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Spirocyclopropane-type sesquiterpene hydrocarbons from *Schinus terebinthifolius* Raddi

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ABSTRACT

The essential oil of *Schinus terebinthifolius* fruits was reinvestigated using GC and GC–MS techniques. Apart from several known compounds three sesquiterpene hydrocarbons with a carbon skeleton exhibiting the rare spiro(cyclopropane) moiety could be isolated by a combination of column chromatography and GLC. Structure assignments were carried out by NMR spectroscopy. These natural products are 9-spiro(cyclopropa)-4,4,8-trimethyl-2-methylenbicyclo[4.3.0]non-1(6)-ene (terebanene), 9-spiro(cyclopropa)-2,4,4,8-tetramethylbicyclo[4.3.0]nona-1,5-diene (teredenene), and (6*R**,8*R**)-9-spiro(cyclopropa)-2,4,4,8-tetramethylbicyclo[4.3.0]non-1-ene (terebinthene).

1. Introduction

Schinus terebinthifolius RADDI is an evergreen shrub or tree of the Anacardiaceae, native to South and Central America. In traditional medicine it has been used for the treatment of inflammations (Gazzaneo et al., 2005). The leaves and reddish fruits are rich in essential oil (1.5–10%) and earlier investigations reported high concentrations of monoterpenes (Stahl et al., 1983; Malik et al., 1994) along with some sesquiterpene hydrocarbons (Singh et al., 1998). The present study describes the reinvestigation of the essential oil obtained from the fruits of *S. terebinthifolius*, which resulted in the isolation and structure elucidation of three sesquiterpene hydrocarbons with a new carbon skeleton exhibiting the rare spiro(cyclopropane) moiety (**10**–**12**).

2. Results and discussion

Dried fruits of *S. terebinthifolius* were hydrodistilled (Sprecher, 1963) to afford the essential oil, which was investigated by GC and GC–MS. Several known constituents could be identified by comparison of their mass spectra and GC retention indices with a spectral library established under identical experimental conditions (König et al., 2004; Hochmuth, 2005). Monoterpenoids constitute major components (85%) with high amounts of α-pinene (16.9%), α-phellandrene (21.1%), β-phellandrene (10.8%) and limonene (23.7%). Enantiomeric purities were determined by enantioselective GC using modified cyclodextrins as stationary phases and found to be highly variable between samples from three different locations (Table 1). Furthermore, small amounts of sesquiterpene hydrocarbons like (−)-β-elemene (**1**), (−)-α-gurjunene (**2**), (−)-(*E*)-β-caryophyllene (**3**), and racemic (±)-germacrene D (**4**) could be identified and their absolute configuration determined by enantioselective GC, along with trace quantities of germacrene B (**5**), α-humulene (**6**), α-bergamotene (**7**), *allo*-aromadendrene (**8**), and δ-cadinene (**9**) (Fig. 1).

Three unknown sesquiterpene hydrocarbons (**10**–**12**), which constituted ca. 0.1–0.3% of the total volatiles, were selected for isolation by a combination of column chromatography on silica gel 60, preparative GC on polysiloxane SE-52, and repeated semipreparative GC using megabore thick film capillary columns coated with DB-1 and DB-1701, respectively.

The mass spectrum of **10** exhibited a signal for the molecular ion at *m/z* 202 which was assigned the molecular formula of $C_{15}H_{22}$. The ^{1}H NMR spectrum in C_6D_6 revealed signals for one exocyclic methylene group at δ 4.61 (1H, *s*) and 4.71 (1H, *s*), two tertiary methyl groups at δ 1.07 (3H, *s*) and 1.09 (3H, *s*), and one secondary methyl group at δ 0.95 (3H, *d*, *J* = 6.9 Hz). The ^{13}C PENDANT spectrum (Homer and Perry, 1994) confirmed the presence

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Table 1
Relative concentrations, enantiomeric excess and sign of optical rotation of monoterpenoids in the hydrocarbon fractions of *S. terebinthifolius* fruits from three different locations; determined by GC–FID and enantioselective GC using [a]: heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin or [b]: heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (nd: not determined).

