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Sesquiterpene constituents of the liverwort *Bazzania trilobata*

Ute Warmers, Wilfried A. König*

Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany

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Abstract

The sesquiterpene constituents of the liverwort *Bazzania trilobata* (L.) Gray were investigated. In addition to many known compounds two new sesquiterpene hydrocarbons, (−)-*cis*-cadina-1(6),4-diene and (+)-*trans*-dauca-4(11),8-diene, could be isolated and identified. The structure elucidation was carried out by NMR spectroscopic techniques and chemical conversions.

Keywords: *Bazzania trilobata*; Jungermanniales; Liverwort; Sesquiterpene; (−)-*cis*-Cadina-1(6),4-diene; (+)-*trans*-Dauca-4(11),8-diene; Enantioselective gas chromatography

1. Introduction

Bazzania trilobata, a leafy liverwort (Hepaticae) of the order Jungermanniales, is widespread in Northern and Central Europe, Eastern Asia, Japan, the USA and Canada (Zinsmeister, Becker & Eicher, 1991; Aichele & Schwegler, 1993). The essential oil of *B. trilobata* has been investigated before (Huneck, 1967; Andersen & Huneck, 1973; Andersen et al., 1977; Asakawa et al., 1979, 1981; Toyota, Asakawa & Takemoto, 1981; Asakawa & Heidelberger, 1982; Huneck et al., 1984; Konecny et al., 1985; Asakawa, 1995; Nagashima et al., 1996). The following sesquiterpenes were described as constituents of *B. trilobata*: α-barbatene, β-barbatene, gymnomitrol, β-bazzanene, isobazzanene, cuparene, δ-cuparenol, β-chamigrene, (+)-*cis*-calamenene, (−)-*cis*-5-hydroxycalamenene, 3-hydroxycalamenene, 2-hydroxycalamenene, 7(10)-peroxycadina-5-ene, ledene, viridiflorol, drimenol and (−)-4(12)-myltaylen-5-ol. A difference was found between the essential oils of *B. trilobata* from Czechoslovakia and from Japan (Konecny et al., 1985). 2-Hydroxycalamenene is solely a constituent of the Czechoslovakian liverwort while δ-cuparenol was only found in the Japanese liverwort.

2. Results and discussion

We have investigated the sesquiterpene constituents of the essential oils of *B. trilobata* collected at several places in Europe and in the USA. Samples of volatile plant constituents were obtained by hydrodistillation or solvent extraction and analysed by gas chromato graphy (GC) and GC–mass spectrometry (GC–MS). There were only small differences in the relative percentages of single constituents in the hydrodistillation and solvent extraction products. Individual components were isolated by preparative GC and investigated by NMR (^{1}H, ^{13}C, ^{1}H–^{1}H-COSY, HMQC, HMBC, NOESY). For structure verification chemical conversions were carried out. The absolute configuration of most compounds was derived by polarimetric measurements and/or enantioselective GC using cyclodextrin phases.

The European and the American liverwort differ in their essential oil constituents. 44 sesquiterpene constituents of the European liverwort and 29 of the American liverwort were identified (Scheme 1 and 2).

* Corresponding author. Tel.: +49-40-4123-2824; fax: +49-40-4123-2893.

E-mail address: wkoenig@chemie.uni-hamburg.de (W.A. König)

