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Sesquiterpene constituents from the essential oil of the liverwort *Plagiochila asplenioides*

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Abstract

The essential oil of the liverwort *Plagiochila asplenioides* from two different locations in Northern Germany were investigated by chromatographic and spectroscopic methods. Seven compounds were isolated by preparative gas chromatography (GC) and their structures investigated by mass spectrometry (MS), NMR techniques and chemical correlations in combination with enantioselective GC. In addition to known constituents, aromadendra-1(10),3-diene, two aromatic sesquiterpene hydrocarbons, bisabola-1,3,5,7(14)-tetraene and bisabola-1,3,5,7-tetraene, three sesquiterpene ethers, muurolan-4,7-peroxide, plagiochilines W and X, in addition to *ent*-4-*epi*-maaliol, could be identified as natural compounds for the first time.

Keywords: *Plagiochila asplenioides*; Liverworts; Sesquiterpene hydrocarbons; Oxygenated sesquiterpenes; *abeo*-Amorphane/muurolane; *seco*-Aromadendrane-type sesquiterpenoids

1. Introduction

Plagiochila species are known to produce a broad and diverse spectrum of secondary metabolites comprising of mono-, sesqui- and diterpenoids as well as bibenzyls. Among the terpene compounds, sesquiterpenoids are the most common (Asakawa et al., 1979, 1980a,b; Matsuo et al., 1981; Spörle et al., 1991; Wu et al., 1993; Valcic et al., 1997; Kraut and Mues, 1999; Rycroft et al., 2001, 2002; Nagashima et al., 2003; Asakawa, 2004). In continuation of our investigations on the chemistry of the liverworts, we describe in this article the isolation of three new sesquiterpene hydrocarbons, bisabola-1,3,5,7(14)-tetraene (**1**), bisabola-1,3,5,7-tetraene (**2**), (−)-aromadendra-1(10),3-diene (**3**), four oxygenated sesquiterpenes (+)-muurolan-4,7-peroxide (**4**), (+)-plagiochiline W (**10**), (+)-plagiochiline X **(11)** and *ent*-4-*epi*-maaliol (**12**). The NMR data of compound **9**, a sesquiterpene peroxide (Nagashima et al., 2003) are also reported for the first time.

2. Results and discussion

GC-MS of the essential oils of the liverwort *Plagiochila asplenioides* collected in Ferbruary 2004 from Northern part of Hamburg and December 2001 in the Harz mountains was obtained by hydrodistillation. In order to exclude artefact formation by hydrodistillation, a diethyl ether extract was prepared which confirmed the representative components except for the relative amount observed. A complex mixture of compounds with many sesquiterpene hydrocarbons including maali-1,3-diene (Warmers et al., 1998), anastreptene,

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✠ W.A. König deceased on 19 November 2004, his scientific achievements keep him among us.

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italicene, α-barbatene, β-funebrene, γ-maaliene, α-maaliene, β-barbatene, β-acoradiene, β-chamigrene, γ-curcumene, (+)-δ-selinene (**16**), (−)-bicyclogermacrene (**13**), α-cuprenene, α-chamigrene and β-bazzanene were identified from the non-oxygenated fraction. In the oxygenated fraction (+)-maalian-5-ol (**18**) (Matsuo et al., 1979), rosifoliol (**26**) (Nagashima et al., 2003), gymnomitr-3(15)-en-4β-ol (**19**) (Kraut and Mues, 1999), plagiochilide (**20**) and 3α-acetoxybicyclogermacrene (**21**) were identified in the essential oils of *P. asplenioides* from both locations. All known compounds were identified by comparing their mass spectra and retention indices with a computer supported spectral library established under identical experimental conditions (Joulain and König, 1998; Hochmuth et al., 2002). Compounds such as (−)-selina-5,7(11)-diene (**14**, 8.0%, ^{13}C NMR data are reported for the first time, see Table 1) in addition to (−)-cascarilladiene (**15**), (+)-δ-selinene (**16**) and (−)-selina-5,11-diene (**17**) were obtained only as thermal transformation products of (+)-maalian-5-ol (**18**, 18.7%) (Matsuo et al., 1979) at 200 °C injector temperature during preparative GC (Scheme 1). (+)-10-*epi*-Selina-5,7(11)-diene, a rearrangement product generated under acidic conditions with identical mass spectrum as **14**, had been elucidated before by comparison with the ^{1}H NMR spectral data of δ-selinene (Adio et al., 2003). In addition, three oxygenated compounds (**4**, **10**, and **11**) were isolated and characterized in the essential oil of *P. asplenioides* from the collection site north of Hamburg which were not present in the oxygenated fraction of *P. asplenioides* collected in the Harz mountains. In contrast, the aromatic sesquiterpene hydrocarbons **1** and **2** which were not present in *P. asplenioides* from north of Hamburg were isolated from the collection site in the Harz mountains. Compounds **3** and **12** were present in the essential oils from both locations.

Bisabola-1,3,5,7(14)-tetraene (**1**, 3.2%), a non-chiral *para*-substituted aromatic compound, exhibited a molecular ion signal at *m*/*z* 202 and an elemental composition of $C_{15}H_{22}$. The ^{1}H NMR spectrum (C_6D_6) showed signals of one doublet and one singlet for methyl groups at δ 0.82 (6H, *d*, H-12, H-13, J = 6.6 Hz) and 2.12 (3H, *s*, H-15), respectively. The olefinic carbon signals at δ 149.1 (*s*) and 111.8 (*t*) suggested a methylene double bond, which was confirmed by two signals in the ^{1}H NMR spectrum at δ 5.05 (1H, *d*, H-14a, J = 1.3 Hz) and 5.34 (1H, *d*, H-14b, J = 1.6 Hz). The downfield shifted signals at δ 7.01 (2H, *d*, H-2, H-4, J = 7.9 Hz) and 7.33 (2H, *d*, H-1, H-5, J = 8.2 Hz) were assigned to the *para*-substituted aromatic fragment. Additional structural information was obtained from the ^{13}C NMR data (Table 1) as well as from ^{1}H–^{1}H-COSY, HMQC and HMBC spectra (Fig. 1) and led to structure **1**.

