

Composition of the essential oil of the liverwort *Radula perrottetii* of Japanese origin

Hailemichael Tesso ^{a,*}, Wilfried A. König ^{a,✱}, Yoshinori Asakawa ^b

^a Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

^b Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan

Received 12 November 2004; received in revised form 21 February 2005

Available online 8 April 2005

Abstract

Analysis of the essential oil of the liverwort *Radula perrottetii* afforded two novel viscidane diterpenes, viscida-3,9,14-triene (**1**), viscida-3,11(18),14-triene (**2**), four bisabolane sesquiterpenes, bisabola-2,6,11-triene (**3**), bisabola-1,3,5,7(14),11-pentaene (**4**), bisabola-1,3,5,7,11-pentaene (**5**), 6,7-epoxybisabola-2,11-diene (**6**), and 1-methoxy-4-(2-methylpropenyl)benzene (**7**) as new natural products. In addition, the known compounds bisabola-1,3,5,7(14),10-pentaene (**8**), *ar*-tenuifolene (**9**), α -helmiscapene (**10**), and β -helmiscapene (**11**) were also isolated. Isolation was carried out by preparative gas chromatography, and the structures were established by extensive NMR analysis. This is the first finding of viscidane diterpenes in liverworts. Compounds **8**, **9** and the rarely encountered eudesmane sesquiterpene hydrocarbons **10** and **11** are reported for the first time from *R. perrottetii*.
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Keywords: *Radula perrottetii*; Liverwort; Essential oil; Viscidane diterpenes; Bisabolane and eudesmane sesquiterpenes; *ar*-Tenuifolene

1. Introduction

The liverwort *Radula perrottetii* Gott. belongs to the Jungermanniales (Hepaticae). Earlier, several prenylated bibenzyls and derivatives thereof have been reported from *R. perrottetii* (Asakawa et al., 1982, 1991; Toyota et al., 1985, 1994). Further investigation of the chemical constituents of *R. perrottetii* resulted in the isolation of two diterpene hydrocarbons with viscidane structure (**1**, **2**), four bisabolane sesquiterpenes (**3–6**) as well as 1-methoxy-4-(2-methylpropenyl)benzene (**7**) as new compounds. In addition, the known compounds bisabola-1,3,5,7(14),10-pentaene (**8**), *ar*-tenuifolene (**9**) as well as two eudesmane sesquiterpenes (**10**, **11**) were isolated (Fig. 1).

2. Results and discussion

The essential oil of *R. perrottetii* was analysed by gas chromatography (GC) and coupled gas chromatography-mass spectrometry (GC/MS). Mass spectra and retention indices on a non-polar stationary phase (CPSil-5) of the components of the essential oil were compared with a library of mass spectra of authentic compounds established under identical experimental conditions (Joulain and König, 1998; Hochmuth et al., 2003): Δ -3-carene, α -terpinene, *p*-cymene, (*Z*)- β -ocimene, γ -terpinene, terpinolene, β -elemene, 7-*epi*- α -cedrene, α -gurjunene, α -cedrene, aristolene, γ -maaliene, eremophila-1(10),6-diene, calarene, valereana-4,7(11)-diene, selina-3,7-diene, β -acoradiene, *allo*-aromadendrene, 4,5-*di-epi*-aristolochene, selina-4,7-diene, β -chamigrene, eremophila-1(10),7-diene, eremophilene, hinesene, cuparene, α -chamigrene, (*E*)- γ -bisabolene, γ -cuprenene and bicyclohumulenone were identified. Eleven components that could not be

* Corresponding author. Tel.: +49 40 42838 2831; fax: +49 40 42838 2893.

E-mail address: michaeltesso@yahoo.com (H. Tesso).

✱ W.A. König passed away on 19th November 2004.

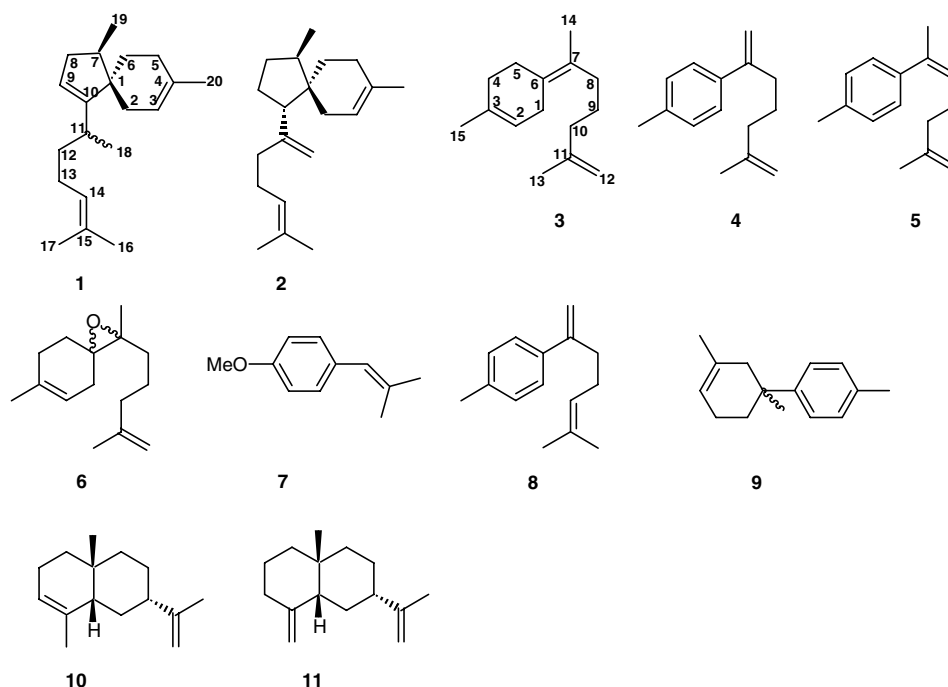


Fig. 1. Structures of compounds isolated from the essential oil of *R. perrottetii*.