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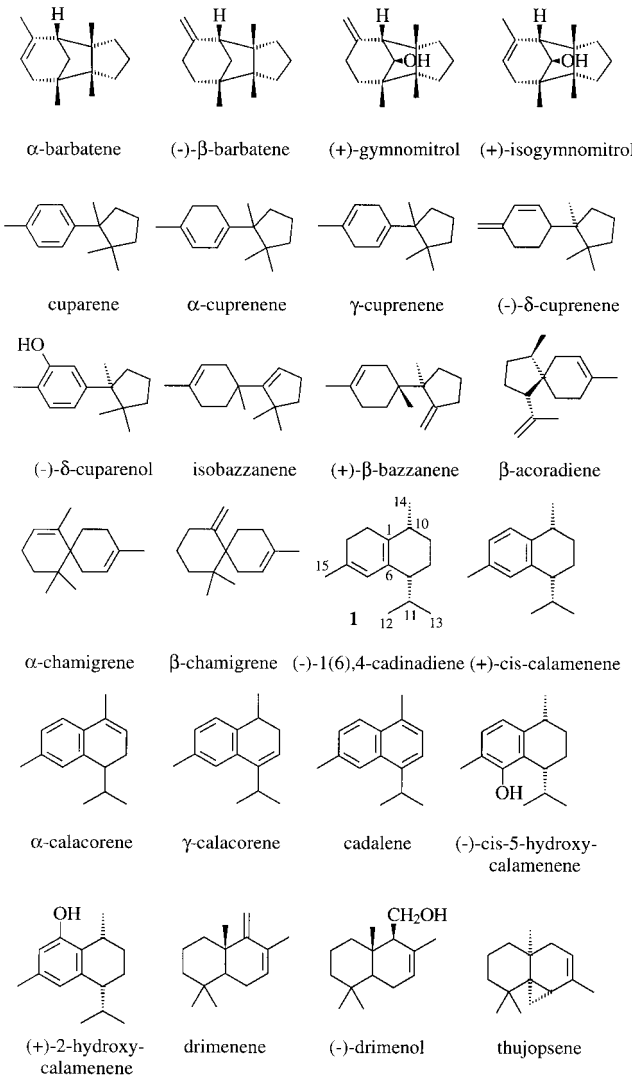
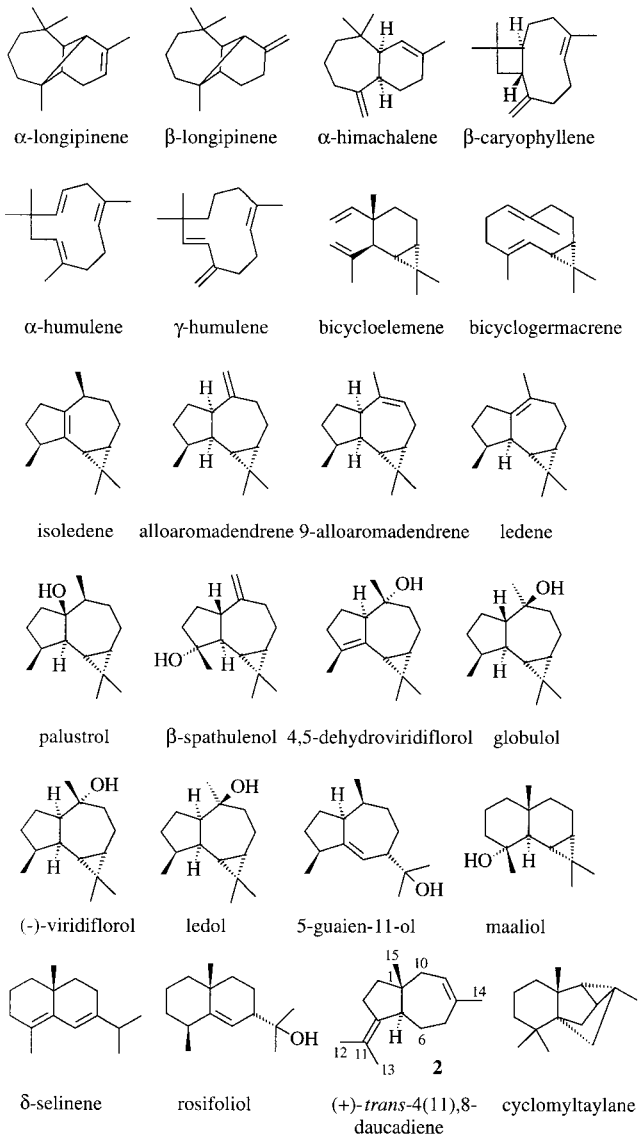
The main difference between the European and the American liverwort is the occurrence of *cis*-2-hydroxycalamenene solely in the European liverwort and of δ-cuparenol solely in the American liverwort (Table 1 and Figs. 1 and 2). This is in agreement with the difference between the Czechoslovakian and the Japanese liverwort.

Most of the identified constituents of *B. trilobata* were already known, but two of them were new: (−)-*cis*-cadina-1(6),4-diene (**1**) and (+)-*trans*-dauca-4(11),8-diene (**2**).