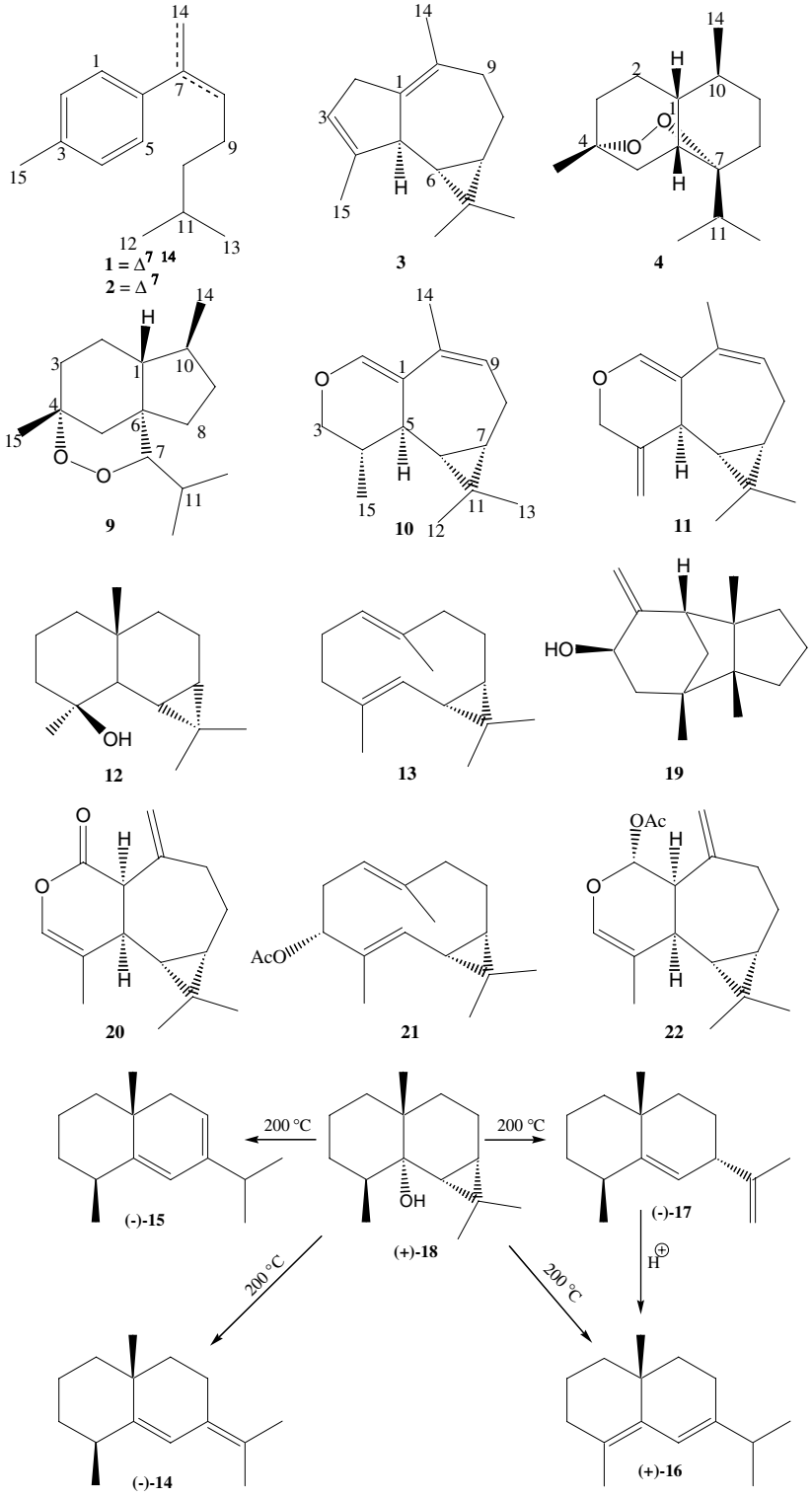
Bisabola-1,3,5,7-tetraene (**2,** 2.3%), a colourless oil and double bond isomer of **1** with a *para*-substituted aromatic fragment, exhibited a molecular ion signal at *m*/*z* 202 and an elemental composition of $C_{15}H_{22}$. The ^{1}H NMR spectrum (C_6D_6) showed signals similar to **1** except for the absence of the methylene protons and the presence of a signal at δ 1.96 (3H, *s*, H-14) typical of a methyl group attached to a double bond and the presence of a methine proton at δ 5.84 (1H, *br.t*, H-8, J = 6.9 Hz). The ^{13}C NMR data of **2** (Table 1) as well as ^{1}H–^{1}H-COSY, HMQC and HMBC spectra (Fig. 2) led to structure **2**.

(−)-Aromadendra-1(10),3-diene (**3**) was isolated in trace amounts (1.2%) as a colourless oil which eluted just before β-bazzanene from the non-polar fraction of the essential oil. The tricyclic compound exhibited a molecular ion signal at *m*/*z* 202 and an elemental composition of $C_{15}H_{22}$. The ^{1}H NMR spectrum (C_6D_6) showed signals of four methyl groups at δ 0.98 (3H, *s*,

Table 1
^{13}C NMR spectral data of compounds **1**, **2**, (−)-**3**, (−)-**14**, (+)-**4**, (+)-**9**, (+)-**10**, and (+)-**11** (125.7 MHz, C_6D_6), δ (ppm)[a]

C no.	**1**	**2**	(−)-**3**	(−)-**14**	(+)-**4**	(+)-**9**	(+)-**10**	(+)-**11**
1	126.7	126.3	136.9	41.9	40.1	56.2	118.2	119.6
2	129.6	129.5	39.1	18.2	29.3	22.7	142.4	143.2
3	137.1	136.1	121.8	33.7	39.4	38.6	67.9	68.8
4	129.6	129.5	143.2	38.7	79.7	78.7	34.2	144.6
5	126.7	126.3	46.8	147.3	41.5	54.6	37.7	36.0
6	139.2	141.8	35.1	121.9	37.7	53.5	33.3	33.5
7	149.1	134.7	26.7	128.8	86.1	93.7	28.9	29.1
8	36.3	128.0	23.1	24.1	24.7	28.9	24.4	24.7
9	26.7	27.3	37.1	42.3	28.2	33.9	124.8	125.4
10	39.2	39.4	125.6	34.6	34.1	37.6	133.3	132.6
11	28.4	28.3	19.2	124.0	37.1	29.3	19.5	19.4
12	23.0	23.0	29.0	20.0	16.3	20.0	28.9	28.9
13	23.0	23.0	16.2	21.0	19.3	22.5	15.6	15.6
14	111.8	16.2	22.0	26.6	21.6	19.2	22.6	23.1
15	21.3	21.3	15.5	23.8	27.8	26.8	16.9	111.6

[a] All assignments were confirmed by HMBC and HMQC NMR spectra.



Scheme 1.

