)	H-4, H-2, H-14	32.2 (*s*)	1.87 (*dd*, 5.1 Hz, 3.2 Hz)	H-2, H-1
4	38.3 (*t*)	1.87–1.81 (*m*)	H-14, H-3	36.3 (*t*)	1.88–1.86 (*m*)	H-14
5	37.4 (*q*)			36.4 (*q*)		
6	26.2 (*t*)	1.55–1.51 (*m*)	H-12a, H-12b	23.9 (*t*)	1.60–1.57 (*m*)	H-12
7	14.2 (*t*)	1.55–1.51 (*m*)	H-8, H-9a, H-9b, H-12a, H-12b	14.3 (*t*)	1.60–1.57 (*m*)	H-8, H-12
8	24.9 (*s*)	1.99–1.84 (*m*)	H-7, H-9a, H-9b	24.1 (*s*)	2.32 (*brd*, 5.7 Hz)	H-7, H-9,
9	28.7 (*s*)	9a: 1.78–1.72 (*m*) 9b: 2.20–2.13 (*m*)	H-7, H-8	120.5 (*t*)	5.14 (*t*, 7.2 Hz)	H-8
10	142.0 (*q*)		H-9a, H-7, H-8	137.6 (*q*)		
11	141.5 (*q*)			140.3 (*q*)		
12	102.0 (*s*)	5.44(*brd*)	H-6, H-7	103.5 (*s*)	5.47 (*d*, 7.2 Hz)	H-6, H-7
13	21.7 (*p*)	1.10(*s*)	H-14	19.0 (*p*)	1.01 (*s*)	
14	16.3 (*p*)	0.99(*d*, 6.6 Hz)	H-4, H-2, H-3, H-13	15.0 (*p*)	0.94 (*d*, 6.6 Hz)	H-4

Table 2
HMBC and NOESY data for compounds **1** and **2** (in C_6D_6)

Atom no.	**1**		**2**	
	HMBC	NOESY	HMBC	NOESY
1	H-9a, H-8, H-3, H-4	H-4	H-14, H-9, H-3	H-13, H-9
2	H-3		H-14, H-4	H-13
3	H-14		H-1, H-14	H-12, H-13, H-14
4	H-13, H-14, H-3, H-2	H-12a	H-1, H-13, H-2	H-12, H-13
5	H-13, H-14, H-3, H-2, H-8		H-14, H-9, H-3	
6	H-13, H-12a, H-12b		H-12, H-13, H-8	H-13, H-14
7	H-12a, H-12b, H-9a,		H-12, H-9	
8	H-9a			H-13, H-6
9	H-6, H-7		H-1	H-1
10	H-13, H-9a, H-2, H-8, H-4		H-8	
11	H-6, H-7		H-12, H-6, H-7, H-8, H-4	
12		H-4		
13		H-9b, H-3, H-7		H-3, H-4, H-6, H-7
14	H-3, H-2	H-12a H-6, H-7		H-3, H-6, H-7

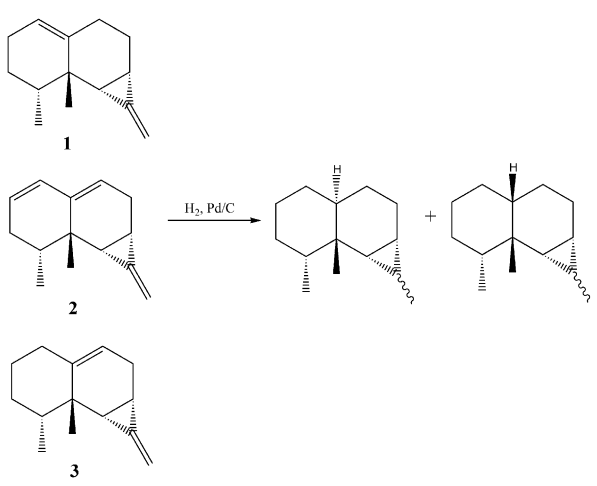
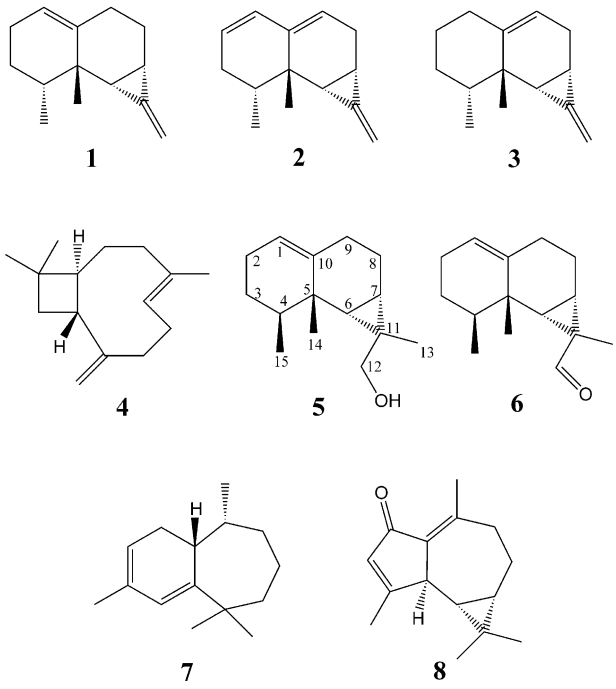


