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Volatile constituents in the liverwort *Tritomaria polita*

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

The essential oil of the liverwort *Tritomaria polita*, collected in Ötztal/Tyrol (Austria), was investigated by chromatographic and spectroscopic methods. Several new compounds were isolated by preparative gas chromatography (GC) and their structures investigated by mass spectrometry (MS) and NMR techniques. In addition to known constituents, the sesquiterpenoids (+)-eudesma-3,11-dien-8-one, (+)-eudesma-3,7(11)-dien-8-one, (+)-6,11-epoxy-eudesmane, (-)-6,7-*seco*-eudesm-7(11)-en-6-al, (+)-6β-hydroxy-eudesm-11-ene, (-)-6α-hydroxy-eudesm-11-ene, (+)-6,11-epoxy-isodaucane could be identified as natural compounds for the first time.

Keywords: *Tritomaria polita*; Liverwort; Sesquiterpene hydrocarbons; Oxygenated sesquiterpenes; *seco*-Eudesmane

1. Introduction

Tritomaria polita (a liverwort), previously taxonomically separated and listed as *Saccobacis polita* (Nees) Buch (Frahm and Frey, 1992), occurs at elevations above 1500 m in the Bavarian and Austrian Alps. In contrast to *Tritomaria quinquedentata*, which has been investigated before by several research groups (Tori et al., 1993; Warmers and König, 1999) the species *Tritomaria polita,* to our knowledge, was not. We now report that in the essential oil of *T. polita* monoterpenes and sesquiterpene hydrocarbons are present only as minor constituents, but major peaks of oxygenated sesquiterpenes with an eudesmane backbone can be detected by GC–MS. This may be an indication of the relationship to *T. quinquedentata*, where the oxygenated eudesmanes 7-*epi*-isojunenol and 7-*epi*-junenol together with α-selinene are major constituents (Warmers and König, 1999).

2. Results and discussion

The essential oil of *T. polita* was prepared by hydrodistillation and analysed by GC and GC–MS. All known constituents were identified by mass spectrometry in conjunction with their gas chromatographic retention indices by comparison with a spectral library established under identical experimental conditions (Joulain and König, 1998; MassFinder software and data bank, Hochmuth et al., 2002). Sesquiterpenoids with unknown mass spectra were selected for isolation by preparative GC and subjected to NMR spectroscopic investigation. The known sesquiterpene hydrocarbons were β-elemene, aromadendrene, *allo*-aromadendrene (1.5%), 4,5-di-*epi*-aristolochene, α-amorphene, eremophilene, (+)-α-selinene (5%) (Williams et al., 1995), δ-amorphene (Melching et al., 1997), selina-3,7(11)-diene identified (relative concentrations of major compounds in parentheses, others below 1%).

The optical rotation of the new compounds was determined by polarimetric measurements. Enantioselective GC using cyclodextrin phases in conjunction with chemical conversions was employed to assign the absolute configuration.

(+)-Eudesma-3,11-dien-8-one (**1**), was isolated for the first time as a natural product. The ^{1}H NMR ($CDCl_3$) and the positive optical rotation of **1** were consistent with the reported oxidation product of *ent*-8β-hydroxy-eudesma-3,11-diene (Kondo et al., 1990). Its relative configuration was derived from the NOESY spectrum (Fig. 1). Treatment of (+)-**1** with activated basic alumina for two days at room temperature afforded

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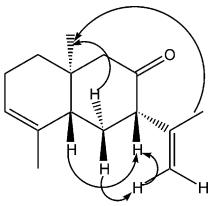
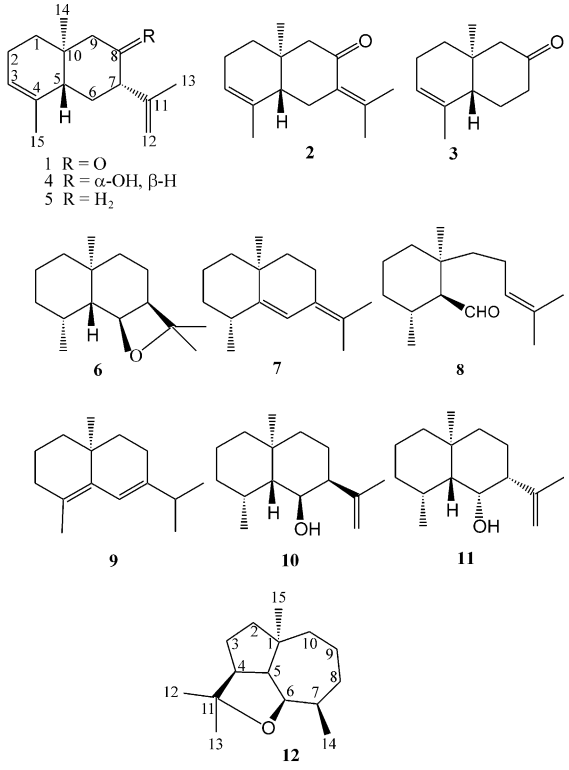


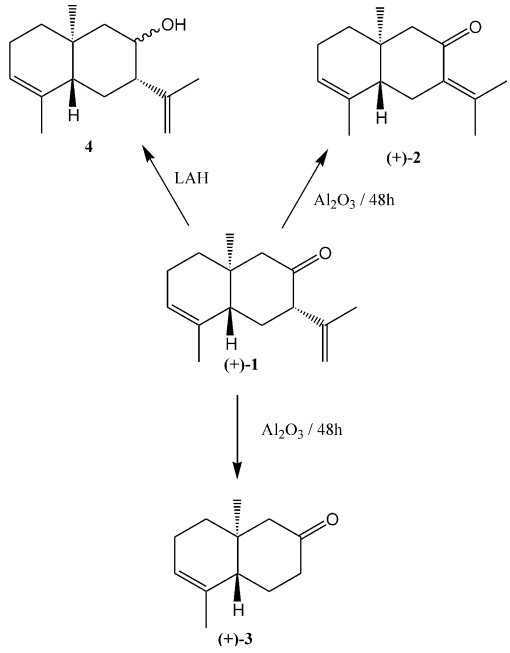
Fig. 1. NOE correlations observed for (+)-**1**.