	Botanical Garden Hamburg		Botanical Garden Hohenheim		France	
	(rel %)	*ee* (%)	(rel %)	*ee* (%)	(rel %)	*ee* (%)
α-Pinene[a]	64.2	82.6 (−)	88.9	100 (+)	28.6	25.0 (+)
β-Pinene[a]	15.7	100 (−)	2.3	100 (−)	6.2	9.0 (+)
α-Phellandrene[b]	trace	nd	trace	nd	36.5	91.2 (+)
β-Phellandrene[b]	13.5	100 (+)	0.6	20.0 (+)	14.7	6.4 (+)
Limonene[b]	2.5	45.6 (−)	6.0	99.8 (+)	13.6	94.8 (−)

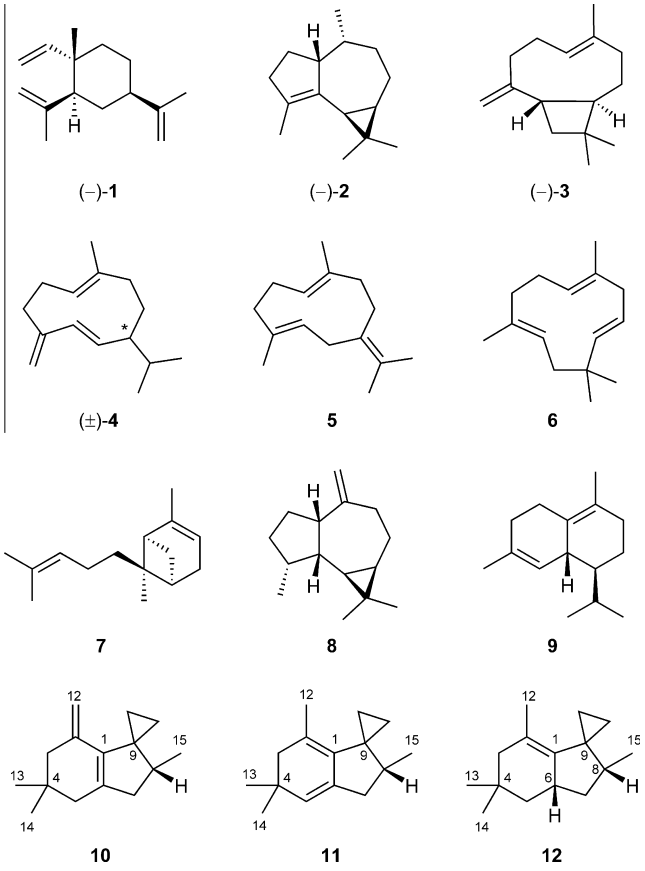

Fig. 1. Sesquiterpene hydrocarbons from *S. terebinthifolius* fruits.

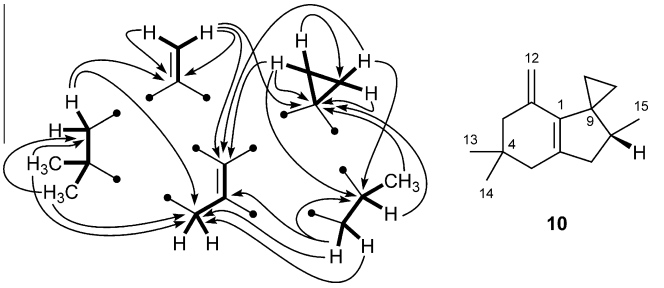

Fig. 2. Relevant HMBC correlations and structure of terebanene (**10**).