Fig. 1. Chemical correlation of compounds **1**, **2**, and **3**.



2.4. (+)-β-Caryophyllene (**4**)

This compound was isolated from the hydrocarbon fraction of the essential oil of *B. japonica* by preparative GC. Polarimetric measurements (benzene) showed positive optical rotation. Its identity was established by comparison of its GC–MS characteristics with (−)-β-caryophyllene (Joulain and König, 1998). Further identification was carried out by comparison of the NMR spectra with literature data (Fricke et al., 1995; Saritas et al., 1998; Weyerstahl et al., 1998). Comparison of **4** with a mixture of (+)- and (−)-β-caryophyllene using enantioselective gas chromatography unambiguously indicated the exclusive presence of the unusual (+)-enantiomer. The occurrence of (+)-β-caryophyllene in the liverworts *Pellia endiviifolia* has been reported before by Fricke et al. (1995) and in *Preissia quadrata* by König et al. (1996).

2.5. (−)-Aristol-1(10)-en-12-ol (**5**)

This new compound was isolated from the oxygenated fraction of the essential oil of *B. japonica* by preparative GC. Its mass spectrum exhibited the molecular ion peak at *m/z* 220, corresponding to elemental composition $C_{15}H_{24}O$. An aristolane skeleton was deduced from its MS and NMR data. The ^1H and ^{13}C NMR spectra of **5** indicated the presence of an *endo*-cyclic double bond (*δ* 120.9, 143.4) and three methyl groups at *δ* 1.13 (*s*), 1.10 (*s*) and 0.97 (*d*, 6.9 Hz, Table 3). The relative configuration was derived from the NOESY spectrum (Table 4). The absolute configuration

Table 3
^{1}H, ^{13}C NMR and H–H COSY data of compounds **5** and **6** (in $CDCl_3$)