(+)-eudesma-3,7(11)-diene-8-one (**2**) and (+)-3,4,4a*R*,7,8,8a*R*-hexahydro-5,8a-dimethylnaphthalen-2(1H)-one (**3**), (Schemes 1 and 2). The latter is assumed to be a degradation product since it exhibited a molecular ion at *m*/*z* 178 corresponding to $C_{12}H_{18}O$. A comparison of the ^{1}H NMR spectral data (C_6D_6) of (+)-**3** with **2** showed the disappearance of the signals at δ1.85 (*s*, 3H, CH_3-12), 4.77 (*s*, 1H, H-13a) and 4.97 (*s*, 1H, H-13b) and thus revealed the loss of an isopropenyl group. This was confirmed by the ^{1}H NMR spectrum in $CDCl_3$. It also showed the disappearance of the signals at δ 1.78 (*s*, 3H, CH_3-12), 4.77 (*s*, 1H) and 4.96 (*s*, 1H) corresponding to the isopropenyl residue. The GC–MS and the ^{1}H NMR spectrum ($CDCl_3$) of (+)-**3** was consistent with the enantiomeric reaction product obtained by Vial et al. (1989) by degradation of podocarpic acid. In addition, the mass spectrometric investigation of a sample of (+)-**3** kept in chloroform solution at room temperature for 24 h showed a compound with a molecular ion at *m*/*z* 288. This suggests further degradation of (+)-**3** in the presence of traces of HCl in the chloroform solution. Compound (+)-**1** was also sensitive to heat. Isolation by prep. GC always resulted in a mixture with (+)-**2** (8%). The instability of (+)-**1** could be due to formation of a resonance stabilized allylic carbonium ion by loss of hydrogen from the α-position to the carbonyl group.

Compound **2**, an α,β-unsaturated ketone with positive optical rotation, was also present in the essential oil of *T. polita*. It showed the identical mass spectrum and same retention times on both achiral and chiral capillary GC columns with the product formed from **1**. The enantiomer of **2** has been reported as a constituent of the liverwort *Bazzania fauriana* (Toyota and Asakawa, 1988) and the racemic compound, resulting from the oxymercuration–demercuration reaction of germacrone was reported by Tsankova and Ognyanov (1980). The ^{1}H NMR data ($CDCl_3$) of (+)-**2** are in agreement with the reported data. The ^{13}C NMR spectral data (C_6D_6) of (+)-**1** and (+)-**2** are reported for the first time (Table 1). The absolute configuration of (+)-**1** and (+)-**2** were deduced by chemical correlation with (+)-α-selinene (**5**) as shown in Schemes 2 and 3. Reduction of (+)-**1** with lithium aluminium hydride gave a sesquiterpene alcohol whose GC–MS data were identical to the isolated *ent*-8α-hydroxy-eudesma-3,11-diene (**4**). All spectral data and the direction of optical rotation were in agreement with a compound described by Kondo et al. (1990) as a constituent of the liverwort *Bazzania spiralis*. The absolute configuration of (+)-**4** from *T. polita* was elaborated by comparison of its hydrogenation products with that of the hydrogenation products of authentic (+)-α-selinene (**5**) by capillary GC with different cyclodextrin derived chiral stationary phases



Scheme 1.



Scheme 2.

Table 1
^{13}C NMR spectral data of compounds **(+)-1** and **(+)-2** (125.7 MHz, C_6D_6); δ (ppm)[a]

Carbon	(+)-1	(+)-2
1	37.3(*t*)	36.8(*t*)
2	23.0(*t*)	22.8(*t*)
3	122.1(*d*)	122.5(*d*)
4	133.5(*s*)	133.9(*s*)
5	45.6(*d*)	43.8(*d*)
6	30.5(*t*)	29.4(*t*)
7	59.1(*d*)	145.1(*s*)
8	207.3(*s*)	200.6(*s*)
9	55.7(*t*)	56.4(*t*)
10	36.9(*s*)	33.4(*s*)
11	144.3(*s*)	131.2(*s*)
12	20.9(*q*)	22.7(*q*)
13	113.2(*t*)	23.8(*q*)
14	16.9(*q*)	17.2(*q*)
15	21.3(*q*)	21.0(*q*)

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

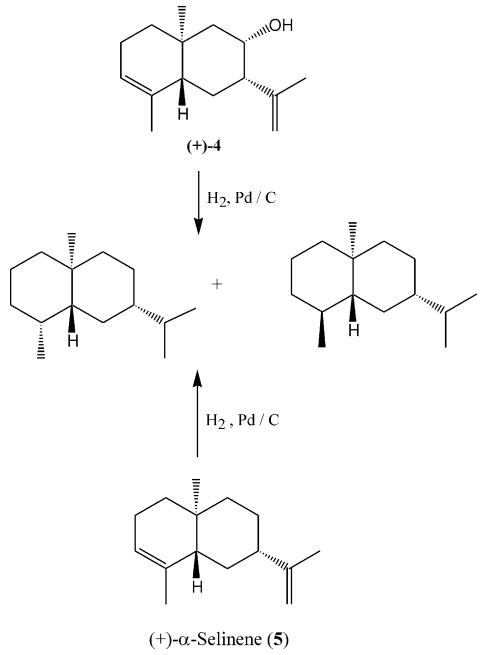
Table 2
^{13}C NMR spectra data for compounds (+)-**6**, (−)-**8**, (+)-**10**, (−)-**11** and (+)**12** (125.7 MHz, C_6D_6), δ (ppm)[a]