H-12), 1.08 (3H, *s*, H-13), 1.55 (3H, *s*, H-14) and 1.68 (3H, *s*, H-15). The two highly shielded protons at δ 0.55–0.60 (1H, *m*, H-7) and 0.68 (1H, *t*, H-6, $J = 10.4$ Hz) together with the methyl group signals at δ 0.98 and 1.08 were assigned to a dimethyl cyclopropane ring fused to a hydroazulene skeleton. The ^{13}C NMR data of **3** (see Table 1) as well as the information derived from ^{1}H–^{1}H-COSY, HMQC and HMBC NMR (Fig. 3) led to structure **3**. Its relative configuration resulted from the NOESY spectrum (Fig. 4). Assignment

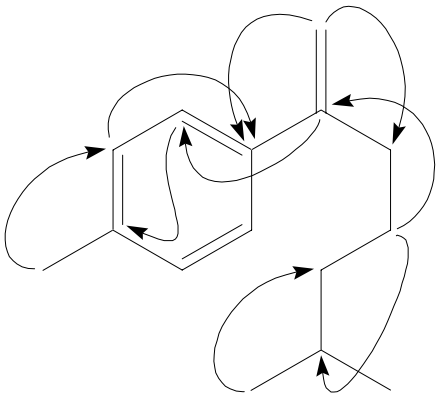


Fig. 1. Long-range ^{1}H–^{13}C of **1**.

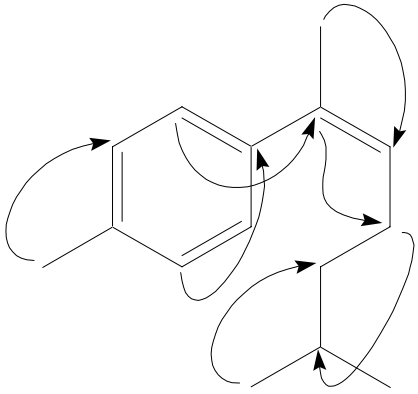


Fig. 2. Long-range ^{1}H–^{13}C of **2**.

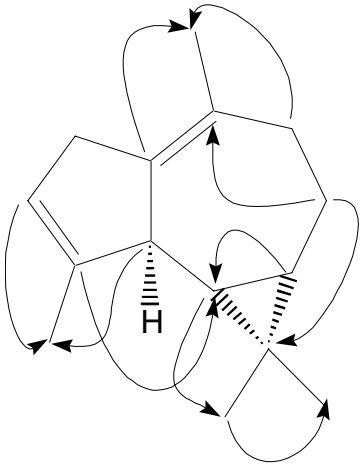


Fig. 3. Long-range ^{1}H–^{13}C of **3**.

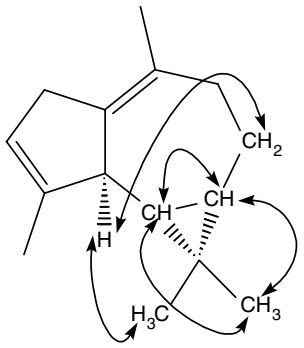
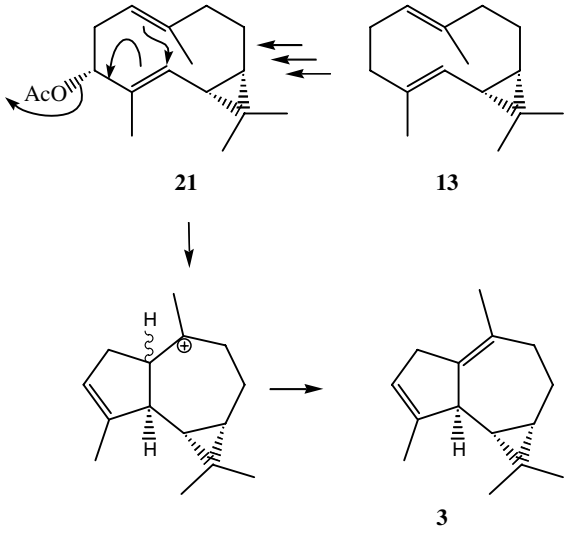


Fig. 4. NOE correlations for **3**.



Scheme 2. Proposed biogenetic relationship of **3**, **13** and **21**.

of the α-orientation of the cyclopropane ring was based on considerations concerning the biogenesis of **3** and its co-occurence with (−)-bicyclogermacrene (**13**) isolated from the same essential oil (Scheme 2). The spatial interactions of proton H-6 with H-7, and one of the methyl group protons, H-12, in addition to the interactions of proton H-5 with H-8a and the second methyl group, H-13, were in agreement with the *cis*-fused cyclopropane ring.

(+)-Muurolan-4,7-peroxide (**4**, 1.4%), a new tricyclic oxygenated muurolane derivative, exhibits a fragmented ion signal at *m/z* 204 as the heaviest ion detected under EIMS. Since the EIMS gave no conclusive indication of the corresponding molecular ions, the chemical ionization was carried out. Compound **4** gave fragmented ion signals at *m/z* 221 ($M^+ + 1\text{-}H_2O$) and *m/z* 223 ($M^+ + 1\text{-}O$) on chemical ionization (CI) using *iso*-butane and ammonia as reactant gases respectively. Hence the molecular formula could not be detected even by CI-MS, however, the structure was concluded from the atmospheric pressure chemical ionization techniques (APCI) which indicated a weak peak at *m/z* 239 $[M + 1]^+$. The elemental analysis experiment of **4** confirmed the absence of nitrogen. The ^{13}C-PENDANT and HMQC spectra revealed the presence of 15 carbon centers, and demonstrated that a total of 26 protons were directly attached to the carbon skeleton. Thus, the molecular formula of **4** should be $C_{15}H_{26}O_2$ to account for the anomaly observed from the chemical ion-

ization fragments at *m/z* 221 and 223. The ^{1}H NMR spectrum (C_6D_6) showed four methyl signals at δ 0.78 (3H, *d*, H-12, $J = 6.9$ Hz), 0.87 (3H, *d*, H-14, $J = 6.9$ Hz), 1.10 (3H, *d*, H-13, $J = 6.6$ Hz) and 1.28 (3H, *s*, H-15). The two quaternary carbon signals at δ 79.7 (*s*) and 86.1 (*s*) were assigned to the oxygen-linked carbons C-4 and C-7, respectively. The ^{13}C NMR data of **4** (Table 1) in addition to the infomation from the ^{1}H–^{1}H-COSY, HMQC and HMBC NMR data (Fig. 5) led to structure **4**. Its relative configuration was derived from the NOESY spectrum (Fig. 6). The α-orientation of the six membered-ring was assigned from the spatial interactions of H-6 with one of the isopropyl methyl protons, H-12.