identified by this method were isolated by preparative GC and their structures were established as viscida-3,9,14-triene (**1**), viscida-3,11(18),14-triene (**2**), bisabola-2,6,11-triene (**3**), bisabola-1,3,5,7(14),11-pentaene (**4**), bisabola-1,3,5,7,11-pentaene (**5**), 6,7-epoxybisabola-2,11-diene (**6**), 1-methoxy-4-(2-methylpropenyl)benzene (**7**), bisabola-1,3,5,7(14),10-pentaene (**8**), *ar*-tenuifolene (**9**), α -helmiscapene (**10**) and β -helmiscapene (**11**) (Fig. 1) from their respective MS, 1D and 2D NMR data and comparison with reported data for the known compounds. Compound **8** was previously reported from *Biota orientalis* wood (Tomita and Hirose, 1969) and synthesized (Vyvyan et al., 2004). Compound **9**, hitherto described only from the East African sandalwood plant *Osyris tenuifolia* as the (–)-enantiomer (Kreipl and König, 2004) was found to exist in the unusual racemic form in the oil of the liverwort *R. perrottetii*. This was determined by enantioselective GC using modified cyclodextrin columns. It was earlier reported (Asakawa, 1995; König, 1998; Toyota et al., 1999) that biosyntheses of racemic mixtures of sesquiterpene hydrocarbons occur in liverworts only occasionally. Both compounds **10** and **11** were previously described as constituents of liverworts (Andersen et al., 1977).

2.1. Viscida-3,9,14-triene (**1**)

Compound **1** was isolated as an oil by preparative GC. Its mass spectrum displayed a molecular ion signal at m/z 272. Its ^1H (Table 1) and HMQC NMR data exhibited the presence of three allylic methyl singlets,

two methyl doublets, five methine proton signals (three of them olefinic) as well as six methylene multiplets. The ^{13}C NMR (Table 1) showed signals of 20 carbon atoms comprising five primary, six secondary, five ter-

Table 1
 ^1H and ^{13}C NMR data of compounds **1** and **2**

Atom no.	1			2		
	δ ^1H	m (J)	δ ^{13}C	δ ^1H	m (J)	δ ^{13}C
1	–	–	50.3	–	–	44.8
2	1.86	m	28.8	1.80	m	32.8
	1.97	m		2.00	m	
3	5.42	bs	121.5	5.40	bs	122.1
4	–	m	133.2	–	–	133.8
5	1.87	m	28.4	1.89	m	28.4
6	1.36	m	32.9	1.34	m	30.8
	1.75	m		1.43	m	
7	2.08	m	40.8	1.77	m	41.3
8	2.45	m	39.1	1.13	m	33.6
	1.85	m		1.82	m	
9	5.35	t (2.2)	120.6	1.68	m	29.6
10	–	–	156.8	2.29	m	55.9
11	1.96	m	31.3	–	–	150.6
12	1.43	m	38.9	2.11	m	37.6
	1.61	m				
13	2.05	m	26.9	2.24	m	28.1
14	5.25	t (7)	125.5	5.25	t (7)	125.3
15	–	–	130.9	–	–	131.5
16	1.59	s	17.8	1.57	s	18.4
17	1.69	s	25.9	1.67	s	26.2
18	1.06	d (7)	23.0	4.87	s	111.6
				5.00	s	
19	0.98	d (7)	18.6	0.91	d (7)	18.0
20	1.62	bs	23.7	1.62	bs	23.8

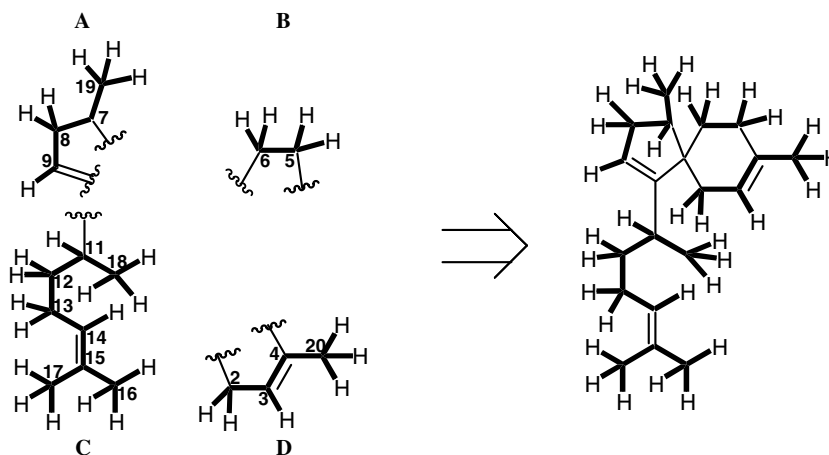


Fig. 2. Key ^1H , ^1H -COSY correlations (bold face bonds) for **1**, and the substructures arising from the COSY data.