Atom no.	**5**			**6**		
	^{13}C (ppm)	^{1}H (ppm)	^{1}H–^{1}H COSY	^{13}C (ppm)	^{1}H (ppm)	^{1}H–^{1}H COSY
1	120.9 (*t*)	5.28 (*br.s*)	H-2, H-3, H-9a, H-9b	122.4 (*t*)	5.33 (*br.s*)	H-9a, H-9b, H-2, H-3
2	25.6 (*s*)	2.02–1.89 (*m*)	H-3, H-1	25.9 (*s*)	2.00–1.95 (*m*)	H-1, H-3, H-9b
3	27.2 (*s*)	1.45–1.38 (*m*)	H-1, H-2, H-4, H-15	27.3 (*s*)	1.46–1.40 (*m*)	H-2, H-6, H-15, H-1
4	36.6 (*t*)	1.76–1.70 (*m*)	H-15, H-3, H-2	36.2 (*t*)	1. 67–1.58 (*m*)	H-15, H-3
5	36.3 (*q*)			36.9 (*q*)		
6	30.5 (*t*)	0.69 (*d*, 9.5 Hz)	H-12, H-7, H-14, H-13	33.2 (*t*)	1.48 (*d*, 9.77 Hz)	H-12, H-7, H-4
7	16.3 (*t*)	0.90 (*td*, 9.5, 3.2 Hz)	H-6, H-8a, H-8b, H-13	20.9 (*t*)	1.68–1.60 (*m*)	H-8a, H-8b, H-6
8	20.2 (*s*)	8a: 1.43–1.39 (*m*) 8b: 2.04–1.98 (*m*)	H-6, H-7, H-9a, H-9b	19.6 (*s*)	8a: 1.48–1.39 (*d*, 9.77 Hz) 8b: 2.15–2.07 (*m*)	H-8b, H-9a, H-9b H-8a, H-9a, 9b, H-7
9	29.6 (*s*)	9a: 1.80–1.75 (*m*) 9b: 2.28–2.23 (*m*)	H-8a, H-8b, H-1	29.3 (*s*)	9a: 1.87 (*dd*, 13.6 Hz, 6.3 Hz) 9b: 2.38–2.30 (*m*)	H-9b, H-8a, H-8b H-1, H-8a, H-8b, H-9a, H-2
10	143.4 (*q*)			142.1 (*q*)		
11	25.56 (*q*)			35.8 (*q*)		
12	11.8 (*p*)	1.13 (*s*)	H-13, H-15, H-6, H-8a, H-9	203.8 (*p*)	8.66 (*s*)	H-6, H-7, H-13, H-15, H-3
13	74.4 (*s*)	3.24 (*dd*, 20.2, 11.0 Hz)	H-6, H-7, H-12, H-14	8.6 (*t*)	1.25 (*s*)	H-14, H-6
14	22.8 (*p*)	1.10 (*s*)	H-13, H-15, H-6, H-3, H-4	23.0 (*p*)	1.17 (*s*)	H-15, H-13, H-12, H-4, H-6
15	16.0 (*p*)	0.97 (*d*, 6.9 Hz)	H-4, H-2, H-14, H-12	16.1 (*p*)	0.90 (*d*, 6.6 Hz)	H-4, H-3, H-14, H-2,

Table 4
HMBC and NOESY data for compounds **5** and **6** (in $CDCl_3$)

Atom no.	**5**		**6**	
	HMBC	NOESY	HMBC	NOESY
1	H-3, H-6, H-9a, H-9b, H-8a, H-8b	H-15, H-6, H-12, H-14	H-3, H-9a, H-2, H-4	H-12
2	H-6, H-4		H-3, H-4	H-8b, H-6, H-7, H-12, H-14
3	H-15, H-6, H-7		H-15, H-4, H-2	H-8b
4	H-14, H-2		H-13, H-14, H-15, H-7, H-8a	
5	H-3, H-6, H-4, H-14, H-15		H-15, H-14, H-9a	
6	H-12, H-13, H-14, H-4		H-12, H-4, H-8a, H-8b, H-14	H-13
7	H-13, H-8a, H-9a, H-13		H-13, H-8a, H-8b, H-9b, H-12	H-13
8	H-6		H-9a, H-6, H-7	H-13 H-14
9	H-7, H-2, H-3, H-8a, H-8b	H-14	H-8a, H-8b, H-6, H-7, H-2,	H-12 H-15
10	H-14, H-6, H-2, H-3, H-8a, H-8b, H-9a, H-9b		H-14, H-8a, H-8b, H-9a, H-9b	
11	H-6, H-7		H-13, H-6, H-7, H-8a, H-8b, H-12	
12	H-13, H-6, H-7	H-1, H-4	H-13, H-6, H-7	H-13, H-6, H-7, H-1
13	H-12, H-6, H-7	H-15	H-12, H-6, H-7	H-14
14	H-6, H-4	H-9b, H-8a, H-9b	H-6, H-7, H-2	H-9b, H-3, H-13
15	H-2, H-3	H-13, H-3, H-6	H-3, H-4	H-6, H-13

of **5** was determined by comparison of the fully hydrogenated products of **5** and (+)- and (−)-aristolene (Fig. 2) by enantioselective GC on a modified cyclodextrin stationary phase. The main peak of the fully hydrogenated product of (+)-aristolene proved identical with the fully hydrogenated product of **5** after co-injection on a modified cyclodextrin stationary phase.