The absolute configuration of **4** was elucidated by both direct rigorous hydrogenation and acid rearrangement reactions (Scheme 3). The comparison of the four fully saturated diastereoisomeric amorphanes (molecular mass 208) of **4** with that of the fully hydrogenated products of authentic (−)-amorpha-4,7(11)-diene (**5**, obtained from the liverwort *Marsupella aquatica*, Adio et al., 2002) by enantioselective GC on a modified cyclodextrin stationary phase confirmed the identity of the products derived from **4** and **5** and the absolute configuration of **4** (Scheme 3). In addition, treatment of **4** with an acidic ion exchange resin (Amberlyst) for 2 h at room temperature afforded (−)-*epi*-zonarene (**6**) (85%) as the major product together with its dehydrogenation products (+)-*trans*-calamenene (**7**) and (−)-*cis*-calamenene (**8**) as minor components (Scheme 3). Compound **4** was resistant to treatment with lithium aluminium hydride ($LiAlH_4$).

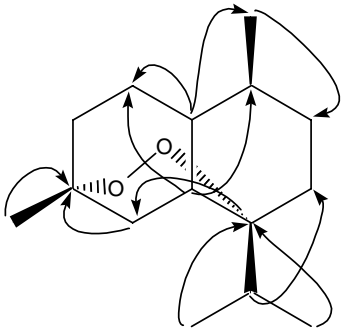


Fig. 5. Long-range ^{1}H–^{13}C of **4**.

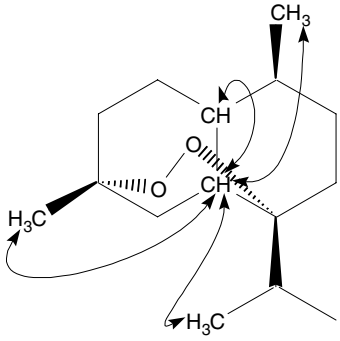
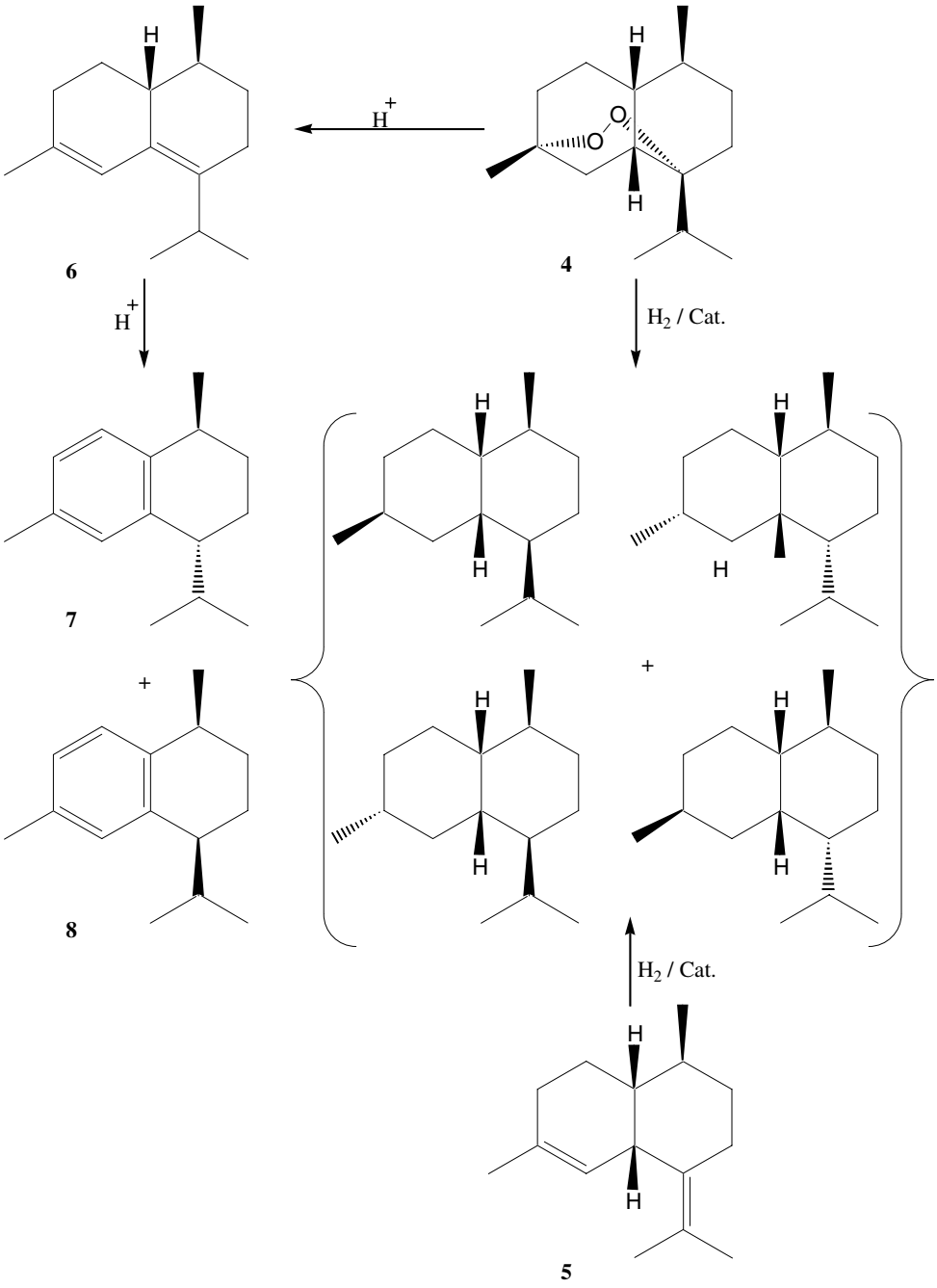


Fig. 6. NOE correlations for **4**.