tiary (three of them olefinic) and four quaternary (three of them olefinic) carbon atoms. The ^1H and ^{13}C NMR data in combination with the mass spectrum confirmed an elemental composition of $\text{C}_{20}\text{H}_{32}$, a diterpene hydrocarbon with five double bond equivalents (rings and/or double bonds). The exhibition of a total of six olefinic carbon signals in the ^{13}C NMR spectrum of the compound indicated a bicyclic diterpene hydrocarbon showing three double bonds. In the 2D ^1H , ^1H -COSY (Fig. 2), key correlations were observed that allowed recognition of four substructures (Fig. 2) of the molecule. Based on HMBC correlations (Table 3), the substructures were assembled together to the depicted viscidane skeleton (Fig. 1). Some of the key HMBC correlations that led to the structure include the correlations observed between a saturated aliphatic quaternary carbon atom at δ 50.3 (C-1) and a methyl doublet at δ 0.98 (H_3 -19), a methylene group multiplets at δ 2.45 (H_a -8) and 1.85 (H_b -8), a methine proton multiplet at δ 2.08 (H-7), an olefinic methine proton triplet at δ 5.35 (H-9) each from substructure **A**, a methylene group multiplets at δ 1.36 (H_a -6) and 1.75 (H_b -6) from substructure **B**, a methine proton multiplet at δ 1.96 (H-11) from substructure **C**, and a methylene group multiplets at δ 1.86 (H_a -2) and 1.97 (H_b -2) from substructure **D**. This indicated that the quaternary carbon at δ 50.3 (C-1) was positioned at a junction where the five and the six membered rings of the molecule were fused. In the same way, several proton groups from the different substructures exhibited HMBC correlations with an olefinic quaternary carbon atom at δ 156.8 (C-10). These were, the methylene protons multiplets at δ 2.45 (H_a -8) as well as 1.85 (H_b -8), the olefinic methine proton triplet at δ 5.35 (H-9) both from the substructure **A**, the methylene group multiplets at δ 1.36 (H_a -6) as well as 1.75 (H_b -6) from substructure **B**, and the methine proton multiplet at δ 1.96 (H-11), a methylene group multiplets at δ 1.43 (H_a -12) and 1.61 (H_b -12), and a methyl protons doublet at δ 1.06 (H_3 -18) each from the substructure **C**. This observation

further substantiated the structure. Furthermore, the depicted connection between C-4 and C-5 that joined the substructures **B** and **D** to form the six membered ring was realized from the HMBC correlations observed between an allylic methyl protons singlet at δ 1.62 (H_3 -20) and a methylene carbon atom at δ 28.4 (C-5). The remaining HMBC correlations of the compound are given in Table 3. These correlations and all the NMR data correspond entirely with the proposed structure of viscida-3,9,14-triene for compound **1**.

2.2. Viscida-3,11(18),14-triene (**2**)

Compound **2** that eluted next to **1** on the CPSil-5 capillary column was isolated as an oil on preparative GC. Its mass spectrum showed a molecular ion peak at m/z 272 similar to **1** but the base peak for the latter was at m/z 122 while that of the former was at m/z 119. Its ^1H (Table 1) and HMQC NMR data indicated presence of three allylic methyl singlets, a methyl doublet, four methine protons signals (two of them olefinic) and eight methylene multiplets (including one exocyclic methylene). The ^{13}C NMR spectrum of **2** (Table 1) displayed signals of 20 carbon atoms comprising four primary, eight secondary (including an exocyclic olefinic), four tertiary (two of them olefinic) and four quaternary (three of them olefinic) carbon atoms. Thus, similar to **1**, the ^1H and ^{13}C NMR data in combination with the mass spectrum revealed an elemental composition of $\text{C}_{20}\text{H}_{32}$, a diterpene hydrocarbon with five double bond equivalents. Compound **2** differed from **1** in that, in place of one of the methyl doublets, **2** exhibited an exocyclic methylene group and in place of one of the olefinic methine protons, it displayed a methylene protons signals. However, like **1**, compound **2** displayed three double bonds (six olefinic carbons) indicating a bicyclic diterpene hydrocarbon. Examination of connectivity of atoms in the ^1H , ^1H -COSY (Fig. 3) and HMBC spectra (Table 3) established that **2** was a viscidane diterpene

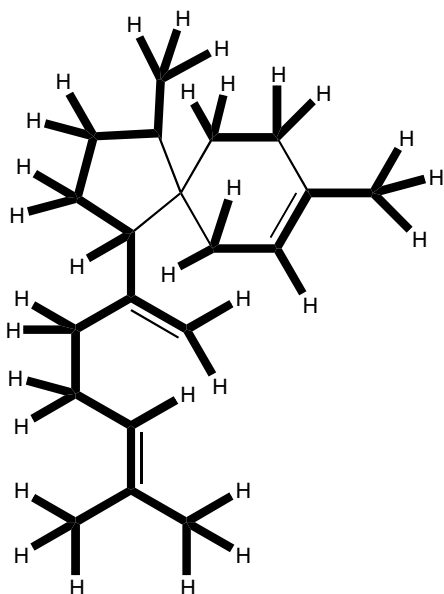


Fig. 3. Important ^1H , ^1H -COSY correlations for **2** shown in bold face bonds.

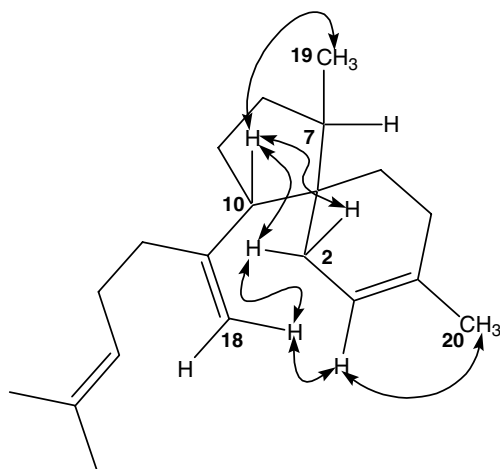


Fig. 4. Key NOESY correlations observed for **2**.

showing one of the double bonds between C-11 and C-18 instead of between C-9 and C-10 as in **1**. The important ^1H , ^1H -COSY and HMBC correlations observed for **2** are given in Fig. 3 and Table 3, respectively.