of three methyl groups at δ 17.8 (*q*), 30.2 (*q*), and 30.9 (*q*), along with six methylene groups at δ 9.7 (*t*), 17.1 (*t*), 33.4 (*t*), 47.6 (*t*), 53.0 (*t*), and 102.6 (*t*), one methine group at δ 37.5 (*d*), and five quaternary carbons at δ 26.0 (*s*), 32.0 (*s*), 134.1 (*s*), 137.9 (*s*), and 145.5 (*s*). Considering the five units of unsaturation required for the molecular formula $C_{15}H_{22}$ the identification of one exocyclic- and one tetrasubstituted double bond (δ 102.6 (*t*), 134.1 (*s*), 137.9 (*s*), 145.5 (*s*)) implied a tricyclic compound. A 1,1-disubstituted cyclopropane unit was deduced from high field shifted ^{1}H NMR signals at δ 0.33 (*m*), 0.46 (*m*), 0.99 (*m*) and 1.06 (*m*), which were assigned to two methylene groups at δ 9.7 (*t*) and 17.1 (*t*) in agreement with the HMQC spectrum. Furthermore, inspection of COSY and HMQC spectra revealed two isolated anisochoric allylic methylene groups and a third anisochoric allylic methylene group adjacent to the chiral methine group carrying the secondary methyl group. These partial structures could be connected according to the long range H,C-coupling correlations in the HMBC spectrum (Fig. 2) to reveal the new 9-spiro(cyclopropa)-4,4,8-trimethyl-2-methylenebicyclo[4.3.0]non-1(6)-ene structure (**10**) which we like to name terebanene.

The mass spectrum of **11** exhibited a signal for the molecular ion at *m/z* 202 which indicated the molecular formula of $C_{15}H_{22}$. The ^{1}H NMR spectrum was similar to that of **1**, thus, suggesting the presence of a double bond isomer. Two isochoric tertiary methyl groups at δ 1.10 (6H, *s*) and one secondary methyl group at δ 0.95 (3H, *d*, *J* = 6.6 Hz), as well as the characteristic spiro(cyclopropane) unit at δ 0.33 (*m*), 0.34 (*m*), 0.57 (*m*), and 0.83 (*m*) were identified. But in contrast to **10**, the ^{1}H NMR and ^{13}C PENDANT signals for the exocyclic- and one allylic methylene group were replaced by those for an olefinic methyl group at δ 1.33 (3H, *s*) and δ 14.5 (*q*), as well as an olefinic methine group at δ 5.40 (1H, *s*) and δ 138.3 (*d*) in its isomer **11**, respectively. Inspection of COSY, HMQC and HMBC spectra confirmed the assignment of a 9-spiro(cyclopropa)-2,4,4,8-tetramethylbicyclo[4.3.0]nona-1,5-diene structure for **11**, which we like to name teredenene.

The mass spectrum of **12** exhibited a signal for the molecular ion at *m/z* 204 which was assigned the molecular formula of $C_{15}H_{24}$. The ^{1}H NMR spectrum showed striking similarities with those of **10** and **11**, thus, suggesting the presence of a dihydro derivative. Two tertiary methyl groups at δ 1.01 (3H, *s*) and 1.06 (3H, *s*) and one secondary methyl group at δ 0.62 (3H, *d*, *J* = 6.9 Hz), as well as the characteristic spiro(cyclopropane) unit at δ 0.51 (*m*), 0.52 (*m*), 0.62 (*m*), and 0.73 (*m*) were identified. The presence of one olefinic methyl group at δ 1.24 (3H, *s*) and one allylic methine group at δ 2.58 (1H, *br.s*, 6-H) suggested a 9-spiro(cyclopropa)-2,4,4,8-tetramethylbicyclo[4.3.0]non-1-ene structure (**12**) which we like to name terebinthene. The structure of **12** was confirmed by inspection of the ^{13}C PENDANT, COSY, HMQC and HMBC spectra, while its relative configuration was derived from the *gp*-NOESY spectrum. NOE interactions between both methine protons 6-CH and 8-CH indicated their *syn*-configuration in (6*R**,8*R**)-terebinthene (**12**) (Fig. 3).