(−)-*cis*-Cadina-1(6),4-diene {(1*R*,4*R*)-1,2,3,4,5,6-hexahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene} (**1**) is a sesquiterpene hydrocarbon with cadinane skeleton. The mass spectrum exhibits a molecular ion signal at *m*/*z* 204 and an elemental composition of $C_{15}H_{24}$. The ^{1}H NMR spectrum shows doublets for three methyl groups at tertiary carbon atoms at δ 0.68, 0.93 and 1.03, a singlet for a methyl group at a quaternary sp^2-carbon atom at δ 1.79 and a singlet for an olefinic proton at δ 5.62. In the ^{13}C- and in the DEPT spectra signals for four primary carbon atoms (δ 17.1, 20.4, 21.3 and 23.6), four secondary carbons (δ 19.0, 28.0, 29.2 and 29.5), four tertiary carbons (δ 29.3, 33.4, 43.0 and 122.1) and three quaternary carbons (δ 129.9, 133.0 and 133.7) are observed. The low field signals were assigned to four olefinic carbons. The 7-epimer of **1**, (−)-*trans*-cadina-1(6),4-diene, has been described as a constituent of manuka essential oil (*Leptospermum scoparium*) (Melching, Bülow, Wihstutz, Jung & König, 1997). The mass spectra of both epimers show only small differences in their relative intensities, but the chemical shifts in the NMR spectra differ markedly. There is also a difference of 12 retention index units



Scheme 1



Scheme 2

Table 1
Sesquiterpene constituents of the liverwort *Bazzania trilobata*

Sesquiterpenes	European liverwort	American liverwort
Bicycloelemene	x	x
α-Longipinene	x	
Cyclomyltaylan	x	
Isoledene	x	x
β-Longipinene	x	
β-Caryophyllene		x
α-Barbatene	x	x
Thujopsene	x	x
Isobazzanene	x	
(−)-β-Barbatene	x	x
α-Himachalene	x	
Alloaromadendrene	x	
α-Humulene	x	x
(−)-*cis*-Cadina-1(6),4-diene	x	x
β-Acoradiene	x	x
β-Chamigrene	x	x
(−)-γ-Humulene	x	x
δ-Selinene	x	
9-Alloaromadendrene	x	
Bicyclogermacrene	x	x
Ledene	x	
Cuparene		x
α-Cuprenene	x	
Drimenene	x	
α-Chamigrene	x	x
(+)-*cis*-Calamenene	x	x
(+)-β-Bazzanene	x	x
γ-Cuprenene		x
α-Calacorene	x	x
(−)-δ-Cuprenene	x	x
γ-Calacorene	x	
(+)-*trans*-Dauca-4(11),8-diene	x	x
Maaliol	x	
Palustrol	x	x
β-Spathulenol	x	x
4,5-Dehydroviridiflorol	x	
Globulol	x	x
(−)-Viridiflorol	x	x
Rosifoliol	x	
Ledol	x	
5-Guaien-11-ol	x	
(+)-Isogymnomitrol	x	x
Cadalene	x	
(+)-Gymnomitrol	x	x
(−)-*cis*-5-Hydroxycalamenene	x	x
(−)-Drimenol	x	x
(+)-*cis*-2-Hydroxycalamenene	x	
(−)-δ-Cuparenol		x

(GC on a non-polar dimethylpolysiloxane stationary phase). The *cis*-isomer (RI = 1460) elutes prior to the *trans*-isomer (RI = 1472).

1 is easily oxidised. The oxidation product was identified as (+)-*cis*-calamenene (**3**) by comparison with both enantiomeric pairs of *cis*- and *trans*-calamenene using enantioselective GC (Fig. 3) (König, Rieck, Hardt & Gehrcke, 1994). **3** is also a constituent of the essential oil of *B. trilobata*.