Carbon	(−)-8	(+)-6	(+)-10	(−)-11	(+)-12
1	37.3(*t*)	41.8(*t*)	44.0(*t*)	42.7(*t*)	42.6(*s*)
2	21.7(*t*)	22.2(*t*)	22.2(*t*)	21.4(*t*)	41.9(*t*)
3	34.5(*t*)	36.6(*t*)	38.0(*t*)	37.6(*t*)	26.4(*t*)
4	27.6(*d*)	33.8(*d*)	33.4(*d*)	30.0(*d*)	54.2(*d*)
5	64.2(*d*)	55.5(*d*)	53.7(*d*)	55.3(*d*)	62.8(*d*)
6	205.3(*d*)	78.0(*d*)	70.5(*d*)	66.6(*d*)	82.7(*d*)
7	125.2(*d*)	40.1(*d*)	46.3(*d*)	51.2(*d*)	41.7(*d*)
8	22.1(*t*)	18.9(*t*)	22.1(*t*)	22.5(*t*)	38.6(*t*)
9	43.9(*t*)	39.9(*t*)	38.8(*t*)	44.2(*t*)	23.5(*t*)
10	36.4(*s*)	33.1(*s*)	34.0(*s*)	34.0(*s*)	41.2(*t*)
11	131.2(*s*)	83.5(*s*)	146.5(*s*)	147.8(*s*)	81.0(*s*)
12	25.9(*q*)	31.9(*q*)	23.9(*q*)	23.0(*q*)	32.2(*q*)
13	17.6(*q*)	25.1(*q*)	113.2(*t*)	111.9(*t*)	23.8(*q*)
14	20.0(*q*)	18.2(*q*)	21.8(*q*)	20.6(*q*)	20.3(*q*)
15	20.9(*q*)	20.7(*q*)	22.8(*q*)	20.0(*q*)	28.1(*q*)

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

(Scheme 3). The co-existence of (+)-**1**, (+)-**2** and (+)-**4** suggested a structural and stereochemical relationship.

The new tricyclic compound (+)-**6** exhibited a molecular ion at *m*/*z* 222 and an elemental composition of $C_{15}H_{26}O$. The ^{13}C NMR spectrum revealed the presence of 15 carbon resonances. ^{1}H NMR and HMQC spectroscopic analysis indicated a total of 26 protons directly attached to the carbon skeleton. The ^{1}H NMR spectrum showed signals of a doublet and three singlets for methyl groups at δ 1.09 (*d*, 3H, *J* = 5.9 Hz), 0.58 (*s*, 3H), 1.30 (*s*, 3H) and 1.31 (*s*, 3H), respectively. The strongly downfield shifted signal at δ 4.28 (*dd*, 1H, *J* = 7.4, 8.7 Hz) was assigned to an oxygenated methine proton at H-6. Information from 2D ^{1}H–^{1}H-COSY, HMBC, HMQC spectra in addition to the ^{13}C NMR and DEPT (Table 2) suggested structure **6** with an eudesmane backbone. Its relative configuration resulted from spatial interactions of H-14 with the protons of H-6, H-7 and H-15 as observed from the NOESY spectrum (Fig. 2). Treatment of **6** with acidic ion exchange resin (Amberlyst) for 2 h at room temperature gave an unknown hydrocarbon (*m*/*z* 204, base peak 108) (4%) and (+)-**7** (7%), (-)-δ-selinene (**9**) (24%) (proved by comparison with an authentic reference compond by enantioselective GC) and compounds (+)-**10** (33%), (−)-**11** (19%) and (−)-**8** (2.1%), which were also present in the essential oil of *T. polita* (Scheme 4). Selcia-5, 7(11)-diene(7) with unspecified configuration was previously mentioned as a constituent of Olibanum essential oil by Maupetit (1985), but no spectrocsopic data were given. Scheme 4 shows a possible biogenetic relationship between compounds (+)-**6**, (−)-**8**, (+)-**10** and (−)-**11**. Thus (−)-**8** may be a direct precursor of (−)-**11** as similar treatment of (−)-**8** with acidic ion exchange resin for 2 hours gave predominantly (−)-**11** (75%) and (−)-δ-selinene (**9**) (13%). Interestingly, no trace of (+)-**10** was observed among the reaction products of (−)-**8**. This suggests that (+)-**10** is formed directly by acid hydrolysis of (+)-**6** via a cationic intermediate. The formation of the isomeric alcohols (+)-**10** and (−)-**11** confirmed the C-O-linkage at C-6 in (+)-**6**. Scheme 4 also suggests that (−)-**8** may be formed directly from



Scheme 3.