The relative configurations of compounds **1** and **2** were determined by 2D-NOESY spectrum of compound **2**. Key NOESY correlations (Fig. 4) were observed between the methyl doublet at δ 0.91 (H_3 -19) and the methine proton multiplet at δ 2.29 (H -10). The latter was also correlated with the methylene protons at δ 1.80 (H_a -2) and 2.00 (H_b -2). This indicated the close proximity of these substituents and their location at the same side of the molecule furnishing the depicted relative configuration. Assuming close biogenetic relationships between **2** and **1**, the configurations at C-1 and C-7

in the latter were suggested to be the same as in the former. This is the first isolation of viscidane diterpene hydrocarbons from liverworts. Diterpenes with viscidane skeleton were hitherto reported from plants of the genus *Eremophila* only (Ghisalberti et al., 1984; Forster et al., 1986, 1993; Syah et al., 1997).

The absolute configuration of the viscidane diterpenes from the *Eremophila* species was determined to be the same as that of the sesquiterpene hydrocarbon, (–)- α -acoradiene (Ghisalberti et al., 1984). It is long recognized that liverworts produce terpenes that are predominantly optical antipodes to those found in higher plants (Hayashi and Matsuo, 1975; König, 1998). Therefore, it is expected that the viscidane diterpenes from the liverwort *R. perrottetii* could show the opposite absolute configuration to those reported from the *Eremophila* species although this remains to be clarified.

2.3. Bisabola-2,6,11-triene (**3**)

The ^1H NMR (Table 2) of compound **3** displayed signals of a total of 24 protons comprising three allylic methyls, seven methylenes including an exocyclic methylene, and an olefinic methine. The ^{13}C NMR (Table 2) exhibited 15 carbon atoms. Mass spectrum of **3** revealed a molecular ion signal at m/z 204 confirming an elemental composition of $\text{C}_{15}\text{H}_{24}$ typical for a sesquiterpene hydrocarbon with four double bond equivalents. The exhibition of six olefinic carbon signals revealed compound **3** to be a triply unsaturated monocyclic sesquiterpene hydrocarbon. Furthermore, analysis of the 2D ^1H , ^1H -COSY (Fig. 5) and HMBC (Fig. 6) spectra of the compound led to the depicted bisabolane skeleton. While this is the first report of the compound from a natural source, its synthesis, ^1H and ^{13}C NMR data with incomplete assignments were earlier reported (Braun et al., 2003).

2.4. Bisabola-1,3,5,7(14),11-pentaene (**4**)

The ^1H NMR (Table 2) of **4** displayed signals of 20 protons consisting of an allylic and an aromatic methyl groups, five methylene groups (including two exocyclic methylene groups) as well as four aromatic methine protons. The aromatic methine signals appeared as a pair of doublets, each representing two protons, a pattern, typical for a *p*-disubstituted benzene system. The ^{13}C NMR (Table 2) indicated the presence of 15 carbon atoms. Mass spectrum of **4** exhibited a molecular ion signal at m/z 200 confirming an elemental composition of $\text{C}_{15}\text{H}_{20}$. Interpretation of the observed correlations in the 2D ^1H , ^1H -COSY (Fig. 5) and HMBC (Fig. 6) spectra of the compound revealed the depicted monocyclic aromatic bisabolane sesquiterpene showing two exocyclic double bonds. This is the first report of the

Table 2
¹H and ¹³C NMR data of compounds **3–6**

Atom no.	3			4			5			6		
	δ ¹ H	<i>m</i> (<i>J</i>)	δ ¹³ C	δ ¹ H	<i>m</i> (<i>J</i>)	δ ¹³ C	δ ¹ H	<i>m</i> (<i>J</i>)	δ ¹³ C	δ ¹ H	<i>m</i> (<i>J</i>)	δ ¹³ C
1	2.75	<i>m</i>	30.4	7.00	<i>d</i> (8)	129.3	7.03	<i>d</i> (8)	129.2	2.00	<i>m</i>	29.3
2	5.39	<i>m</i>	121.5	7.31	<i>d</i> (8)	126.5	7.32	<i>d</i> (8)	126.0	2.24	<i>m</i>	120.2
3	—	—	134.2	—	—	137.1	—	—	136.3	5.31	<i>m</i>	134.5
4	2.35	<i>bs</i>	27.2	7.31	<i>d</i> (8)	126.5	7.32	<i>d</i> (8)	126.0	1.67	<i>s</i>	27.3
5	1.97	<i>bs</i>	32.3	7.00	<i>d</i> (8)	129.3	7.03	<i>d</i> (8)	129.2	1.86	<i>m</i>	31.9
6	—	—	128.9	—	—	138.9	—	—	141.6	2.24	<i>m</i>	120.2
7	—	—	126.3	—	—	148.7	—	—	135.3	—	—	63.3
8	2.08	<i>t</i> (7.5)	34.2	2.45	<i>t</i> (7)	35.3	5.82	<i>t</i> (8)	127.2	—	—	64.0
9	1.54	<i>m</i>	27.3	1.58	<i>m</i>	26.5	2.26	<i>q</i> (7)	27.3	1.40–1.58	<i>m</i>	34.7
10	1.98	<i>m</i>	38.3	1.96	<i>t</i> (7)	37.7	2.06	<i>t</i> (8)	37.8	1.40–1.63	<i>m</i>	24.18
11	—	—	146.1	—	—	145.6	—	—	145.4	1.92	<i>t</i> (7)	38.5
12	4.82	<i>bs</i>	110.5	4.76	<i>s</i>	110.6	4.81	<i>s</i>	110.7	—	—	146
13	1.66	<i>s</i>	22.8	4.77	<i>s</i>	—	4.82	<i>s</i>	—	4.78	<i>s</i>	110.8
14	1.62	<i>s</i>	18.7	1.58	<i>s</i>	22.4	1.65	<i>s</i>	22.6	4.80	<i>s</i>	—
15	1.64	<i>s</i>	23.9	5.03	<i>s</i>	111.7	1.93	<i>s</i>	15.9	1.62	<i>s</i>	22.6
				5.32	<i>s</i>	—	—	—	—	1.18	<i>s</i>	18.8
				2.12	<i>s</i>	21.1	2.16	<i>s</i>	21.0	1.61	<i>s</i>	23.7