2.6. (−)-Aristol-1(10)-en-12-al (6)

This compound was also isolated from the oxygenated fraction of the essential oil of *B. japonica* by preparative GC. The mass spectrum of **6** exhibited a molecular ion signal at *m*/*z* 218 and elemental composition $C_{15}H_{22}O$. The structure was deduced from its MS and NMR data (see Experimental). The ^{13}C NMR signals of compound **6** are similar to those of **5** except C-6, 7, 11, 12, and 13. The absolute configuration of **6** was derived by comparison of the fully hydrogenated products of **6** and (+)-aristolene (Fig. 2) by enantioselective GC on a modified cyclodextrin stationary phase (Fig. 3). This compound was isolated from a higher plant before (Rodriguez et al., 1995), although the reported NMR and MS data are slightly different from our own data.

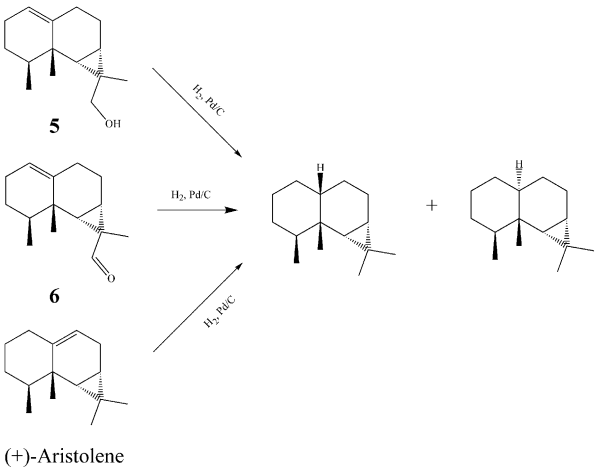


Fig. 2. Chemical correlation of compounds **5** and **6** with (+)-aristolene.

2.7. Other sesquiterpenes

From the essential oil of *B. japonica*, (+)-himachala-2,4-diene (**7**) and tridensenone (**8**) were also isolated. Identification was achieved by comparing their mass and NMR spectra with literature data. **7** has never before been found in a liverwort. It was first characterized as an acid rearrangement product of α- and β-himachalene (Mehta and Singh, 1977). Later it was described as a constituent of *Abies alba* (Khan et al., 1988; Khan and Pentegove, 1988). Tridensenone is a common liverwort constituent and was isolated from the ether extract of *Bazzania tridens* (Toyota et al., 1981). It was also found in the soft coral *Parerythropodium fulvum* (Wessels et al., 2001).

Norsesquiterpenes have been found quite frequently in nature. Although details of their biosynthesis are not known, it can be assumed that they are formed by enzymatic degradation of a parent sesquiterpene which usually has the same stereochemistry as in the case of cyprotene and cyperene in *Cyperus alopecuroides* (Mekem Sonwa et al., 1997) or albene and petasitene in *Petasites hybridus* (Saritas et al., 2002). In the case of the norsesquitepenes **1**–**3** there is definitely no stereochemical relationship to the aristolane type constituents present in *B. japonica*.