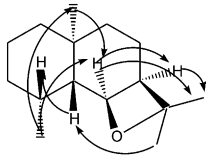
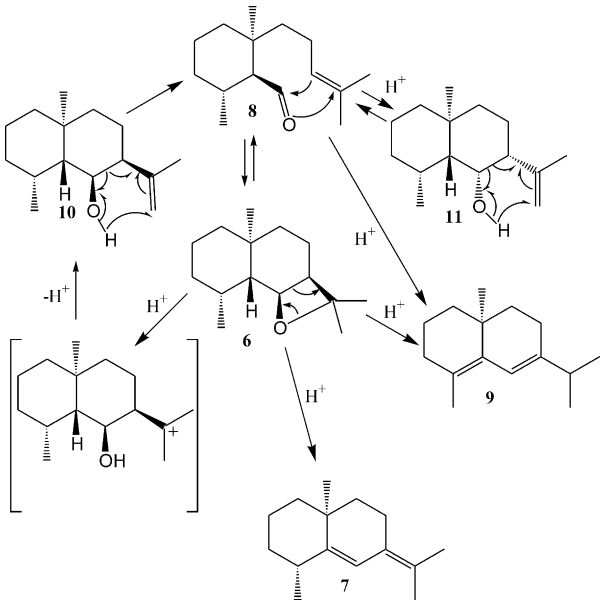


Fig. 2. NOE correlations observed for (+)-**6**.



Scheme 4.

(+)-**6** via a concerted mechanism involving the opening of the oxetane ring in the absence of protons ([2+2] cycloreversion). From the co-occurrence of (+)-**6** and (−)-**8** it cannot be excluded that the latter is an artefact formed during the isolation procedure. In analogy to reactions described by Coxon et al. (1972) it is also possible that one of the isomeric alcohols (+)-**10** or (−)-**11** is a precursor of (−)-**8**. The presence of (−)-δ-selinene (**9**) in the acid hydrolysis products confirmed the α-orientation of the methyl group at C-10.

The new sesquiterpene hydrocarbon (+)-**7**, an acid hydrolysis product of (+)-**6**, exhibited a molecular ion at *m/z* 204 and an elemental composition of $C_{15}H_{24}$. The ^{1}H NMR spectrum (C_6D_6) showed signals of a doublet and three singlets for methyl groups at δ 1.09 (*d*, 3H, *J* = 6.6 Hz), 1.07 (*br.s*, 3H), 1.70 (*br.s*, 3H) and 1.80 (*br.s*, 3H), respectively. The strongly downfield shifted signal at δ 6.32 (*br.s*, 1H) was assigned to the methine proton H-6. (+)-**7** has a mass spectrum almost identical to (−)-δ-selinene (**9**). The structure of (+)-**7** was derived from the ^{1}H NMR spectral data by comparison with the known data of (-)-(**9**). Its absolute configuration was deduced from its relationship to (+)-**6**.

(−)-6,7-*seco*-Eudesma-7(11)-en-6-al (**8**), a trace component isolated as an oil by preparative GC, has a new *seco*-eudesmane skeleton. The mass spectrum exhibited a molecular ion at *m/z* 222 and an elemental composition of $C_{15}H_{26}O$. The ^{1}H NMR spectrum (C_6D_6) showed signals of a doublet and three singlets for methyl groups at δ 0.68 (3H, *d*, *J* = 6.0 Hz), 0.81 (3H, *s*), 1.60 (3H, *s*) and 1.68 (3H, *s*), respectively. The singlets of methyl groups at δ 1.60 and 1.68 indicate that they are attached to a double bond. The downfield signal at δ 9.46 (1H, *d*, H-6, *J* = 4.4 Hz) was assigned to the aldehyde proton. The ^{13}C NMR spectral data, confirmed by HMBC and HMQC, are listed in Table 2. The 2D ^{1}H–^{1}H-COSY, HMBC (Table 3) and HMQC spectra confirmed the structure of (−)-**8**. In the NOESY spectrum of (−)-**8**, spatial interactions between the aldehyde proton and the methyl protons H-14 and H-15 were observed. This indicates that H-14 and H-15 are on the same side of the molecule. Treatment of (−)-**8** with acidic ion exchange resin afforded (−)-δ-selinene (**9**) (13%) and (−)-**11** (75%) (Scheme 4). The latter, which is isomeric to (+)-**10** was also isolated. The correlation with (−)-**9** suggests α-orientation for the methyl group at C-10. Hence, the methyl group at C-4 should also be in the α-orientation in agreement with the NOESY spectrum.

Table 3
Important ^{1}H-^{13}C long-range HMBC couplings of (−)-6,7-*seco*-eudesm-7(11)-ene-6-al (**8**)

Carbon	Protons
C-4	H-2a, H-2b, H-3a, H-5, H-15.
C-5	H-1a, H-3a, H-4, H-6, H-9a, H-9b, H-14, H-15.
C-6	H-4/5, H-15.
C-7	H-8a, H-8b, H-9a, H-9b, H-12, H-13.
C-11	H-8a, H-8b, H-12, H-13.

(+)-6β-Hydroxy-eudesm-11-ene (**10**), a sesquiterpene alcohol with eudesmane skeleton exhibited a molecular ion at *m/z* 222 and an elemental composition $C_{15}H_{26}O$. The ^{1}H NMR (C_6D_6) spectrum showed signals of a doublet and two singlets for methyl groups at δ 1.31 (*d*, 3H, *J* = 6.3 Hz), 0.82 (*s*, 3H) and 1.63 (*s*, 3H), respectively. The olefinic carbon signals at 113.2 (*t*) and 146.5 (*s*), suggested an exomethylene double bond, which was confirmed by two signals in the ^{1}H NMR spectrum at δ 4.81 (*s*, 1H) and 4.88 (*s*, 1H). The signal at δ 70.5 was assigned to a secondary carbon with the hydroxyl group. All this information from ^{13}C NMR and DEPT spectroscopic (Table 2) as well as from 2D ^{1}H–^{1}H-COSY, HMQC, and HMBC analysis were consistent with the assigned structure (+)-**10**. Its relative configuration was derived from the NOESY spectrum (Fig. 3). Rigorous catalytic hydrogenation of (+)-**10** resulted in a simultaneous hydrogenation and dehydration to give two fully saturated diastereoisomeric eudesmanes (molecular mass 208), which were compared with the fully hydrogenated products of authentic (+)-α-selinene (**5**). Enantioselective GC showed that one of the hydrogenation products of (+)-**10** gave identical retention times with a hydrogenation product

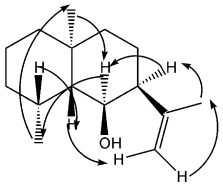


Fig. 3. NOE correlations observed for (+)-**10**.