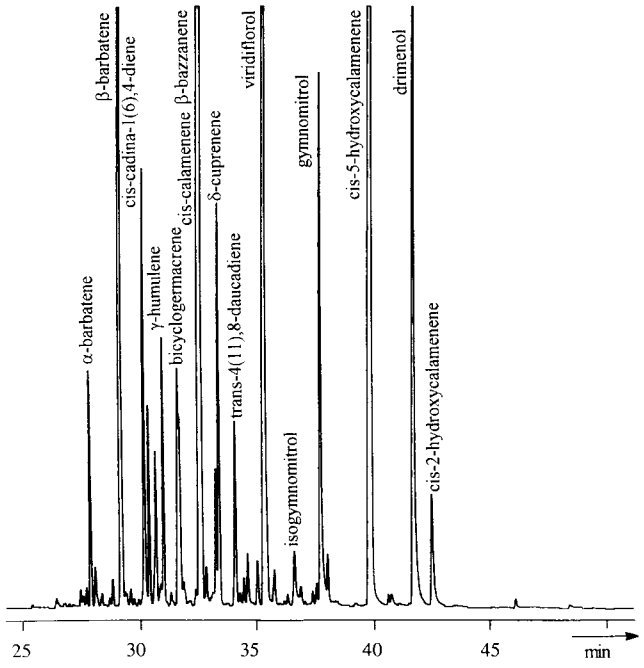


Fig. 1. Partial gas chromatogram of the essential oil of the European liverwort *B. trilobata* on a 25 m fused silica capillary with polysiloxane CpSil5; column temp. 50°C, temp. program 3°C/min to 230°C; carrier gas 0.5 bar H_2.

(+)-*trans*-Dauca-4(11),8-diene {(3a*R*,8a*R*)-1,2,3,3a,4,7,8,8a-octahydro-3a,6-dimethyl-1-(1-methylethylidene)-azulene} (**2**) is a sesquiterpene hydrocarbon

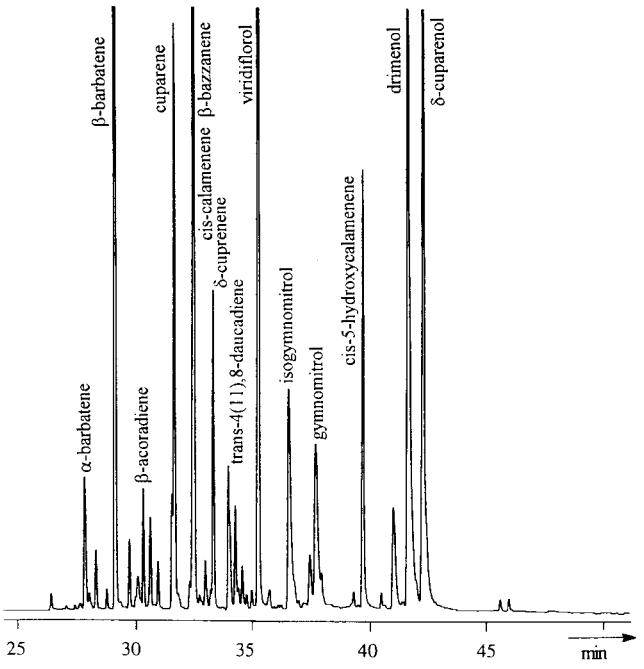


Fig. 2. Partial gas chromatogram of the essential oil of the American liverwort *B. trilobata* on a 25 m fused silica capillary with polysiloxane CpSil5; column temp. 50°C, temp. program 3°C/min to 230°C; carrier gas 0.5 bar H_2.

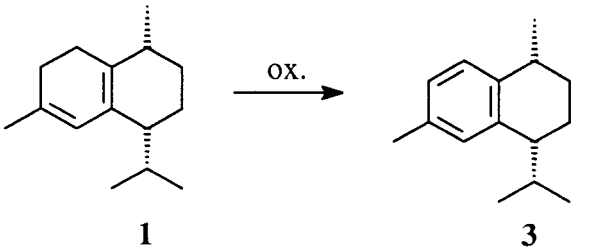


Fig. 3. Oxidation of (−)-*cis*-cadina-1(6),4-diene (**1**) to (+)-*cis*-calamenene (**3**).

with daucane skeleton. The mass spectrum shows a molecular ion signal at *m/z* 204 and an elemental composition of $C_{15}H_{24}$. In the ^1H NMR spectrum singlets for four methyl groups at quaternary carbon atoms are found at δ 0.93, 1.70, 1.76 and 1.81. The three low field resonances indicate methyl groups bonded to sp^2-carbon atoms. At δ 5.61 a multiplet for an olefinic proton can be observed. The ^{13}C- and the DEPT spectra show signals for four primary carbon atoms (δ 17.4, 20.8, 23.0 and 28.7), five secondary carbons (δ 30.0, 30.2, 31.0, 40.2 and 41.2), two tertiary carbons (δ 52.4 and 124.2) and four quaternary carbons (δ 44.7, 109.6, 136.1 and 139.0). The low field signals were assigned to four olefinic carbons.