Spiro(cyclopropane)-type sesquiterpenoids like the derivatives of terebinthane (**13**) found in *S. terebinthifolius* are extremely rare in nature (Fig. 4). Only oxygenated derivatives with the illudane (**15**) skeleton have previously been described from fungi of the basidomycetes (Mc Morris et al., 2002; Rasser et al., 2002; Burgess

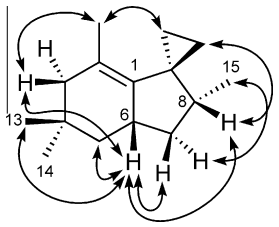

Fig. 3. Relevant NOE interactions in terebinthene (**12**).

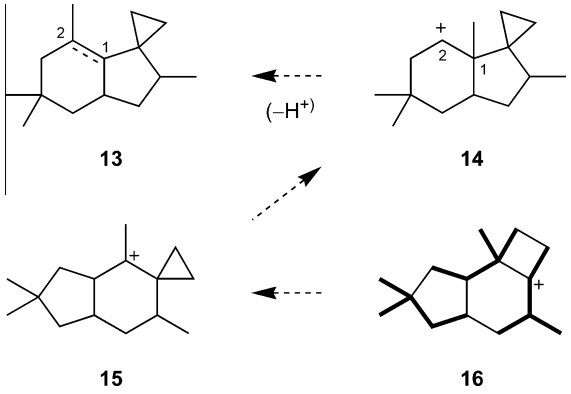

Fig. 4. Terebinthane (**13**), illudane (**15**), and protoilludane (**16**) skeletons and proposed biosyntheses of spiro(cyclopropanes) **13** and **15** by ring contraction of cyclobutane **16** (continuous head–tail fused isoprene units drawn bold).

et al., 1999), while related glucosides are known from ferns of the Pteridaceae (Niwa et al., 1983; Saito et al., 1990; Koyama et al., 1991; Castillo et al., 1997, 1998). The biosynthesis of the spiro(cyclopropane) moiety in **13** might proceed via ring contraction of an (unidentified) cyclobutane intermediate (**16**), comparable to the conversion of a protoilludane (**16**) precursor to the illudane (**15**) skeleton (Fig. 4). Formation of the new terebinthane skeleton (**13**) requires an additional Wagner–Meerwein rearrangement to **14**, followed by migration of a methyl group from 1-C to 2-C during its biosynthesis from **15**.

3. Experimental

3.1. General experimental procedures

Gas chromatography was performed using an Orion Micromat 412 double column GC equipped with 25 m fused silica capillaries (0.25 mm i.d. × 0.25 μm) coated with polysiloxanes CPSil-5 CB and CPSil-19 CB (Chrompack), split injection; split ratio approx. 1:30; FID; carrier gas 0.5 bar H_2; injector and detector temperatures at 200 °C and 250 °C, respectively. Temp. progr. 50–230 °C, 3 °C/min. Enantioselective GC with the isolated compounds was performed using a Carlo Erba 2150 instrument equipped with a 25 m fused silica capillary (0.25 mm i.d.) coated with 50% heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin or 50% heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin in OV-1701 (Agilent J&W). For preparative GC a modified Varian 1400 instrument was used, equipped with a stainless steel column (1.85 m × 4.3 mm) filled with 10% polydimethylsiloxane SE-52 on Chromsorb W-HP; FID; He served as the carrier gas at a flow rate of 120 ml/min; injector and detector temperatures at 200 and 250 °C, respectively. Temp. progr. 90–140 °C, 1 °C/min. Fractions were collected in Teflon tubes cooled with liquid nitrogen. Semipreparative GC was carried out with a Hewlett Packard HP 6890 gas chromatograph (FID) with autosampler, equipped with a 30 m DB-1 or DB-1701 megabore capillary column (0.53 mm i.d., film thickness 5 μm) with He (60 ml/min) as the carrier gas, coupled to an automatic fraction collector PFC system (Gerstel, Mühlheim, Germany). Temp. progr. 100–135 °C, 2 °C/min. GC–MS investigations (EI, 70 eV) were carried out with a VG Analytical 70-250S mass spectrometer (ion source temperature: 250 °C) coupled to a Hewlett Packard HP 5890 gas chromatograph (25 m fused silica capillary column with polydimethylsiloxane CPSil-5 CB, film thickness 0.25 μm). One- and two-dimensional NMR experiments were carried out with a Bruker WM 400 or WM 500 instrument. Benzene-d_6 was used as solvent, and TMS served as internal standard.