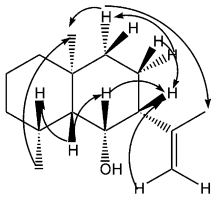


Fig. 4. NOE correlations observed for (−)-**11**.

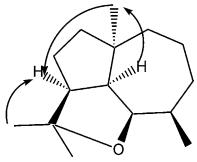


Fig. 5. NOE correlations observed for (+)-**12**.

of (+)-**5**. This is possible if an intermediate with a C-6-C-7 double bond is formed, which generates two diastereoismeric products upon hydrogenation. Thus the absolute configuration at the stereogenic centers C-5 and C-10 could be assigned and was in agreement with the NOESY spectrum. The β-orientation of the hydroxyl group at C-6 and of the isopropenyl group at C-7 was derived from the spatial interactions of H-6 and H-7 with α-H-14. Interestingly, compound (−)-**11**, a sesquiterpene alcohol with a molecular ion at *m*/*z* 222 and an elemental composition of $C_{15}H_{26}O$ showed identical GC- and mass spectra as well as ^{1}H NMR, ^{13}C NMR (Table 2), 2D ^{1}H–^{1}H-COSY, HMBC and HMQC spectral data as (+)-**10**. (−)-**11** also gave similar results when subjected to catalytic hydrogenation, but in this case the different absolute configuration at C-7 was clearly shown by the NOESY spectrum (Fig. 4), which indicated spatial interactions between H-5, H-6 and H-7, thus suggesting that H-7 is in β-orientation with respect to H-5. The structures of the two isomeric alcohols (+)-**10** and (−)-**11** are in agreement with the correlations shown in Scheme 4.

(+)-**12**, a new tricyclic skeleton with isodaucane backbone was isolated with the hydrocarbon fraction of the liverwort. (+)-**12** exhibited a molecular ion at *m*/*z* 222 and an elemental composition of $C_{15}H_{26}O$. The ^{1}H NMR showed signals of a doublet and three singlets for methyl groups at δ 1.21 (*d*, 3H, *J* = 6.6 Hz), 0.85 (*s*, 3H), 1.17 (*s*, 3H) and 1.30 (*s*, 3H), respectively. A signal at δ 3.22 (*t*, 1H, *J* = 9.8 Hz) was assigned to the oxygenated methine H-6 which coupled with an adjacent proton H-5 at δ 1.99 (*t*, 1H, *J* = 9.5 Hz). The 2D ^{1}H–^{1}H-COSY, HMQC and HMBC spectra in addition to the ^{13}C NMR (Table 2) confirmed the structure. Its relative configuration resulted from the NOESY spectrum (Fig. 5). No visible changes were observed in the GC and GC–MS of (+)-**12** after treatment with acidic ion exchange resin for 2–8 h. This suggests that (+)-**12** is a relatively stable compound. Its absolute configuration remains undetermined.

Considering the major sesquiterpenes constituents in the investigated *Tritomaria* species, the inclusion of *T. polita* into the *Tritomaria* family appears chemotaxonomically justified as eudesmane type structures are predominant in both *T. polita* and *T. quinquedentata*. (+)-α-Selinene as a major constituent in both species may serve as a precursor for the oxygenated secondary metabolites formed, however, the site of oxygenation is different. No sesquiterpene lactones are present in *T. polita* but quite abundant in *T. quinquedentata*.

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).

3.1.3. GC–MS

Electron impact (70 eV) and chemical ionization (NH_3) GC–MS were carried out on a Hewlett Packard HP 5890 gas chromatograph coupled with a VG Analytical 70-250S magnetic field mass spectrometer.

3.1.4. NMR spectroscopy

NMR spectra were recorded with either 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 inaccuracies.

3.1.6. Thin layer chromatography

Thin layer chromatography was effected using glass or aluminum supported 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:ethylacetate (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.

Dehydration was carried out with ca. 1 mg of sample in 0.5 ml of pyridine and 1 drop of phosphoryl chloride under ice cooling. After 1 h of stirring at room temp. the reaction was quenched by adding a few drops of water and the mixture was extracted three times with *n*-hexane. The organic phase was washed several times with water and dried over Na_2SO_4.

3.2. Plant material and essential oil

Tritomaria polita was collected on wet rocks in July 2001 close to Obergurgl in the Ötztal, Tyrol/Austria at an elevation of approx. 2300 m. A voucher specimen is kept at the Abteilung für Systematische Botanik und Ökologie, University of Ulm. 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 using SE-30- and/or SE-52-columns combined with at least one cyclodextrin phase column.