The relative configuration of the stereogenic centres at C-1 and C-5 could be derived from a NOESY spectrum. The saturation of the resonance of H-15 results in an increase of the signal intensities of H-6b and H-7b but not of H-5. H-5 interacts with H-7a. This suggests *trans*-connection of the two rings (Fig. 4).

Acid rearrangement of **2** yields (+)-daucene (**4**) (Fig. 5). **4** was identified by GC–MS and enantioselective GC. Hydrogenation of **2** and **4** yields identical products: two partially and five completely hydrogenated compounds, which have the same MS and retention times on achiral and chiral GC phases with cyclodextrin derivatives. Under *cis*-addition of hydrogen we expect only four diastereomeric daucanes for **2**

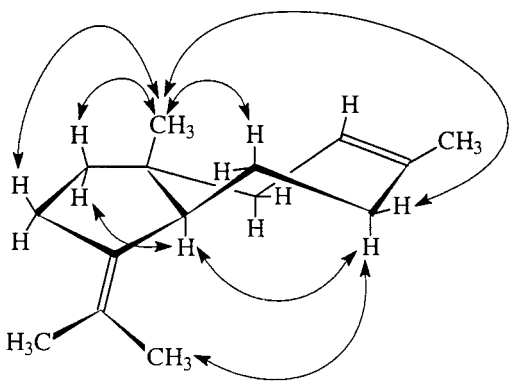


Fig. 4. NOE effects of *trans*-dauca-4(11),8-diene (**2**).

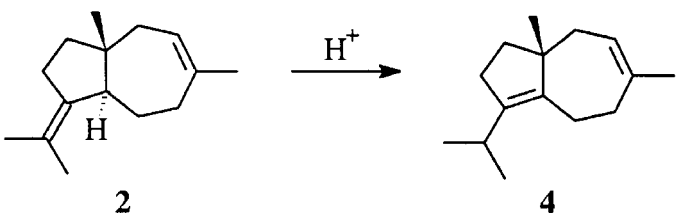


Fig. 5. Acid rearrangement of (+)-*trans*-dauca-4(11),8-diene (**2**) to (+)-daucene (**4**).

and **4**: two identical and two different daucanes (Fig. 6). **4** yields mainly the two saturated daucanes (41 and 33%) formed by *cis*-addition of hydrogen at C-4 and C-5 from the backside of the molecule. **2** yields mainly three saturated daucanes (46, 29 and 21%); two of them were identical with the major hydrogenation products of **4**. The occurrence of the other minor products (1–8%) could be explained by an isomerisation of **2** to **4** and a small amount of *anti*-addition. The correlation of **2** and **4** indicates the same configuration at C-1.

3. Experimental

3.1. Plant material

B. trilobata was collected in Adelberg/Göppingen (Germany), in Bad Herrenalb/Black Forest (Germany), near Göteborg (Sweden) and in the Pickett-National Forest (USA). It was identified by

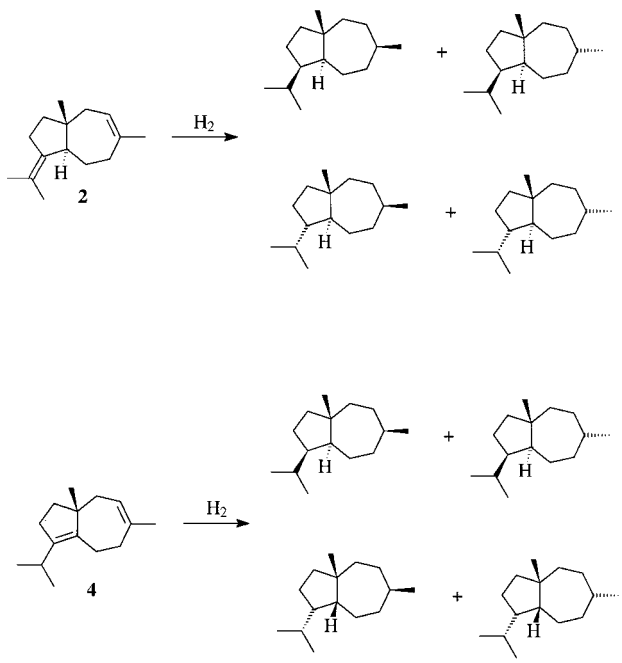


Fig. 6. Catalytic hydrogenation of (+)-*trans*-dauca-4(11),8-diene (**2**) and (+)-daucene (**4**) (expected products).