3.2. Plant material and isolation of the essential oil

Air-dried fruits (ca. 3 g) from *S. terebinthifolius* were collected in the botanical gardens of Hamburg and Hohenheim (Germany) in February 2007. The essential oil was obtained by hydrodistillation (2 h) with a clevenger type apparatus and collected in 1 ml *n*-pentane (Merck, Darmstadt, Germany). In addition, a partially enriched sesquiterpene fraction (4 ml) was provided by D. Joulain (Grasse, France).

3.3. Isolation of single components

The isolation of single constituents of the crude oil was carried out by column chromatography on silica gel 60 (60 g, ICN) using *n*-pentane as the eluent. Fractions of 20 ml were collected. Sesquiterpene containing fractions were concentrated to 5 ml and subjected to preparative GC using a SE-52 column (90–140 °C; 1 °C/min). Compounds **10**–**12** were finally isolated by repeated semipreparative GC using a megabore DB-1 column (115 °C, 30 min, isothermal) and a megabore DB-1701 column (110 °C, 30 min, isothermal), respectively.

3.4. Identification

3.3.1. Terebanene (**10**): 9-spiro(cyclopropa)-4,4,8-trimethyl-2-methylenbicyclo[4.3.0]non-1(6)-ene

R.I.$_{CPSil\ 5}$: 1356; ^{1}H NMR (400.1 MHz, C_6D_6): δ 0.33 (1H, *m*, H-10a), 0.46 (1H, *m*, H-11a), 0.51 (1H, *m*, H-11b), 0.95 (3H, *d*, *J* = 6.9 Hz, CH_3-15), 0.99 (1H, *m*, H-10b), 1.05 (1H, *m*, H-8), 1.07 (3H, *s*, CH_3-13), 1.09 (3H, *s*, CH_3-14), 1.76 (1H, *d*, *J* = 17.5 Hz, H-7a), 2.1 (1H, *d*, *J* = 16.7 Hz, H-3a), 2.23 (1H, *d*, *J* = 17.3 Hz, H-3b), 2.27 (1H, *d*, *J* = 16.7 Hz, H-5a), 2.36 (1H, *m*, H-5b), 2.36 (1H, *m*, H-7b), 4.61 (1H, *s*, H-12a), 4.71 (1H, *s*, H-12b); ^{13}C NMR (100.1 MHz, C_6D_6): δ 9.7 (*t*, C-10), 17.1 (*t*, C-11), 17.8 (*s*, C-15), 26.0 (*s*, C-9), 30.2 (*s*, C-13), 30.9 (*s*, C-14), 32.0 (*s*, C-4), 33.4 (*t*, C-7), 37.5 (*d*, C-8), 47.6 (*t*, C-5), 53.0 (*t*, C-3), 102.6 (*t*, C-12), 134.1 (*s*, C-2), 137.9 (*s*, C-6), 145.5 (*s*, C-1); MS (EI, 70 eV), *m/z* (%) = 202 (21) $[M]^+$, 187 (45), 173 (12), 159 (35), 145 (41), 131 (100), 115 (24), 105 (18), 91 (33), 77 (16), 65 (10), 51 (8), 40 (51).