3.4. (+)-Eudesma-3,11-dien-8-one (1)

Colourless oil; $RI_{CPSIL\ 5}$ = 1668; R_f = 0.81; sense of optical rotation (chloroform): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.67 (*s*, 3H, CH_3-14), 1.06 (*dd*, 1H, H-1a, *J* = 6.0, 12.6 Hz), 1.23 (*dt*, 1H, H-1b, *J* = 6.6,12.6 Hz), 1.50 (*s*, 3H, CH_3-15), 1.59 (*dd*, 1H, H-6a, *J* = 12.6, 25.5 Hz), 1.78–1.83 (*m*, 2H, H-9a, H-2a), 1.85 (*s*, 3H, CH_3-12), 1.87–1.93 (*m*, 2H, H-6b, H-2b), 2.02 (*br.d*, 1H, H-5, *J* = 12.9 Hz), 2.08 (*d*, 1H, H-9b, *J* = 13.2 Hz), 2.68 (*dd*, 1H, H-7, *J* = 6.3, 12.6 Hz), 4.77 (*s*, 1H, H-13a), 4.97 (*s*, 1H, H-13b), 5.26 (*s*, 1H, H-3). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.79 (3H, *s*), 1.25 (1H, *br. s*), 1.42 (1H, *dd*, *J* = 6.0, 12.9 Hz), 1.57 (1H, *dt*, J = 6.9, 12.6 Hz), 1.68 (3H, *s*), 1.72 (1H, *dd*, 12.9, 25.9 Hz), 1.78 (3H, *s*), 1.99–2.11 (2H, *m*), 2.16 (1H, *ddd*, 3.8, 6.6, 13.7 Hz), 2.22 (1H, *dd*, *J* = 13.9, 29.6 Hz), 2.49 (1H, *br. d*, *J* = 13.9 Hz), 3.03 (1H, *dd*, *J* = 6.3, 12.3 Hz), 4.77 (1H, *s*), 4.96 (1H, *s*), 5.42 (1H, *br. s*). ^{13}C NMR (125.7 MHz, C_6D_6) data see Table 2; MS (EI, 70eV), *m*/*z* (rel. int.) 218 [M^+] (45), 203 (12), 200 (12), 190 (3), 175 (9), 159 (14), 147 (22), 121 (33), 108 (100), 93 (74), 83 (30), 77 (38), 67 (26), 53 (37), 41 (74). HRMS calc. for $C_{15}H_{22}O$ [M]$^+$: *m*/*z* 218.1671, found: 218.1672.

3.5. (+)-Eudesma-3,7(11)-dien-8-one (2)

Colourless oil; $RI_{CPSIL\ 5}$ = 1745 ; R_f = 0.82; sense of optical rotation (chloroform): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.76 (*s*, 3H, CH_3-14), 1.12–1.17 (*m*, 2H, H-1), 1.48 (*s*, 3H, CH_3-12), 1.56 (*br.s*, 3H, CH_3-15), 1.74–1.83 (*m*, 1H, H-2a), 1.87–1.96 (*m,* 3H, H-6a, H-2b, H-5), 2.01 (*d*, 1H, H-9a, *J* = 16.3 Hz), 2.20 (*s*, 3H, CH_3-13), 2.30 (*d*, 1H, H-9b, *J* = 16.2 Hz), 2.57 (*dd*, 1H, H-6b, *J* = 3.8, 14.5 Hz), 5.29–5.34 (*m*, 1H, H-3). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (*s*, 3H), 1.39–1.45 (*m*, 2H), 1.70 (*br.s*, 3H), 1.84 (*s*, 3H), 1.94–2.07 (*m*, 3H), 2.09 (*s*, 3H), 2.12–2.29 (*m*, 3H), 2.80 (*dd*, 1H, *J* = 3.8, 14.2 Hz), 5.42 (*br.s*, 1H). ^{13}C NMR (125.7 MHz, C_6D_6) see Table 2. MS (EI, 70eV), *m*/*z* (rel. int.): 218 [M^+] (100), 203 (22), 189 (4), 175 (17), 161 (19), 147 (30), 135 (17), 121 (55), 108 (47), 98 (20), 93 (46), 91 (44), 83 (40), 77 (33), 67 (32), 53 (35), 41 (73). HRMS calcd for $C_{15}H_{22}O$ [M]$^+$: *m*/*z* 218.1671, found: 218.1667.

3.6. (+)-3,4,4aR,7,8,8aR-Hexahydro-5,8a-dimethyl-naphthalen-2(1H)-one (3)

Colourless oil; $RI_{CPSIL\ 5}$ = 1461; sense of optical rotation (chloroform): (+); ^{1}H NMR (500 MHz, C_6D_6), δ 0.63 (*s*, 3H), 1.04 (*dd*, 1H, *J* = 6.0, 12.6 Hz), 1.15–1.22 (*m*, 3H), 1.46 (*br.s*, 3H), 1.76 (*d,* 1H, *J* = 13.5 Hz), 1.77–1.85 (*m*, 4H), 2.07 (*dd*, 1H, *J* = 2.0, 13.9 Hz), 2.29 (*dt*, 1H, J = 2.5, 14.8 Hz), 5.25 (*br.s*, 1H). ^{1}H NMR (500 MHz, $CDCl_3$), δ 0.78 (*s*, 3H), 1.41 (*dd*, 1H, *J* = 5.67, 12.6 Hz), 1.67 (*s*, 3H), 2.02–2.10 (*m*, 3H), 2.13–2.22 (*m*, 3H), 2.28–2.39 (*m*, 3H), 2.44–2.50 (*m*, 1H), 5.41 (*br.s*, 1H). MS (EI, 70eV) *m*/*z* (rel. int.): 178 [M^+] (45), 163 (100), 150 (47), 145 (10), 135 (18), 121 (40), 107 (41), 105 (37), 95 (40), 93 (61), 91 (42), 81 (82), 79 (63), 77 (39), 67 (37), 53 (38), 41 (63).