Compound **9**, named plagio-4,7-peroxide (19.5%) is an *abeo*-muurolane or *abeo*-amorphane. **9** showed fragmented ion signals at *m/z* 220 (1.7), 221 (1), 222 (<1), as the heaviest ions detected under direct inlet EIMS and a chemical ionization fragmented ion signals at *m/z* 221 ($M^+ + 1\text{-}H_2O$) and *m/z* 223 ($M^+ + 1\text{-}O$), similar to compound **4** using *iso*-butane and ammonia as reactant gases respectively. The molecular formula was concluded from the atmospheric pressure chemical ionisation (APCI) techniques which indicated a very weak peak at *m/z* 239 $[M + 1]^+$. The elemental analysis of **9** also confirmed the absence of nitrogen. The ^{1}H NMR spectrum (C_6D_6) of **9** revealed signals of four methyl groups at δ 0.81 (3H, *d*, H-12, $J = 6.3$ Hz), 0.91 (3H, *d*, H-14, $J = 6.3$ Hz), 1.23 (3H, *d*, H-13, $J = 6.6$ Hz) and 1.30 (3H, *s*, H-15). A well resolved longrange ^{4}J-coupling was observed for methylene proton H-5b at δ 1.94 (1H, *dd*, $J = 1.9$, 10.4 Hz) and H-7 at δ 3.30 (1H, *d*, $J = 10.4$ Hz). Additional useful information was obtained from the ^{13}C NMR data (Table 1) as well as from the ^{1}H–^{1}H-COSY, HMQC and HMBC spectra and led to structure **9** (Fig. 7(a)). The proposed relative configuration at C-1, C-4, C-6 and C-10 was established by the NOESY spectrum (Fig. 7(b)). Compound **9** was sufficiently stable to rearrangements on addition of acidic ion exchange resin (Amberlyst) and few drops of concentrated hydrochloric acid (HCl) in methanol for 2 h respectively. The peroxy bridge of compound **9** was also resistant to catalytic hydrogenation and treatment with lithium aluminium hydride ($LiAlH_4$). Thus, compound **9** was assumed to be a peroxide based on the interpretation of the atmospheric pressure chemical ionisation (APCI) mass spectrometry only which does not correlate with an epoxide function with a molecular mass of $M = 222$.

Nagashima et al. (2003) have isolated the peroxy compound **9** from *Plagiochila asplenioides* in addition to several known aromadendrane- and gymnomitrane-type compounds. The NMR data of **9** are reported for the first time. In addition, another compound with the same skeletal backbone has also recently been isolated from the seeds of *Artemisia annua* and this structure was proposed to be derived from amorphanes/muurolanes after rearrangement of the decalin ring by Brown et al. (2003).

Compounds **10** (0.8%) and **11** (0.5%) are *seco*-aromadendranes isolated as minor components in addition to the known plagiochiline H (**22**, an acetylated hemiacetal isolated from the liverwort *Plagiochila yokogurensis* by Asakawa et al., 1980a). These colourless compounds were isolated from the oxygenated fraction of the essential oil by preparative GC using a γ-cyclodextrin derived



Scheme 3. Transformations and hydrogenation products of **4**.

chiral stationary phase. The assigned names, plagiochiline W (**10**) and plagiochiline X (**11**) are proposed in accordance with *seco*-aromadendranes of the structurally related plagiochilines A–V isolated from *Plagiochila* species (Asakawa et al., 1979, 1980a,b; Matsuo et al., 1981; Valcic et al., 1997).

(+)-Plagiochiline W (**10**), a tricyclic ether, exhibited a molecular ion signal at *m/z* 218 and the elemental composition of $C_{15}H_{22}O$. The ^1H NMR spectrum (C_6D_6) of **10** indicated signals of four methyl groups at δ 0.89 (3H, *d*, H-15, J = 7.3 Hz), 0.92 (3H, *s*, H-12), 0.99 (3H, *s*, H-13) and 1.77 (3H, *s*, H-14). The highly deshielded signal at δ 6.78 (1H, *s*) was assigned to the proton on C-2 (δ, 142.4) of the dihydropyran ring. Plagiochiline X (**11**), a dehydrogenated form of **10** with a 4(15)-exomethylene group, gave a molecular formula of $C_{15}H_{20}O$ as shown by HRMS (M^+: *m/z* 216.1488). The ^1H NMR spectrum (C_6D_6) of **11** indicated signals similar to **10**. Compound **11** showed three methyl groups at δ 0.91 (3H, *s*, H-12), 1.02 (3H, *s*, H-13) and 1.73 (3H, *s*, H-14). The secondary methyl signal at δ 0.89 (3H, *d*, H-15, J = 7.3 Hz) of **10** was replaced by the exomethylene protons δ 4.83 (1H,

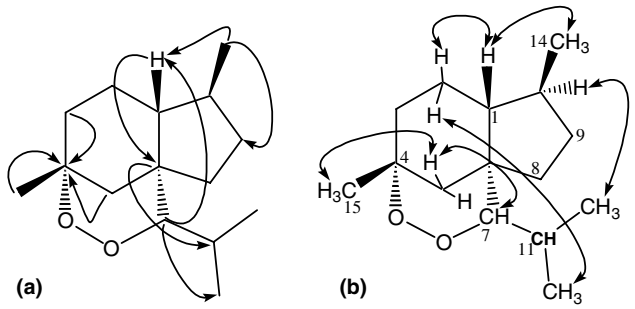


Fig. 7. (a) Long-range ^{1}H–^{13}C of **9** and (b) NOE correlations for **3**.

s, H-15a) and 4.95 (1H, *br.s*, H-15b). This was confirmed by ^{13}C NMR signals at δ 111.6 (*t*) and 144.6 (*s*). The methylene protons at C-3 of **10** at δ 3.49 (1H, *dd*, $J = 5.1$, 10.4 Hz) and 3.78 (1H, *dd*, $J = 2.5$, 10.4 Hz) were also slightly deshielded to δ 4.08 (1H, *d*, $J = 11.3$ Hz) and 4.27 (1H, *d*, $J = 11.3$ Hz) in **11**. Additional structural information was obtained from the ^{13}C NMR data of **10** and **11** (see Table 1). The information from ^{13}C NMR as well as from ^{1}H–^{1}H-COSY, HMQC and HMBC (Fig. 8) led to structures **10** and **11**. The proposed relative configuration at C-4, C-5, C-6 and C-7 were established by the NOESY spectrum of **10** (Fig. 9) and is supported by the co-occurence of (−)-**3**, (−)-**13** and (+)-**22**.

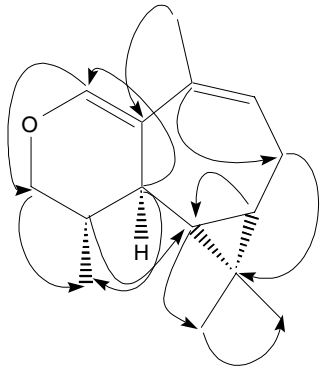


Fig. 8. Long-range ^{1}H–^{13}C of **10**.

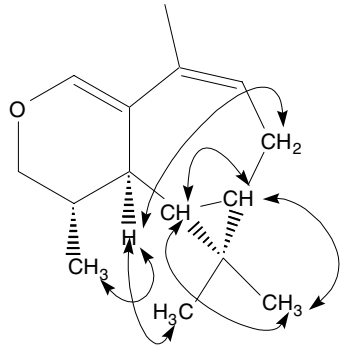


Fig. 9. NOE correlations for **10**.