Table 3
Important HMBC correlations observed for compounds **1** and **2**

1		2	
Proton(s) correlated with carbon	C-	Proton(s) correlated with carbon	C-
H ₃ -19, H-7, H ₂ -8, H-9, H-11, H ₂ -6, H ₂ -2	C-1	H ₃ -19, H-10, H ₂ -9, H ₂ -8, H-7, H ₂ -6, H ₂ -2	C-1
H ₂ -6	C-2	H-10, H ₂ -6	C-2
H ₃ -20	C-3	H ₃ -20	C-3
H ₂ -2, H ₂ -5, H ₂ -6, H ₃ -20	C-4	H ₂ -2, H ₂ -6, H ₃ -20	C-4
H ₃ -20	C-5	H ₃ -20, H ₂ -6	C-5
H-7	C-6	H-7	C-6
H ₃ -19, H ₂ -8, H-9, H ₂ -6	C-7	H ₃ -19, H ₂ -9, H ₂ -8, H ₂ -6, H ₂ -2	C-7
H ₃ -19, H-9	C-8	H ₃ -19, H ₂ -9, H-7	C-8
H ₂ -8, H-11	C-9	H ₂ -6, H-10	C-9
H ₂ -8, H-9, H-11, H ₃ -18, H ₂ -6, H ₂ -12	C-10	H ₂ -8, H ₂ -9, H ₂ -6, H ₂ -12, H ₂ -18	C-10
H ₂ -12, H ₃ -18, H-9	C-11	H ₂ -12, H ₂ -18, H-10	C-11
H-11, H ₃ -18	C-12	H ₂ -18, H ₂ -13, H-10	C-12
H-11, H ₂ -12, H-14	C-13	H ₂ -12, H-14	C-13
H ₂ -12, H ₂ -13, H ₃ -16, H ₃ -17	C-14	H ₂ -12, H ₂ -13, H ₃ -16, H ₃ -17	C-14
H ₂ -13, H ₃ -16, H ₃ -17	C-15	H ₃ -16, H ₃ -17	C-15
H ₃ -17, H-14	C-16	H ₃ -17, H-14	C-16
H ₃ -16	C-17	H ₃ -16, H-14	C-17
H-11, H ₂ -12	C-18	H-10, H ₂ -12	C-18
H ₂ -8, H-7	C-19	H ₂ -8, H-7	C-19

compound from a natural source. Synthesis of the compound was earlier described where ¹H NMR data with partial assignments was reported (Zhang et al., 1995).

2.5. Bisabola-1,3,5,7,11-pentaene (**5**)

¹H NMR spectrum of **5** (Table 2) indicated the presence of two allylic and one aromatic methyl singlet, three methylene groups (including one exocyclic methylene group), four aromatic methine protons as well as one olefinic methine proton. Again, the aromatic methine sig-

nals appeared in a pattern typical for a *p*-disubstituted benzene system. The ¹³C NMR (Table 2) indicated signals of 15 carbon atoms. Mass spectrum of **5** exhibited a molecular ion signal at *m/z* 200 confirming an elemental composition of C₁₅H₂₀. Interpretation of the 2D ¹H, ¹H-COSY (Fig. 5) and HMBC (Fig. 6) spectra of the compound led to the indicated aromatic bisabolane sesquiterpene. This is the first report of the compound from a natural source. The synthesis of the compound was described earlier where partial ¹H NMR data was reported (Kuznetsov et al., 1971; Wu et al., 1992).