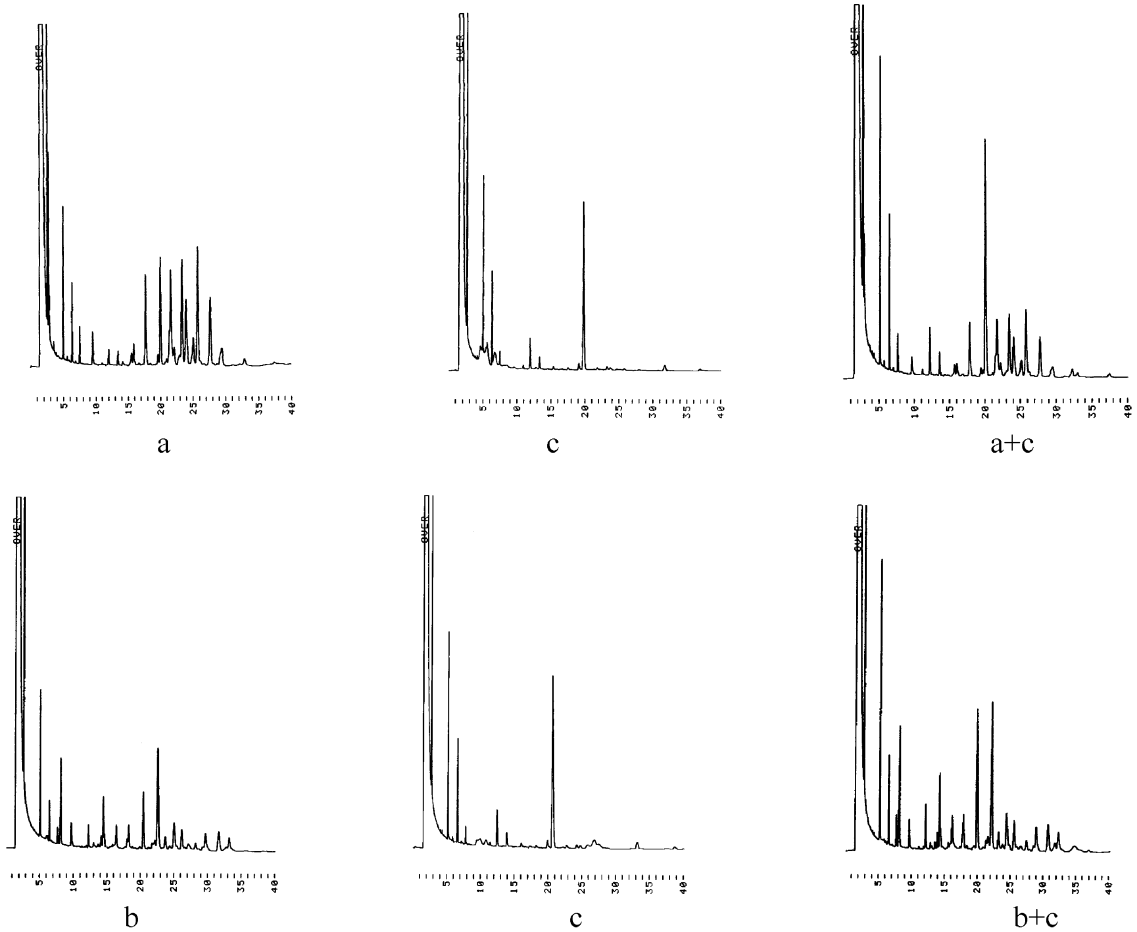


Fig. 3. GC comparison of total hydrogenation products of compounds **5** (a), **6** (b), and (+)-aristolene (c) by co-injection (a+c and b+c) on a capillary column with 6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl-β-cyclodextrin (50% in OV 1701, w/w) at 110 °C.

3. Experimental

3.1. General experimental procedures

3.1.1. Gas chromatography

Orion Micromat 412 double column instrument with 25 m fused silica capillaries with polysiloxanes CPSil-5 and CPSil-19 (Chrompack); Carlo Erba Fractovap 2150 or 4160 gas chromatographs with 25 m fused silica capillaries with octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin, heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin or heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in OV 1701 (50%, w/w), split injection; split ratio approx. 1:30; FID; carrier gas 0.5 bar H_2; injector and detector temperatures were 200 and 250 °C, respectively.

3.1.2. Preparative GC

Modified Varian 1400 and 2800 instruments, equipped with stainless steel columns (1.85 m×4.3 mm) with 10% polydimethylsiloxane SE-30 on Chromosorb W-HP or with 2.5% octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin in OV-1701 (50%, w/w) on Chromosorb G-HP or with 6% heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in SE-52 (50%, w/w) on Chromosorb W-HP; FID; helium as carrier gas at a flow rate of 240 ml/min.; injector and detector temperatures were 200 and 250 °C, respectively (Hardt and König, 1994). Compounds **1**–**3** were isolated by preparative GC on a Gerstel PFC 1 system consisting of a HP 6890 gas chromatograph with autosampler and automatic fraction collector (−30 °C) by using a 30 m megabore capillary column (0.53 mm i.d., film thickness 5 μm) DB-1 at 140 °C with He (60 ml/min) as carrier gas by repetitive injections of a hexane solution of a low boiling fraction of the essential oil of *B. japonica*.