(−)-4-*epi*-Maaliol (**12**), a tricyclic sesquiterpene alcohol which exhibited a molecular ion signal at *m*/*z* 222 and the elemental composition of $C_{15}H_{26}O$ was isolated as a minor component (1.3%). This is the first occurence of **12** in the Hepaticae. The (+)-enantiomer of 4-*epi*-maaliol (**12**) has been isolated from Brazilian vassoura oil (*Baccharis dracunculifolia*, Asteraceae) by Weyerstahl et al. (1995) and its relative configuration was determined by ^{1}H-, ^{13}C- and NOESY NMR-spectra. (+)-(**12**) had also been synthesized from 4-*nor*-maali-4-one by Büchi et al. (1959) but it could only be characterized by its melting point and its infrared spectrum at that time. The stereochemistry of **12** was further confirmed by treatment with an acidic ion exchange resin (Amberlyst 15) at room temperature for two hours. The acid rearrangement reaction of **12** gave β-maaliene (**23**, 9.0%), β-gorgonene (**24**, 4.0%), selina-5,11-diene (**17**, 2.5%), (+)-δ-selinene (**16**, 75.0%, proved by comparison with an authentic reference compound by enantioselective GC), maalioxide (**25**, 1.0%), selina-5,7(11)-diene (**14**, 0.9%), and a trace of rosifoliol (**26**, 0.2%). The epimerisation of the 4-methyl group from α-to β-orientation during the dehydration process of (−)-**12** can be rationalized by the more favourable stereochemistry after formation of the transient carbocation (Scheme 4).

3. Experimental

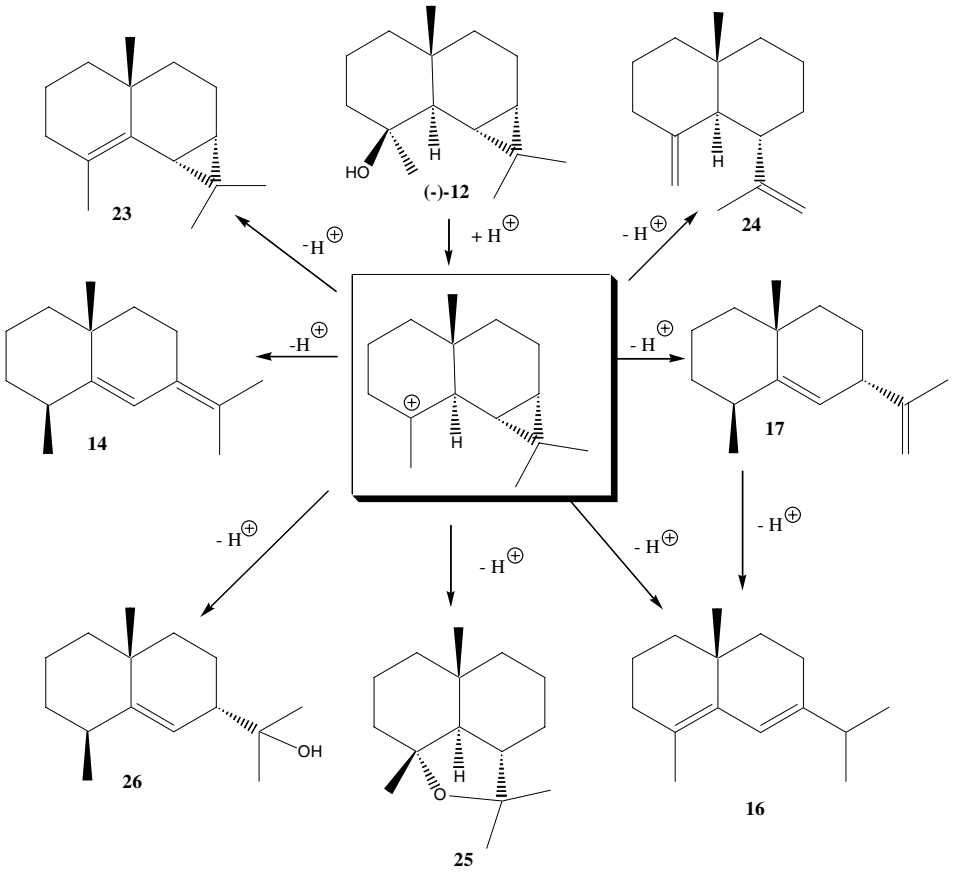
3.1. General experimental procedures

3.1.1. Gas chromatography

Orion Micromat 412 double column instrument with 25 m fused silica capillaries with polysiloxane CPSil-5 and polysiloxane 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).



Scheme 4.

3.1.3. GC-MS

Electron impact (70 eV) and chemical ionization (using NH_3 and *iso*-butane as reagent gases) GC-MS was carried out using a Hewlett-Packard HP 5890 gas chromatograph coupled with a VG Analytical 70-250S magnetic field mass spectrometer. The APCI probe was carried out using MAT 95XL Thermo Quest Finnigan and the corona discharge was kept at 4.43 kV.

3.1.4. NMR spectroscopy

NMR spectra were recorded with a Bruker WM 400 or a Bruker WM 500 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 direction of optical rotation is given to avoid inaccurracies.

3.1.6. Thin layer chromatography

Thin layer chromatography was effected using glass and aluminum plates of silica 60 F_{254} (Merck). An ethanolic solution of sulfuric acid (10%) and anisaldehyde was used as spray reagent. The solvent system used was *n*-hexane:ethyl acetate (3:1, v/v).

3.1.7. Reactions

Hydrogenation reactions were 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 temperature for 1 h. The reaction mixture was filtered and the reaction products were analyzed by GC-MS and by GC on several capillary columns with cyclodextrin derivatives.

Reduction reactions were performed by adding a suspension of $LiAlH_4$ (2 mg) in dry Et_2O to the sample in dry Et_2O and stirred at room temperature for 1 h. The reaction mixture was quenched with water and partitioned with diethyl ether. The mixture was filtered and the reaction products were analyzed by GC-MS and by GC on several capillary columns with cyclodextrin derivatives.

3.2. Plant material and essential oil

The essential oil was prepared by hydrodistillation (2 h) of aqueous homogenates of partially air-dried plants using *n*-hexane as collection solvent. Because of

the greatly differing weight the plant material was not weighed.

3.3. Isolation of single constituents of the essential oils

All isolations were carried out by prep. GC using SE-30- and/or SE-52-columns combined with at least one cyclodextrin derived stationary phase.