Dr. H. Muhle, Universität Ulm, Germany. Plant samples are deposited at the herbarium of the Institut für Allgemeine Botanik, Universität Hamburg, Germany. Because of the greatly differing drying state the fresh plant material was not weighed.

3.2. Hydrodistillation

The essential oil was prepared by hydrodistillation (2 h) of aqueous homogenates of fresh and green plants using *n*-hexane as collection solvent.

3.3. Extraction

Samples of plant volatiles were prepared by extraction (48 h, 20°C) of fresh and green plants with diethyl ether or ethyl acetate.

3.4. Gas chromatography

Orion Micromat 412 double column instruments with (a) 25 m fused silica capillaries with polysiloxane CpSil 5 and polysiloxane CpSil 19 (Chrompack) and (b) 25 m fused silica capillaries with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin and heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in OV 1701 (50%, w/w); split injection; flame ionization detection; carrier gas 0.5 bar H_2.

3.5. Isolation

The isolation was carried out using different preparative GC columns.

3.6. Preparative GC

Modified Varian 1400 instrument, equipped with a stainless steel column (1.85 m × 4.3 mm) with 10% polydimethylsiloxane SE 30 on Chromosorb W-HP, or equipped with a stainless-steel column (1.85 m × 4.3 mm) with 5% heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin/OV 1701 (1:1, w/w) on Chromosorb W-HP, or equipped with a stainless-steel column (1.95 m × 5.3 mm) with 2.5% heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin/SE 52 (1:1, w/w) on Chromosorb G-HP; flame ionization detection; helium as carrier gas at a flow rate of 240 ml/min (Hardt & König, 1994).

3.7. NMR spectroscopy

NMR measurements were carried out with a Bruker WM 400- or a Bruker WM 500 instrument using TMS as internal standard.

3.8. Mass spectrometry

GC–MS measurements were carried out with a VG Analytical 70–250S instrument and a HP 5890 gas chromatograph.

3.9. (−)-cis-Cadina-1(6),4-diene (**1**)

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (3H, d, J = 6.6 Hz, H-12/13), 0.93 (3H, d, J = 6.6 Hz, H-12/13), 1.03 (3H, d, J = 7.1 Hz, H-14), 1.40–1.68 (4H, m, H-8a, H-8b, H-9a, H-9b), 1.79 (3H, s, H-13), 1.88–2.22 (7H, m, H-2a, H-2b, H-3a, H-3b, H-7, H-10, H-11), 5.62 (1H, s, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.1 (q, C-12/13), 19.0 (t, C-8/9), 20.4 (q, C-14), 21.3 (q, C-12/13), 23.6 (q, C-15), 28.0 (t, C-8/9), 29.2 (t, C-2/3), 29.3 (d, C-11), 29.5 (t, C-2/3), 33.4 (d, C-10), 43.0 (d, C-7), 122.1 (d, C-5), 129.9 (s, C-1/4/6), 133.0 (s, C-1/4/6), 133.7 (s, C-1/4/6); MS (EI, 70 eV): m/z (rel. int.) 204 (52) [M^+], 189 (16) [$M^+ - CH_3$], 162 (19), 161 (100), 145 (6), 134 (12), 119 (16), 105 (24), 91 (11), 81 (19), 69 (5), 55 (6), 41 (11).

3.10. Oxidation of (−)-cis-cadina-1(6),4-diene (**1**)

1 mg of **1** was kept in $CHCl_3$ at room temp. for 7 days. The reaction product was identified as (+)-*cis*-calamenene (**3**) by GC–MS and by comparison with a sample of both enantiomeric pairs of *cis*- and *trans*-calamenene on diverse capillary GC columns with cyclodextrin derivatives.