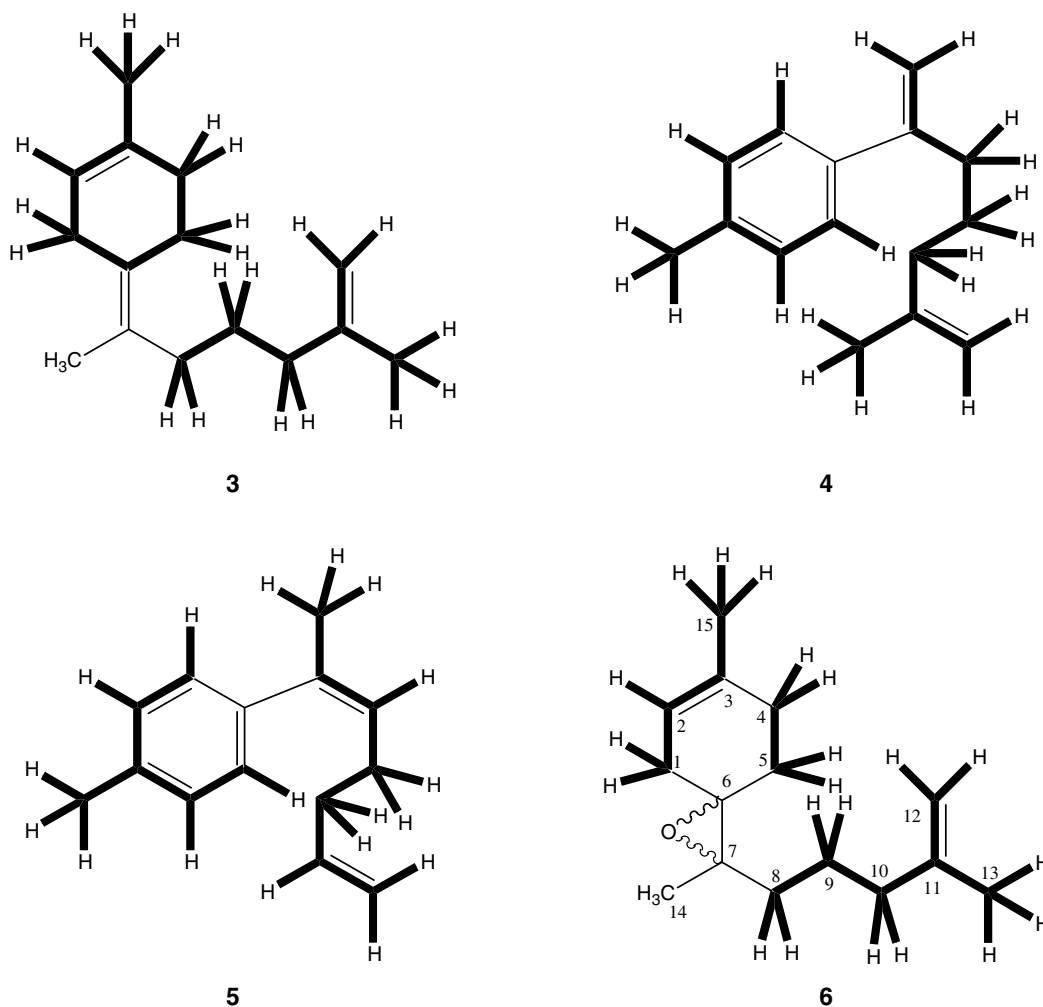


Fig. 5. Important ^1H , ^1H -COSY correlations (bold face bonds) for 3–6.

2.6. 6,7-Epoxybisabola-2,11-diene (6)

The ^1H NMR spectrum (Table 2) of compound 6 indicated the presence of a tertiary methyl singlet connected to an oxygenated carbon atom and two allylic methyl groups, seven methylene groups (including one exocyclic methylene group) as well as one olefinic methine proton. Presence of 15 carbon atoms was concluded from ^{13}C NMR spectrum (Table 2) of the compound. As the EI-MS of 6 failed to show a molecular ion signal, CI-MS measurement was carried out, and the corresponding spectrum exhibited a strong $[\text{M} + 1]^+$ signal at m/z 221, confirming an elemental composition of $\text{C}_{15}\text{H}_{24}\text{O}$. Analysis of the 2D ^1H , ^1H -COSY (Fig. 5) and HMBC (Fig. 6) spectra of the compound led to the depicted bisabolane skeleton having two C–C double bonds and an epoxide ring as indicated. The presence of the epoxide ring was evident by the two up-field shifted signals at δ 63.3 and 63.4 indicating oxygenated quaternary carbon atoms in the ^{13}C NMR spectrum (Table 2). The fact that the tertiary methyl singlet

protons at δ 1.18 (H_3 -14) were strongly correlated with these two carbons in the HMBC spectrum (Fig. 6) further substantiated the position of the epoxy function. This is the first report of the compound from a natural source. Synthesis of 6 and its ^1H as well as ^{13}C NMR data with partial assignments were earlier reported (Braun et al., 2003).

2.7. 1-Methoxy-4-(2-methylpropenyl)benzene (7)

The ^1H NMR (see Section 3) of 7 indicated the presence of two allylic methyl groups, one methoxy singlet, an olefinic methine, and four aromatic methine protons that appeared in a pattern characteristic of a *p*-disubstituted benzene system. The ^{13}C NMR (see Section 3) indicated the presence of a total of eleven carbon atoms. The MS of 7 exhibited a molecular ion peak at m/z 162 confirming an elemental composition of $\text{C}_{11}\text{H}_{14}\text{O}$. Interpretation of the spectra of the compound led to the indicated structure. This is the first isolation of the compound from a natural source.

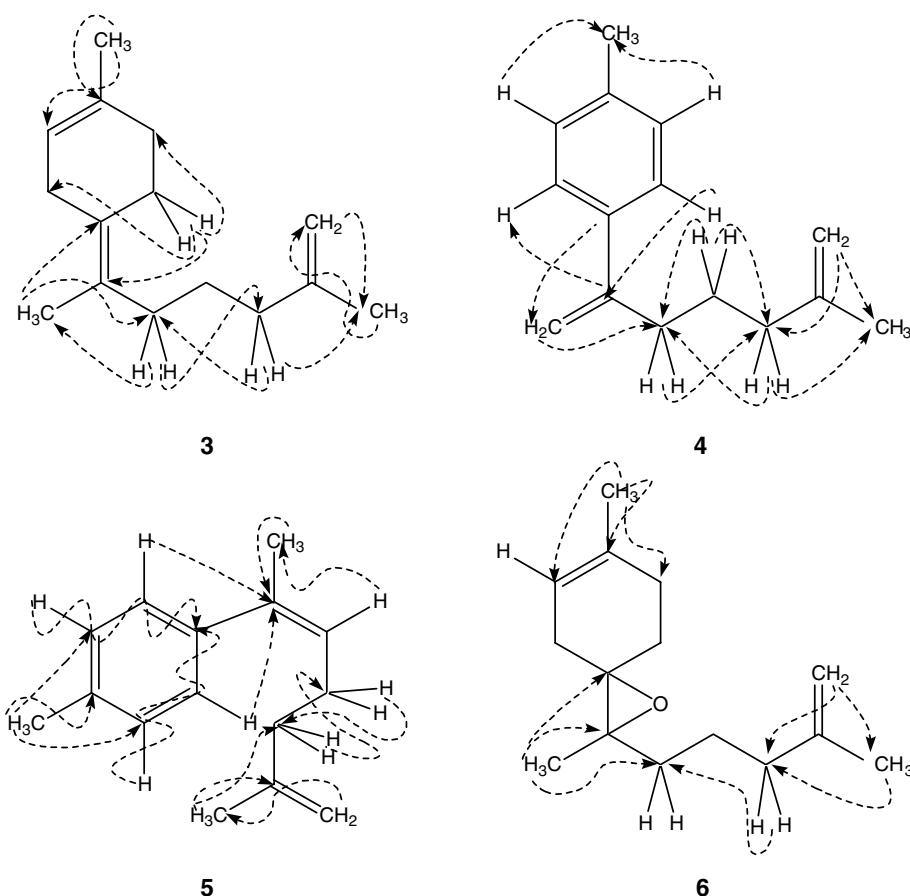


Fig. 6. Important HMBC correlations shown in dashed arrows for **3–6**.