3.3.2. Teredenene (**11**): 9-spiro(cyclopropa)-2,4,4,8-tetramethylbicyclo[4.3.0]nona-1,5-diene

R.I.$_{CPSil\ 5}$: 1393; ^{1}H NMR (400.1 MHz, C_6D_6): δ 0.33 (1H, *m*, H-11a), 0.34 (1H, *m*, H-10a), 0.57 (1H, *m*, H-11b), 0.83 (1H, *m*, H-10b), 0.95 (3H, *d*, *J* = 6.6 Hz, CH_3-15), 1.02 (1H, *m*, H-8), 1.11 (3H, *s*, CH_3-13), 1.11 (3H, *s*, CH_3-14), 1.33 (3H, *s*, CH_3-12), 2.2 (1H, *d*, *J* = 15.0 Hz, H-7a), 2.3 (2H, H-3a, b), 2.6 (1H, *d*, *J* = 16.0 Hz, H-7b), 5.4 (1H, *s*, H-5); ^{13}C NMR (100.1 MHz, C_6D_6): δ 9.6 (*t*, C-10), 13.0 (*t*, C-11), 14.5 (*q*, C-12), 17.6 (*q*, C-15), 26.4 (*s*, C-9), 30.0 (*q*, C-13), 30.4 (*q*, C-14), 31.7 (*t*, C-7) 38.4 (*d*, C-8), 43.5 (*s*, C-4), 44.3 (*t*, C-3), 123.0 (*s*, C-1), 138.0 (*s*, C-2), 138.5 (*d*, C-5), 139.0 (*s*, C-6); MS (EI, 70 eV), *m/z* (%) = 202 (42) $[M]^+$, 187 (100), 173 (21), 159 (21), 145 (33), 128 (14), 119 (14), 105 (12), 91 (14), 77 (12), 65 (7), 53 (9), 41 (21).

*3.3.3. Terebinthene (**12**): 9-spiro(cyclopropa)-2,4,4,8-tetramethylbicyclo[4.3.0]non-1-ene*

R.I.$_{CPSil\ 5}$: 1447; ^{1}H NMR (400.1 MHz, C_6D_6): δ 0.51 (1H, *m*, H-10a), 0.52 (1H, *m*, H-11a), 0.62 (3H, *d*, *J* = 6.8 Hz, CH_3-15), 0.62 (1H, *m*, H-10b), 0.73 (1H, *m*, H-11b), 1.01 (3H, *s*, CH_3-13), 1.06 (3H, *s*, CH_3-14), 1.09 (1H, H-5a), 1.10 (1H, *m*, H-7a), 1.24 (3H, *s*, CH_3-12), 1.57 (1H, *m*, *J* = 18.5 Hz, H-5b), 1.72 (1H, *m*, H-7b), 1.97 (1H, *m*, H-8), 2.07 (2H, *m*, H-3a, -3b), 2.58 (1H, H-6); ^{13}C NMR (100.1 MHz, C_6D_6): δ 6.5 (*t*, C-10), 7.3 (*t*, C-11), 13.8 (*q*, C-12), 17.3 (*q*, C-15), 24.7 (*s*, C-9), 29.7 (*q*, C-13), 31.4 (*q*, C-14), 34.5 (*d*, C-8), 37.2 (*s*, C-4), 38.6 (*t*, C-7), 41.5 (*d*, C-6), 46.3 (*t*, C-3), 48.7 (*t*, C-5), 126.1 (*s*, C-2), 139.0 (*s*, C-1); MS (EI, 70 eV), *m/z* (%) = 204 (31) $[M]^+$, 189 (64), 175 (56), 161 (42), 147 (28), 133 (68), 119 (100), 105 (86), 91 (76), 77 (33), 67 (17), 55 (27), 41 (57).

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