3.1.3. GC–MS

Electron impact (70 eV) GC–MS was conducted with a Hewlett–Packard HP 5890 gas chromatograph (25 m fused silica capillary with polydimethylsiloxane CPSil-5) coupled to a VG Analytical 70-250S mass spectrometer (ion source temp. 250 °C).

3.1.4. NMR-spectroscopy

NMR measurements were carried out with a Bruker WM 400 (^{1}H, 400 MHz; ^{13}C, 100.6 MHz) or a Bruker WM 500 (^{1}H, 500 MHz, ^{13}C, 125.8 MHz) instrument in C_6D_6 and/or $CDCl_3$ using TMS as internal standard.

3.1.5. Polarimetry

Measurements were performed with a polarimeter 341 (Perkin-Elmer) at 589 nm at 20 °C. Due to the very small amounts of isolated compounds only the sense of optical rotation is given to avoid inaccurracies.

3.1.6. Chemical transformations

Hydrogenation was performed by bubbling hydrogen gas through a stirred solution of ca. 1 mg of sample in 1 ml *n*-hexane and 0.5 mg Pd/C at room temp. for 30 min. The reaction mixture was filtered and the reaction products were analysed by GC–MS and GC on several capillary columns with cyclodextrin derivatives.

3.2. Plant material and essential oils

B. japonica was collected at Aioi-cho, Nakagun, a mountainous area near Tokushima, (Japan), in August 2001. The essential oil was prepared by hydrodistillation (2–3 h) of aqueous homogenates of fresh and green plants using *n*-hexane as collection solvent.

3.3. 4-epi-11-nor-Aristola-1(10),11-diene (**1**)

Colourless oil; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): $\delta = 0.99$ (3H, *d*, 6.6Hz), 1.10 (3H, *s*), 1.42–1.32 (2H, *m*), 1.55–1.51 (2H, *m*), 1.78–1.72 (1H, *m*), 1.87–1.81 (1H, *m*), 1.99–1.83 (4H, *m*), 2.20–2.12 (1H, *m*), 5.35 (1H, *br.s*), 5.44 (2H, *brd*); ^{13}C NMR (125.7 MHz, C_6D_6): $\delta = 14.2$ (C-7, *t*), 16.3 (C-14, *p*), 21.7 (C-13, *p*), 24.9 (C-8, *s*), 25.7 (C-2, *s*), 26.2 (C-6, *t*), 27.0 (C-3, *s*), 28.7 (C-9, *s*), 37.4 (C-5, *q*), 38.3 (C-4, *t*), 102.0 (C-12, *s*), 122.2 (C-1, *t*), 141.5 (C-11, *q*), 142.1 (C-10, *q*); MS (EI) *m*/*z* (rel. int.): 188 (M^+, 18), 173 (72), 159 (62), 145 (52), 131 (80), 117 (60), 105 (70), 91 (100), 77 (47), 65 (26), 53 (32), 41 (69).

3.4. 4-epi-11-nor-Aristola-1,9,11-triene (**2**)

Colourless oil; sense of optical rotation (benzene): (+) ; ^{1}H NMR (500 MHz, C_6D_6): $\delta = 0.94$ (3H, *d*, 6.62 Hz), 1.01 (3H, *s*), 1.60–1.57 (2H, *m*), 1.87 (3H, *dd*, 5.04 Hz, 3.15 Hz), 2.32 (2H, *brd*, 5.67 Hz), 5.14 (1H, *t*, 7.25 Hz), 5.47 (2H, *d*, 7.25 Hz), 5.52 (1H, *dd*, 9.77 Hz, 4.1 Hz), 5.94 (1H, *d*, 9.77 Hz); ^{13}C NMR (125.7 MHz, C_6D_6): $\delta = 130.3$ (C-1, *t*), 125.4 (C-2, *t*), 14.3 (C-7, *t*), 15.0 (C-14, *p*), 19.0 (C-13, *p*), 23.9 (C-6, *t*) 24.1 (C-8, *s*), 32.2 (C-3, *s*), 36.3 (C-4, *t*), 36.4 (C-5, *q*), 103.5 (C-12, *s*), 120.5 (C-9, *t*), 137.6 (C-10, *q*), 140.3 (C-11, *q*); MS(EI) *m*/*z* (rel. int.): 186 (M^+, 39), 171 (64), 157 (53), 143 (62), 129 (100), 115 (58), 105 (35), 91 (69), 77 (51), 65 (25), 51 (25), 39 (45).