3. Experimental

3.1. General

The liverwort used in this study was collected in Aioicho, Nakagun, Tokushima, Japan, in October 2003. The NMR spectra were recorded on a Bruker WM 400 or 500 MHz spectrometers in deuteriobenzene (C_6D_6). The chemical shift values are reported with reference to TMS and the coupling constants are given in Hz. Optical rotations were measured as solutions in benzene on a Perkin–Elmer 341 or Jasco DIP 370 polarimeter at 589 nm and 20 °C.

3.2. Hydrodistillation and isolation procedure

Cleaned and pulverized plant material was homogenized and hydrodistilled in a Clevenger type apparatus for 2.5 hours, and a slightly greenish oil was collected in HPLC grade hexane. The oil was analysed on a HRGC 5300 MEGA series equipped with double columns: a 25 m/0.25 mm i.d. non-polar CPSil-5-CB and a more polar CPSil-19-CB column of identical dimensions (Chrompack). The oven temperature was pro-

grammed from 50 to 230 °C at a rate of 3 °C/min. The injector and detector temperatures were kept at 200 and 250 °C, respectively. The oil was further analysed using GC/MS with a HP 5890 GC coupled to a VG Analytical 70-250S mass spectrometer with electron impact (70 eV) ionisation. Retention indices and mass spectra of the components were compared with library spectra generated under identical experimental conditions (Joulain and König, 1998; Hochmuth et al., 2003). Subsequently, the oil was fractionated on a modified Varian 1400 preparative gas chromatograph, equipped with a stainless steel column (1.85 m × 4.3 mm) packed with 10% polydimethylsiloxane SE 30 on Chromosorb W-HP (Sigma–Aldrich). Compounds **1**, **2** and **7** were isolated from their respective fractions using the same preparative GC equipped with a column (1.95 m × 5.3 mm) packed with 6.5% 6-*O*-TBDMS-2,3-di-*O*-Me- β -cyclodextrin in SE-52 (1:1, w/w) on chromosorb W-HP (Sigma–Aldrich). All the other compounds were isolated on an Agilent 6890N Network preparative GC System equipped with a DB-1 column (30 m, 0.53 mm i.d. and 5 μ m film thickness), and coupled to a preparative fraction collector (PFC-1) (Gerstel, Mülheim an der Ruhr, Germany).

3.3. Determination of enantiomeric ratio of **9**

Determination of enantiomeric ratio of **9** was carried out on two Carlo Erba GC series 2150 instruments equipped with two 25 m fused silica capillaries coated with modified cyclodextrins, viz., 3'-*O*-acetyl-6-*O*-TBDMS-2,3-di-*O*-methyl- β -cyclodextrin, and 6-*O*-methyl-2,3-*O*-pentyl- γ -cyclodextrin both in 50% OV 1701 as stationary phases at oven temperatures of 120 °C. The remaining GC conditions were the same as that described under Section 3.1.

3.4. *Viscida-3,9,14-triene (1)* (*1-(1,5-dimethylhex-4-enyl)-4,8-dimethylspiro[4.5]deca-1,7-diene*)

Colourless oil (ca. 1 mg), RI_{CPSil-5} = 1862, sense of optical rotation (benzene): (–); MS (EI, 70 eV), *m/z* (rel. inten. %): 272 [M^+] (12), 229 (3), 215 (2), 204 (21), 190 (40), 175 (4), 161 (66), 147 (56), 134 (15), 122 (100), 107 (72), 91 (35), 79 (24), 67 (26), 55 (32), 41 (65). ¹H and ¹³C NMR data, see Table 1.

3.5. *Viscida-3,11(18),14-triene (2)* (*1,8-dimethyl-4-(5-methyl-1-methylenehex-4-enyl)spiro[4.5]dec-7-ene*)

Colourless oil (ca. 1 mg), RI_{CPSil-5} = 1911, sense of optical rotation (benzene): (–) MS (EI, 70 eV), *m/z* (rel. inten. %): 272 [M^+] (21), 257 (4), 243 (1), 229 (5), 215 (5), 203 (14), 187 (16), 175 (4), 161 (31), 147 (18), 132 (34), 119 (100), 105 (44), 93 (81), 81 (40), 69 (92), 55 (27), 41 (40). ¹H and ¹³C NMR data, see Table 1.

3.6. *Bisabola-2,6,11-triene (3)* (*4-(1,5-dimethylhex-5-enylidene)-1-methylcyclohexene*)

Colourless oil (ca. 1 mg), RI_{CPSil-5} = 1525: MS (EI, 70 eV), *m/z* (% rel. inten.): 204 [M^+] (26), 189 (5), 171 (4), 161 (9), 148 (20), 133 (35), 119 (89), 105 (50), 93 (100), 79 (45), 69 (20), 55 (42), 41 (74). ¹H and ¹³C NMR data, see Table 2.

3.7. *Bisabola-1,3,5,7(14),11-pentaene (4)* (*2-methyl-6-(4-methylphenyl)-1,6-heptadiene*)

Colourless oil (ca. 0.5 mg), RI_{CPSil-5} = 1509: MS (EI, 70 eV), *m/z* (% rel. inten.): 200 [M^+] (11), 185 (3), 172 (8), 157 (6), 145 (6), 132 (100), 117 (22), 105 (8), 91 (13), 65 (5), 41 (11). ¹H and ¹³C NMR data, see Table 2.