3.4. Bisabola-1,3,5,7(14)-tetraene (**1**)

Colourless oil; $RI_{CPSIL\ 5} = 1483$; ^1H NMR (500 MHz, C_6D_6): δ 0.82 (6H, *d*, H-12, H-13, $J = 6.6$ Hz), 1.16 (2H, *q*, H-10, $J = 6.9$ Hz), 1.40–1.50 (3H, *m*, H-11, H-9), 2.12 (3H, *s*, H-15), 2.44 (2H, *t*, H-8, $J = 7.5$ Hz), 5.05 (1H, *d*, H-14a, $J = 1.3$ Hz), 5.34 (1H, *d*, H-14b, $J = 1.6$ Hz), 7.01 (2H, *d*, H-2, H-4, $J = 7.9$ Hz), 7.33 (2H, *d*, H-1, H-5, $J = 8.2$ Hz); ^{13}C NMR (125.7 Hz, C_6D_6): see Table 1; MS (EI, 70 eV), *m/z* (rel. int.): 202 [M^+] (18), 187 (5), 159 (8), 145 (32), 132 (100), 131 (35), 119 (17), 117 (40), 115 (26), 105 (17), 77 (8), 65 (10), 55 (9), 41 (21). HREIMS calcd. for $C_{15}H_{22}$ [M^+] *m/z* 202.1721 found [M^+] *m/z* 202.1723.

3.5. Bisabola-1,3,5,7-tetraene (**2**)

Colourless oil; $RI_{CPSIL\ 5} = 1557$; ^1H NMR (500 MHz, C_6D_6): δ 0.88 (6H, *d*, H-12, H-13, $J = 6.9$ Hz), 1.28 (2H, *q*, H-10, $J = 6.9$ Hz), 1.48–1.60 (1H, *m*, H-11, $J = 6.9$ Hz), 1.96 (3H, *s*, H-14), 2.14(2H, *q*, H-9, $J = 7.6$ Hz), 2.16 (3H, *s*, H-15), 5.84 (1H, *br. t*, H-8, $J = 6.9$ Hz), 7.04 (2H, *d*, H-2, H-4, $J = 8.2$ Hz), 7.34 (2H, *d*, H-1, H-5, $J = 8.2$ Hz); ^{13}C NMR (125.7 Hz, C_6D_6): see Table 1; MS (EI, 70 eV), *m/z* (rel. int.): 202 [M^+] (28), 145 (100), 132 (71), 131 (40), 115 (20), 105 (27), 91 (16), 77 (8), 65 (5), 53 (6), 41 (18). HREIMS calcd. for $C_{15}H_{22}$ [M^+] *m/z* 202.1721 found [M^+] *m/z* 202.1726.

3.6. (−)-Aromadendra-1(10),3-diene (**3**)

Colourless oil; $RI_{CPSIL\ 5} = 1512$; sense of optical rotation (benzene): (−); ^1H NMR (500 MHz, C_6D_6): δ 0.55–0.60 (1H, *m*, H-7), 0.68 (1H, *t*, H-6, $J = 10.4$ Hz), 0.98 (3H, *s*, H-12), 1.08 (3H, *s*, H-13), 1.55 (3H, *s*, H-14), 1.56–1.62 (1H, *m*, H-8a), 1.66–1.73 (1H, *m*, H-8b), 1.68 (3H, *s*, H-15), 2.13–2.27 (2H, *m*, H-9), 3.00 (1H, *d*, H-2a, $J = 20.5$ Hz), 3.06 (1H, *d*, H-5, $J = 10.7$ Hz), 3.14 (1H, *d*, H-2b, $J = 20.5$ Hz), 5.34–5.39 (1H, *m*, H-3); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI, 70 eV), *m/z* (rel. int.): 202 [M^+] (22), 187 (10), 159 (44), 145 (26), 133 (79), 132 (100), 131 (30), 117 (18), 105 (58), 91 (21), 77 (16), 67 (8), 53 (8), 41 (26). HREIMS calcd. for $C_{15}H_{22}$ [M^+] *m/z* 202.1721 found [M^+] *m/z* 202.1729.

3.7. (+)-Muurolan-4,7-peroxide (**4**)

Colourless oil; $RI_{CPSIL\ 5} = 1485$; sense of optical rotation (benzene): (+); ^1H NMR (500 MHz, C_6D_6): δ 0.78 (3H, *d*, H-12, $J = 6.9$ Hz), 0.81–0.85 (1H, *m*, H-9a), 0.87 (3H, *d*, H-14, $J = 6.9$ Hz), 1.10 (3H, *d*, H-13, $J = 6.6$ Hz), 1.15 (1H, *d*, H-5a, $J = 11.4$ Hz), 1.28 (3H, *s*, H-15), 1.30–1.44 (3H, *m*, H-1, H-3a, H-10), 1.58–1.62 (2H, *m*, H-2), 1.66–1.78 (5H, *m*, H-3b, H-8, H-9b, H-11), 1.82 (1H, *ddd*, H-5b, $J = 2.2$, 4.7, 11.4 Hz), 2.00 (1H, *t*, H-6, $J = 4.7$ Hz); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI, 70 eV), *m/z* (rel. int.): 204 (4), 180 (14), 179 (100), 162 (12), 161 (90), 135 (10), 119 (18), 105 (55), 95 (17), 81 (29), 67 (9), 55 (14), 43 (26). MS (CI, NH_3 gas), *m/z* (rel. int.): 223 ($M^+ + 1 - O$) (3), 221 ($M^+ + 1 - H_2O$) (1), 205 (100), 179 (45), 161 (24) 149 (2) 135 (5). MS (CI, *iso*-butane gas), *m/z* (rel. int.): 223 ($M^+ + 1 - O$) (2) 221 ($M^+ + 1 - H_2O$) (10), 205 (85) 179 (100), 161 (47), 149 (12), 135 (8).

3.8. (+)-Plagio-4,7-peroxide (**9**)

Colourless oil; $RI_{CPSIL\ 5} = 1420$; sense of optical rotation (benzene): (+); ^1H NMR (500 MHz, C_6D_6): δ 0.81 (3H, d, H-12, $J = 6.3$ Hz), 0.91 (3H, *d*, H-14, $J = 6.3$ Hz), 1.01 (1H, *dt*, H-1, $J = 4.7$, 12.6 Hz), 1.10–1.18 (1H, *m*, H-9a), 1.21–1.28 (2H, *m*, H-5a, H-3a), 1.23 (3H, *d*, H-13, $J = 6.6$ Hz), 1.30 (3H, *s*, H-15), 1.44–1.58 (3H, H-8, H-2a), 1.64 (1H, *dd*, H-3b, $J = 5.7$, 13.2 Hz), 1.68–1.76 (1H, *m*, H-10), 1.77–1.91 (2H, *m*, H-9b, H-2b), 1.94 (1H, *dd*, H-5b, $J = 1.9$, 10.4 Hz), 2.12–2.20 (1H, *m*, H-11), 3.30 (1H, *d*, H-7, $J = 10.4$ Hz); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI, 70 eV), *m/z* (rel. int.): 222 (<1), 221 (1), 220 (1.7), 207 (3), 179 (8), 161 (5), 150 (47), 135 (100), 121 (13), 107 (21), 95 (28), 94 (35), 93 (21), 79 (17), 67 (10), 55 (16), 43 (27). MS (CI, NH_3 gas), *m/z* (rel. int.): 223 ($M^+ + 1 - O$) (3), 221 ($M^+ + 1 - H_2O$) (1), 205 (100), 179 (3), 161 (2) 149 (2) 150 (22) 135 (34). MS (CI, *iso*-butane gas), *m/z* (rel. int.): 223 ($M^+ + 1 - O$) (2) 221 ($M^+ + 1 - H_2O$) (18), 205 (100) 179 (12), 161 (8), 150 (76), 135 (67).