3.5. 4-epi-11-nor-Aristola-9,11-diene (**3**)

Colourless oil; sense of optical rotation (benzene): (+) ; ^{1}H NMR (500 MHz, C_6D_6): δ 0.95 (3H, *d*, 6.62 Hz), 1.06 (3H, *s*), 1.37–1.20 (3H, *m*), 1.43–1.38 (1H, *m*), 1.64–1.52 (2H, *m*), 1.71–1.64 (1H, *m*), 1.99–1.93 (1H, *m*), 2.17–2.08 (1H, *m*), 2.24–2.20 (2H, *m*), 5.04 (1H, *br.s*), 5.50 (2H, *brd*, 5.4 Hz); ^{13}C NMR (127.5 MHz, C_6D_6, incomplete, from HMBC): δ 13.7, 16.3, 20.1, 25.1, 31.7, 37.7, 40.7, 116.4, 140.6; MS (EI), *m*/*z* (rel. int.): 188

(M^+, 19), 173 (45), 159 (15), 145 (56), 131 (81), 117 (100), 105 (63), 91 (96), 77 (42), 65 (23), 55 (32), 41 (57).

3.6. (−)-Aristol-1(10)-en-12-ol (5)

Colourless oil; sense of optical rotation ($CDCl_3$): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.69 (1H, *d*, 9.46 Hz), 0.90 (1H, *td*, 9.46, 3.15 Hz), 0.97 (3H, *d*, 6.94 Hz), 1.10 (3H, *s*), 1.13 (3H, *s*), 1.38–1.45 (4H, *m*), 1.70–1.81 (2H, *m*), 1.89–2.02 (3H, *m*), 2.23–2.28 (1H, *m*), 3.24 (2H, *dd*, 20.18, 11.03 Hz), 5.28 (1H, *br.s*); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 120.9 (C-1, *t*), 25.6 (C-2, *s*), 27.2 (C-3, *s*), 36.6 (C-4, *t*), 36.3 (C-5, *q*), 30.5 (C-6, *t*), 16.3 (C-7, *t*), 20.2 (C-8, *s*), 29.6 (C-9, *s*), 143.4 (C-10, *q*), 25.6 (C-11, *q*), 11.8 (C-12, *p*), 74.4 (C-13, *s*), 22.8 (C-14, *p*), 16.1 (C-15, *p*); MS (EI), *m*/*z* (rel. int.): 220 (M^+, 6), 202 (22), 187 (20), 173 (8), 161 (100), 147 (53), 133 (22), 119 (43), 105 (72), 91 (57), 79 (134), 67 (20), 55 (34), 41 (58).

3.7. (−)-Aristol-1(10)-en-12-al (6)

Colourless oil; sense of optical rotation ($CDCl_3$): (−); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90 (3H, *d*, 6.62 Hz), 1.17 (3H, *s*), 1.25 (3H, *s*), 1.46–1.40 (2H, *m*), 1.48 (2H, *d*, 9.8 Hz), 1.68–1.58 (2H, *m*), 1.87 (1H, *dd*, 13.6 Hz, 6.3 Hz), 2.00–1.95 (2H, *m*), 2.15–2.07 (1H, *m*), 2.38–2.30 (1H, *m*), 5.33 (1H, *br.s*), 8.66 (1H, *s*); ^{13}C NMR (127.5 MHz, $CDCl_3$): δ 122.4 (C-1, *t*), 25.9 (C-2, *s*), 27.3 (C-3, *s*), 36.2 (C-4, *t*), 36.9 (C-5, *q*), 33.2 (C-6, *t*), 20.9 (C-7, *t*), 19.6 (C-8, *s*), 29.3 (C-9, *s*), 142.1 (C-10, *q*), 35.8 (C-11, *q*), 8.6 (C-12, *p*), 203.8 (C-13, *t*), 23.0 (C-14, *p*), 16.1 (C-15, *p*); MS: 218 [M^+] (58), 203 (70), 189 (23), 176 (30), 161 (75), 145 (35), 133 (35), 119 (78), 105 (86), 91 (89), 77 (55), 67 (29), 55 (51), 41 (100).

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