3.7. (+)-8α-Hydroxy-eudesma-3,11-diene (4)

Colourless oil; $RI_{CPSIL\ 5}$ = 1669; R_f = 0.77; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): δ = 1.09 (*dd*, 1H, H-9a, *J* = 3.4, 13.9 Hz), 1.20–1.28 (*m*, 1H, H-1a), 1.31 (*s*, 3H, CH_3-14), 1.38 (*dd*, 1H,

H-1b, $J=6.6$, 12.6 Hz), 1.55 (*s*, 3H, CH_3-12), 1.57–1.59 (*m*, 1H, H-6a), 1.62–1.70 (*m*, 1H, H-6b), 1.64 (*s*, 3H, CH_3-15), 1.81–1.88 (*br.d*, 2H, H-5, H-7, $J=11.4$ Hz), 1.92 (*dd*, 1H, H-9b, $J=2.2$, 13.9 Hz), 1.95–1.99 (*m*,1H, H-2a), 2.09–2.16 (*m*, 1H, H-2b), 3.90 (*br.d*, 1H, H-8, $J=2.5$ Hz), 4.79 (*s*, 1H, H-13a), 4.85 (*s*, 1H, H-13b), 5.35 (*br.s*, 1H, H-3). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.04 (*s*, 3H), 1.23-1.28 (*m*, 1H), 1.32 (*dd*, 2H, $J=4.1$, 13.6 Hz), 1.41 (*dd*, 1H, $J=6.3$, 12.3 Hz), 1.61 (*m*, 1H), 1.66 (3H, *br.s*), 1.81 (*s*, 3H), 1.90 (*dd*, 1H, $J=2.5$, 14.5 Hz), 1.96–2.00 (*m*, 2H), 2.06–2.12 (*m*, 1H), 2.12–2.17 (*m*, 1H), 4.05 (*br.d*, 1H, $J=2.52$ Hz), 4.87 (*s*, 1H), 5.02 (*s*, 1H), 5.32 (*br.s*, 1H). ^{13}C NMR (125.7MHz, C_6D_6): δ 18.1 (*q*, C-14), 21.4 (*q*, C-15), 22.6 (*q*, C-12), 23.1 (*t*, C-2), 23.3 (*t,* C-6), 32.3 (*s*, C-10), 38.7 (*t*, C-1), 45.9 (*t*, C-9), 47.6 (*d*, C-5), 51.3 (*d*, C-7), 67.1 (*d*, C-8), 111.9 (*t*, C-13), 121.5 (*d*, C-3), 134.7 (*s,* C-4), 147.2 (*s*, C-11). MS (EI, 70eV) *m*/*z* (rel. int.): 220 [M^+] (33), 205 (12), 202 (31), 187 (41), 177 (17), 161 (44), 147 (34), 131 (22), 123 (23), 121 (54), 119 (49), 108 (47), 107 (78), 105 (67), 95 (31), 93 (84), 9 1(77), 81 (54), 79 (51), 77 (49), 67 (35), 55 (54), 41 (100).

3.8. (+)-6,11-Epoxy-eudesmane (**6**)

Colourless oil; $RI_{CPSIL\ 5}=1534$; $R_f=0.86$; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.58 (*s*, 3H, CH_3-14), 0.90–1.00 (*m*, 1H, H-3a) 1.05–1.12 (*m*, 1H, H-1a), 1.09 (*d*, 3H, CH_3-15, $J=5.9$ Hz), 1.14–1.21 (*m*, 2H, H-4, H-9a), 1.22–1.28 (*m*, 2H, H-1b, H-5), 1.30 (*s*, 3H, CH_3-13), 1.31 (*s*, 3H,CH_3-12), 1.36–1.48 (*m*, 4H,H-9b, H-8a, H-2), 1.49–1.53 (*m*, 1H, H-8b), 1.55–1.62 (*m*, 1H, H-3b), 2.55–2.60 (*m*, 1H, H-7), 4.28 (*dd*, 1H, H-6, $J=7.4$, 8.7 Hz). ^{13}C NMR (125.7 MHz, C_6D_6): see Table 2. MS (EI, 70eV), *m*/*z* (rel. int.): 222 [M^+] (4), 207 (5), 189 (3), 180 (3), 164 (34), 149 (77), 137 (40),123 (50), 109 (90), 95 (35), 81 (50), 69 (46), 55 (52), 41 (100). HRMS calcd for $C_{15}H_{22}O$ [M]$^+$: *m*/*z* 222.1984, found: 222.1971.

3.9. (+)-Eudesma-5,7(11)-diene (**7**)

Colourless oil; $RI_{CPSIL\ 5}=1543$; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 1.07 (*br.s*, 3H), 1.09 (*d*, 3H, $J=6.6$ Hz), 1.70 (*br.s*, 3H), 1.80 (*br.s*, 3H), 6.32 (*br.s*, 1H). MS (EI, 70eV), *m*/*z* (rel. int.): 204 [M^+] (90), 189 (100), 175 (5), 161 (38), 149 (23), 148 (25), 147 (21), 133 (57), 119 (30), 107 (26), 105 (52), 91 (56), 77 (25), 65 (18), 55 (31), 41 (69).