3.9. (+)-Plagiochiline W (**10**)

Colourless oil; $RI_{CPSIL\ 5} = 1627$; sense of optical rotation (benzene): (+); ^1H NMR (500 MHz, C_6D_6): δ 0.49 (1H, *dd*, H-6, $J = 9.5$, 11.0 Hz), 0.89 (3H, *d*, H-15, $J = 7.3$ Hz), 0.92 (3H, *s*, H-12), 0.97–0.99 (1H, *m*, H-7), 0.99 (3H, *s*, H-13), 1.52–1.58 (1H, *m*, H-4), 1.77 (3H, *s*, H-14), 1.96 (1H, *dd*, H-5, $J = 4.10$, 11.0 Hz), 2.30 (2H, *t*, H-8, $J = 7.6$ Hz), 3.49 (1H, *dd*, H-3a, $J = 5.05$, 10.4 Hz), 3.78 (1H, *dd*, H-3b, $J = 2.5$, 10.4 Hz), 5.44 (1H, *t*, H-9, $J = 6.3$ Hz), 6.78 (1H, *s*, H-2); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI, 70 eV), *m/z* (rel. int.): 218 [M^+] (100), 203 (18),

175 (25), 161 (27), 147 (30), 133 (28), 119 (42), 110 (22), 105 (57), 95 (52), 91 (56), 77 (35), 69 (37), 55 (46), 41 (62). HREIMS calcd. for $C_{15}H_{22}O_1$ [M^+] *m/z* 218.1671 found [M^+] *m/z* 218.1656.

3.10. (+)-Plagiochiline X (**11**)

Colourless oil; $RI_{CPSIL\ 5}$ = 1657; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.71 (1H, *dd*, H-6, *J* = 9.8, 11.0 Hz), 0.91 (3H, *s*, H-12), 1.01–1.03 (1H, *m*, H-7), 1.02 (3H, *s*, H-13), 1.73 (3H, *br.s*, H-14), 2.09 (2H, *br.t*, H-8, *J* = 7.9 Hz), 2.99 (1H, *d*, H-5, *J* = 11.0 Hz), 4.08 (1H, *d*, H-3a, *J* = 11.3 Hz), 4.27 (1H, *d*, H-3b, *J* = 11.4 Hz), 4.83 (1H, *s*, H-15a), 4.95 (1H, *br.s*, H-15b), 5.44 (1H, *t*, H-9, *J* = 6.0 Hz), 6.78 (1H, *s*, H-2); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI, 70 eV), *m/z* (rel. int.): 216 [M^+] (91), 201 (26), 187 (16), 173 (43), 159 (45), 147 (72), 145 (68), 131 (36), 119 (48), 105 (58), 93 (45), 91 (100), 77 (52), 65 (26), 53 (28), 41 (79). HREIMS calcd. for $C_{15}H_{20}O_1$ [M^+] *m/z* 216.1514 found [M^+] *m/z* 216.1488.

3.11. (−)-4-epi-Maaliol (**12**)

Colourless oil; $RI_{CPSIL\ 5}$ = 1548; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.54–0.59 (2H, *m*, H-5, H-6), 0.64-0.71 (2H, *m*, H-9a, H-7), 0.86 (3H, *s*, H-12), 0.90 (1H, *dt*, H-1a, *J* = 3.5, 12.9 Hz), 1.02 (6H, *s*, H-13, H-15), 1.10 (1H, *dd*, H-3a, *J* = 8.8, 13.2 Hz), 1.17 (3H, *s*, H-14), 1.20 (1H, *dt*, H-3b, *J* = 4.4, 13.8 Hz), 1.32–1.40 (2H, *m*, H-2a, H-1b), 1.52 (1H, *dd*, H-8a, *J* = 7.9, 15.1 Hz), 1.56–1.61 (1H, *m*, H-9b), 1.79–1.88 (1H, *m*, H-8b), 1.92–2.01 (1H, *m*, H-2b); ^{13}C NMR (125.7 MHz, C_6D_6): δ 16.2 (*q*, C-12), 16.3 (*t*, C-8), 17.5 (*s*, C-11), 18.8 (*t*, C-2), 19.4 (*q*, C-14), 20.0 (*d*, C-6), 21.7 (*d*, C-7), 29.0 (*q*, C-13), 30.4 (*q*, C-15), 32.9 (*s*, C-10), 40.7 (*t*, C-1), 41.6 (*t*, C-9), 41.7 (*t*, C-3), 47.3 (*d*, C-5), 71.2 (*s*, C-4); MS (EI, 70 eV), *m/z* (rel. int.): 222 [M^+] (6), 204 (40), 189 (72), 179 (8), 161 (55), 147 (24), 133 (42), 123 (39), 121 (38), 109 (58), 107 (55), 105 (56), 91 (55), 81 (73), 67 (44), 55 (46), 43 (100). HREIMS calcd. for $C_{15}H_{26}O_1$ [M^+] *m/z* 222.1984 found [M^+] *m/z* 222.1981.

3.12. (−)-Selina-5,7(11)-diene (**14**)

Colourless oil; $RI_{CPSIL\ 5}$ = 1558; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 1.14 (3H, *s*, H-14), 1.17–1.25 (2H, *m*, H-1a, H-9a), 1.19 (3H, *d*, H-15, 7.6 Hz), 1.35–1.60 (5H, *m*, H-3, H-1b, H-2a, H-9b), 1.67 (3H, *br.s*, H-13), 1.75–1.80 (1H, *m*, H-2b), 1.76 (3H, *br.s*, H-12), 2.24 (1H, *br.t*, H-8a, *J* = 14.2 Hz), 2.44–2.55 (2H, *m*, H-4, H-8b), 6.32 (1H, *s*, H-6); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI, 70 eV), *m/z* (rel. int.): 204 [M^+] (98), 189 (100), 161 (40), 148 (21), 147 (20), 133 (43), 119 (22), 105 (30), 91 (31), 77 (14), 55 (16), 41 (32).

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