3.11. (+)-trans-Dauca-4(11),8-diene (**2**)

^{1}H NMR (400 MHz, C_6D_6): δ 0.93 (3H, s, H-15), 1.30–1.42 (1H, m, H-3b), 1.43–1.54 (2H, m, H-2b, H-3a), 1.70 (3H, s, H-12), 1.67–1.77 (1H, m, H-2a), 1.76 (3H, s, H-13), 1.81 (3H, s, H-14), 1.98–2.07 (1H, m, H-10a), 2.03–2.14 (1H, m, H-7b), 2.18–2.29 (1H, m, H-10b), 2.24–2.34 (1H, m, H-6a), 2.29–2.41 (1H, m, H-6b), 2.35–2.44 (1H, m, H-5), 2.86 (1H, ddd, J = 17.0, 6.1, 2.5 Hz, H-7a), 5.61 (1H, dd, J = 7.6, 1.0 Hz, H-9); ^{13}C NMR (125 MHz, C_6D_6): δ 17.4 (q, C-15), 20.8 (q, C-13), 23.0 (q, C-12), 28.7 (q, C-14), 30.0 (t, C-6), 30.2 (t, C-7), 31.0 (t, C-10), 40.2 (t, C-2), 41.2 (t, C-3), 44.7 (s, C-1), 52.4 (d, C-5), 109.6 (s, C-11), 124.2 (d, C-9), 136.1 (s, C-8), 139.0 (s, C-4); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84 (3H, s, H-15), 1.23–1.34 (1H, m, H-3), 1.36–1.47 (2H, m, H-2b, H-3), 1.62 (3H, s, H-12), 1.68 (3H, s, H-13), 1.72 (3H, s, H-14), 1.66–1.78 (2H, m, H-2a, H-6), 1.84 (1H, bt, J = 14.9 Hz, H-7b), 2.03 (1H, dt, J = 17.7, 3.8 Hz, H-10), 2.16–2.32 (3H, m, H-5, H-6, H-10), 2.66 (1H, ddd, J = 17.0, 6.1, 2.5 Hz, H-7a), 5.45 (1H, dd, J = 7.6, 1.0 Hz, H-9); ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.4 (q, C-15), 20.9 (q, C-13), 23.1 (q, C-12), 28.7 (q, C-14),

29.7 (t, C-7), 30.1 (t, C-6), 31.0 (t, C-10), 40.1 (t, C-2), 41.0 (t, C-3), 44.7 (s, C-1), 52.2 (d, C-5), 122.7 (s, C-11), 123.9 (d, C-9), 136.6 (s, C-8), 139.1 (s, C-4); MS (EI, 70 eV): *m/z* (rel. int.) 204 (54) [M^+], 189 (32) [$M^+ - CH_3$], 175 (5), 161 (95), 147 (15), 136 (39), 135 (61), 121 (83), 119 (65), 107 (94), 105 (74), 93 (100), 91 (63), 81 (33), 79 (48), 77 (41), 67 (26), 55 (44), 41 (76).

*3.12. Acid rearrangement of (+)-trans-dauca-4(11),8-diene (**2**)*

To a sol. of 1 mg of **2** in 1 ml n-hexane, 0.5 mg Amberlyst 15 were added. The reaction mixture was stirred at room temp. for 2 days. The main reaction product was identified as (+)-daucene (**4**) by GC–MS and by comparison with a sample of (+)-daucene on diverse capillary GC columns with cyclodextrin derivatives.

*3.13. Hydrogenation of (+)-trans-dauca-4(11),8-diene (**2**)*

To a sol. of 1 mg of **2** in 1 ml *n*-hexane, 0.5 mg Pd/C were added. The suspension was treated with H_2 and stirred under H_2 at room temp. for 1 h. The reaction mixture was filtered and the reaction products were analysed by GC–MS and by GC on diverse capillary columns with cyclodextrin derivatives.

*3.14. Hydrogenation (+)-daucene (**4**)*

The hydrogenation of **4** was performed analogously to the hydrogenation of **2**. The reaction products were analysed by GC–MS and by GC on diverse capillary columns with cyclodextrin derivatives. The hydrogenated compounds were identical with the reaction products of the hydrogenation of **2**.

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