3.8. *Bisabola-1,3,5,7,11-pentaene (5)* (*2-methyl-6-(4-methylphenyl)-1,5-heptadiene*)

Colourless oil (ca. 0.5 mg), RI_{CPSil-5} = 1576, MS (EI, 70 eV), *m/z* (% rel. inten.): 200 [M^+] (8), 185 (5), 171 (3), 161 (5), 145 (100), 130 (12), 115 (9), 105 (13), 91 (7). ¹H and ¹³C NMR data, see Table 2.

3.9. *6,7-Epoxybisabola-2,11-diene (6)* (*2,6-dimethyl-2-(4-methylpent-4-enyl)-1-oxaspiro[2.5]oct-5-ene*)

Colourless oil (ca. 0.5 mg), RI_{CPSil-5} = 1142, sense of optical rotation (benzene): (–); MS (EI, 70 eV), *m/z* (% rel. inten.): 205 (1), 152 (4), 137 (3), 110 (33), 95 (100), 79 (59), 68 (17), 55 (31), 41 (35). ¹H and ¹³C NMR data, see Table 2.

3.10. *1-Methoxy-4-(2-methylpropenyl)benzene (7)*

Colourless oil (ca. 0.5 mg), RI_{CPSil-5} = 1325, MS (EI, 70 eV), *m/z* (% rel. inten.): 162 [M^+] (100), 147 (73), 131 (13), 121 (18), 115 (16), 103 (14), 91 (39), 77 (21), 65 (10), 51 (10), 41 (14). ¹H NMR: (500 MHz, C₆D₆): 1.74 (3H, *s*, H₃₋₉), 1.76 (3H, *s*, H₃₋₁₀), 3.33 (3H, *s*, H₃₋₁₁), 6.3 (2H, *s*, H-7), 6.81 (2H, *d*, *J* = 9, H-2, H-6), 7.16 (2H, *d*, *J* = 9, H-3, H-5). (400 MHz, C₆D₆): 26.8 (C-9, C-10), 54.8 (C-11), 114.0 (C-2, C-6), 125.5 (C-7), 130.3 (C-3, C-5), 131.7 (C-4), 133.5 (C-8), 158.5 (C-1).

3.11. *Bisabola-1,3,5,7(14),10-pentaene (8)* (*2-methyl-6-(4-methylphenyl)-2,6-heptadiene*)

Colourless oil (ca. 0.2 mg), RI_{CPSil-5} = 1517, MS (EI, 70 eV), *m/z* (% rel. inten.): 200 [M^+] (2), 185 (2), 157 (100), 142 (4), 132 (10), 115 (12), 91 (11), 69 (67), 41 (52). ¹H NMR: same as the data reported in Vyvyan et al. (2004).

3.12. *ar-Tenuifolene (9)*

Colourless oil (ca. 0.2 mg), RI_{CPSil-5} = 1531, MS (EI, 70 eV), *m/z* (% rel. inten.): 200 [M^+] (13), 185 (1), 171 (1), 157 (2), 143 (2), 132 (100), 117 (11), 105 (6), 91 (8), 39 (5). ¹H NMR: same as the data reported in Kreipl and König (2004).

3.13. *α-Helmiscapene (10)*

Colourless oil (ca. 0.5 mg), RI_{CPSil-5} = 1451, sense of optical rotation (benzene): (–); MS (EI, 70 eV), *m/z* (rel. inten. %): 204 [M^+] (54), 189 (100), 175 (13), 161 (46), 147 (27), 133 (38), 121 (40), 107 (96), 93 (84), 81 (49), 67 (32), 55 (41), 41 (61). ¹H and ¹³C NMR data: same as the data reported in Adio et al. (2004).

3.14. *β-Helmiscapene (11)*

Colourless oil (ca. 0.5 mg), RI_{CPSil-5} = 1446, sense of optical rotation (benzene): (–); MS (EI, 70 eV), *m/z* (% rel. inten.): 204 [M^+] (12), 189 (73), 176 (62), 161 (33), 147 (100), 133 (46), 121 (40), 107 (57), 93 (71), 79 (61), 67 (44), 55 (38), 41 (52). ¹H NMR: (500 MHz, C₆D₆): 0.77–0.78 (1H, *m*, H_{a-3}), 0.92 (3H, *s*, H₃₋₁₄), 1.13–1.23 (1H, *m*, H_{a-1}), 1.37–1.57 (6H, *m*, H_{b-1}, H₂₋₂, H_{a-6},

H₂-8), 1.62–1.64 (1H, *m*, H_b-1, H₂-2, H_a-6, H₂-8), 1.67 (3H, *s*, H₃-14), 1.72–1.94 (3H, *m*, H-5, H-7, H_a-9), 1.97–2.12 (2H, *m*, H_b-3, H_b-9), 4.74 (2H, *bs*, H₂-15), 4.81 (1H, *s*, H_a-12), 4.83 (1H, *s*, H_b-12). ¹³C NMR: (400 MHz, C₆D₆): 21.3 (C-13), 23.5 (C-2), 27.4 (C-8), 28.2 (C-14), 29.9 (C-9), 30.5 (C-3), 33.9 (C-10), 34.2 (C-6), 41.0 (C-1), 45.8 (C-7), 52.6 (C-5), 108.6 (C-15), 108.7 (C-14), 150.3 (C-4, C-11).

Acknowledgements

We gratefully acknowledge financial support by DAAD (scholarship for H. Tesso) and the *Fonds der Chemischen Industrie*. We thank Dr. V. Sinnwell for his support in recording NMR spectra and Mrs. A. Meiners and Mr. M. Preusse for GC/MS measurements.

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