3.10. (−)-6,7-seco-Eudesm-7(11)-en-6-al (**8**)

Colourless oil, $RI_{CPSIL\ 5}=1614$; $R_f=0.95$; sense of optical rotation (benzene): (−);^{1}H NMR (500 MHz, C_6D_6): δ 0.55–0.65 (*m*, 1H, H-3a), 0.68 (*d*, 3H, CH_3-15, $J=6.0$ Hz), 0.81 (*s*, 3H, CH_3-14), 0.93–1.03 (*m*, 1H, H-1a), 1.12–1.17 (*m*, 1H, H-1b), 1.20–1.32 (*m*, 4H, H-2, H-9), 1.42–1.47 (*m*, 1H, H-3b), 1.60 (*s*, 3H, CH_3-13), 1.61–1.65 (*m*, 2H, H-4, H-5), 1.68 (*s*, 3H, CH_3-12), 1.85–1.94 (*m*, 1H, H-8a), 2.06–2.13 (*m*, 1H, H-8b), 5.16 (*dt*, 1H, H-7, $J=1.6$, 7.3 Hz), 9.46 (*d*, 1H, H-6, $J=4.4$ Hz). ^{13}C NMR (125.7 MHz, C_6D_6): see Table 2. MS (EI, 70eV), *m*/*z* (rel. int.): 222 [M^+] (4), 207(4), 180 (3), 161 (2), 147 (2), 137 (38), 123 (7), 109 (35), 95 (21), 81 (22), 69 (53), 55 (39), 43 (23), 41 (100). HRMS calc. for $C_{15}H_{26}O$ [M]$^+$: *m*/*z* 222.1984, found: 222.1961.

3.11. (+)-6β-Hydroxy-eudesm-11-ene (**10**)

Colourless oil; $RI_{CPSIL\ 5}=1643$; $R_f=0.91$; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6),: δ 0.82 (*s*, 3H, CH_3-14), 0.97–1.03 (*m*, 1H, H-3a), 1.04–1.09 (*m*, 2H, H-1a, H-5), 1.15–1.21 (*m*, 1H, H-9a), 1.29–1.34 (*m*, 1H, H-1b), 1.31 (*d*, 3H, CH_3-15, $J=6.3$ Hz), 1.35–1.42 (*m*, 2H, H-9b, H-8a), 1.44–1.53 (*m*, 3H, H-8b, H-2a, H-4), 1.57–1.62 (*m*, 1H, H-2b), 1.63 (*s*, 3H, CH_3-12), 1.64–1.67 (*m*, 1H, H-3b), 2.42–2.47 (*m*, 1H, H-7), 3.61–3.66 (*m*, 1H, H-6), 4.81 (*s*, 1H, H-13a), 4.88 (*s*, 1H, H-13b). ^{13}C NMR (125.7 MHz, C_6D_6): see Table 2. MS (EI, 70eV) *m*/*z* (rel. int.): 222 [M^+] (3), 207 (7), 189 (6), 180 (3), 161 (2), 149 (2), 137 (47), 123 (57), 109 (39), 95 (27), 81 (48), 69 (44), 55 (56), 41 (100). HRMS calc. for $C_{15}H_{26}O$ [M]$^+$: *m*/*z* 222.1984, found: 222.1965.

3.12. (−)-6α-Hydroxy-eudesm-11-ene (**11**)

Colourless oil; $RI_{CPSIL\ 5}=1598$; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.57 (*dd*, 1H, H-5, $J=2.2$, 10.7 Hz), 0.88–0.93 (*m*, 1H, H-3a), 0.98 (*d*, 3H, $CH_3=15$, $J=6.3$Hz), 1.02–1.08 (*m*, 2H, H-1), 1.23 (*s*, 3H, CH_3-14), 1.26–1.31 (*m*, 4H, H-2, H-9), 1.46–1.49 (*m*, 2H, H-8), 1.59 (*s*, 3H, CH_3-12), 1.71–1.78 (*m*, 2H, H-7, H-3b), 1.89–1.95 (*m*, 1H, H-4), 3.92 (*br.s*, 1H, H-6), 4.76 (*s*, 1H, 13a), 4.83 (*s*, 1H, 13b); MS (EI, 70eV), *m*/*z* (rel. int.): 222 [M^+] (6), 207 (8), 204 (16), 189 (14), 180 (5), 161 (6), 139 (35), 137 (56), 123 (100), 109 (40), 95 (32), 81 (52), 69 (41), 55 (53), 41 (79). HRMS calc. for $C_{15}H_{26}O$ [M]$^+$: *m*/*z* 222.1984, found: 222.1974.

3.13. (+)-6,11-Epoxy-isodaucane (**12**)

Colourless oil; $RI_{CPSIL\ 5}=1468$; $R_f=0.97$; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.85 (*s*, 3H, CH_3-15), 0.94 (*dq*, 1H, H-8a, $J=2.5$,12.0 Hz), 1.17 (*s*, 3H, CH_3-13), 1.15–1.23 (*m*, 1H, H-10a), 1.21 (*d*, 3H, CH_3-14, $J=6.6$ Hz), 1.22–1.27 (*m*, 1H, H-9a), 1.30 (*s*, 3H, CH_3-12), 1.40–1.49 (*m*, 3H, H-2, H-9b), 1.57–1.60 (*m*, 4H, H-7, H-3, H-10b), 1.66–1.72 (*m*, 1H, H-8b), 1.99 (*t*, 1H, H-5, $J=9.5$Hz), 2.35–2.40 (*m*, 1H, H-4), 3.22 (*t*, 1H, H-6, $J=9.8$ Hz); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 2. MS (EI, 70eV), *m*/*z* (rel. int.): 222 [M^+] (4), 208 (16), 207 (100), 189 (16),

164 (10), 151 (17), 149 (30), 135 (9), 123 (33), 109 (22), 107 (24), 93 (26), 81 (64), 79 (28), 67 (26), 55 (42), 43 (83), 41 (77). HRMS calc. for $C_{15}H_{26}O$ $[M]^+$: m/z 222.1984, found: 222.1974.

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