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Sesquiterpenes of the liverwort *Scapania undulata*

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Abstract

The essential oil of the liverwort *Scapania undulata*, collected in the Harz mountains, Northern Germany, was analysed by gas chromatography (GC), GC–mass spectrometry (MS) and several new components were isolated and investigated by various NMR techniques. As new natural compounds the sesquiterpene hydrocarbons (+)-helminthogermacrene (**1**) [the 4*Z*-isomer of germacrene A (**9**)], (−)-*cis*-β-elemene (**2**) as a *Cope*-rearrangement product of **1**, (+)-β-isolongibornene (**3**) and (−)-perfora-1,7-diene (**4**) could be identified. **1** has an identical mass spectrum and identical GC retention time on a non-polar stationary phase as germacrene A (**9**) but is considerably more stable than the latter. The Cope-rearrangement of **1** proceeds slowly at 350 °C and (−)-*cis*-β-elemene (**2**) is formed together with small amounts of other diastereoisomers.

Keywords: *Scapania undulata*; Liverworts; Sesquiterpene hydrocarbons; Cope rearrangement; (+)-Helminthogermacrene; (+)-β-Isolongibornene; (−)-*cis*-β-Elemene; (−)-Perfora-1,7-diene

1. Introduction

Liverworts produce a large variety of sesquiterpenes, particularly sesquiterpene hydrocarbons, which are important intermediates in the biosynthesis of functionalized sesquiterpenes and may be useful reference compounds in studying the product specificity of sesquiterpene synthases, particularly after site-specific mutagenesis. Several chemotypes of *Scapania undulata* have been characterized (Asakawa, 1995). Two of them predominate in the German Harz mountains: a chemotype with the major sesquiterpene constituents possessing a cadinane skeleton (''*ent*-1-*epi*-cubenol-type'') and a chemotype with (−)-longiborneol and longipinane derivatives as major constituents (''longiborneol type''). The constituents of the liverwort *S. undulata* have been investigated before (Andersen et al., 1977). Besides some known sesquiterpene hydrocarbons, a few compounds were discussed which could not be fully identified. A recent screening of the volatile constituents of the longiborneol-type *S. undulata* prepared by hydrodistillation or extraction with diethylether revealed the presence of a component with the mass spectrum of β-elemene, however eluting clearly before β-elemene. Usually β-elemene is formed by Cope-rearrangement of the thermally extremely labile germacrene A (**9**). Although a component with the mass spectrum and the retention time of germacrene A was detected, an increase of the injection port temperature of the gas chromatograph did not generate β-elemene, but an ''isomer'' eluting before β-elemene. The stereochemical analysis of the β-elemene isomer (**2**) and its precursor **1** and the identification of 2 new sesquiterpene hydrocarbons (+)-β-isolongibornene (**3**) and (−)-perfora-1,7-diene (**4**) is described. Interestingly, **3** seems to be identical with ''scapanene'' and **4** with ''undulatene''. Both compounds were discussed in the work of Andersen et al. (1977), and their (incomplete) spectroscopic data are in agreement with **3** and **4**, respectively, but their structures could not be identified at that time.

2. Results and discussion

The essential oil of *Scapania undulata*, a liverwort which is very abundant in the Harz mountains in

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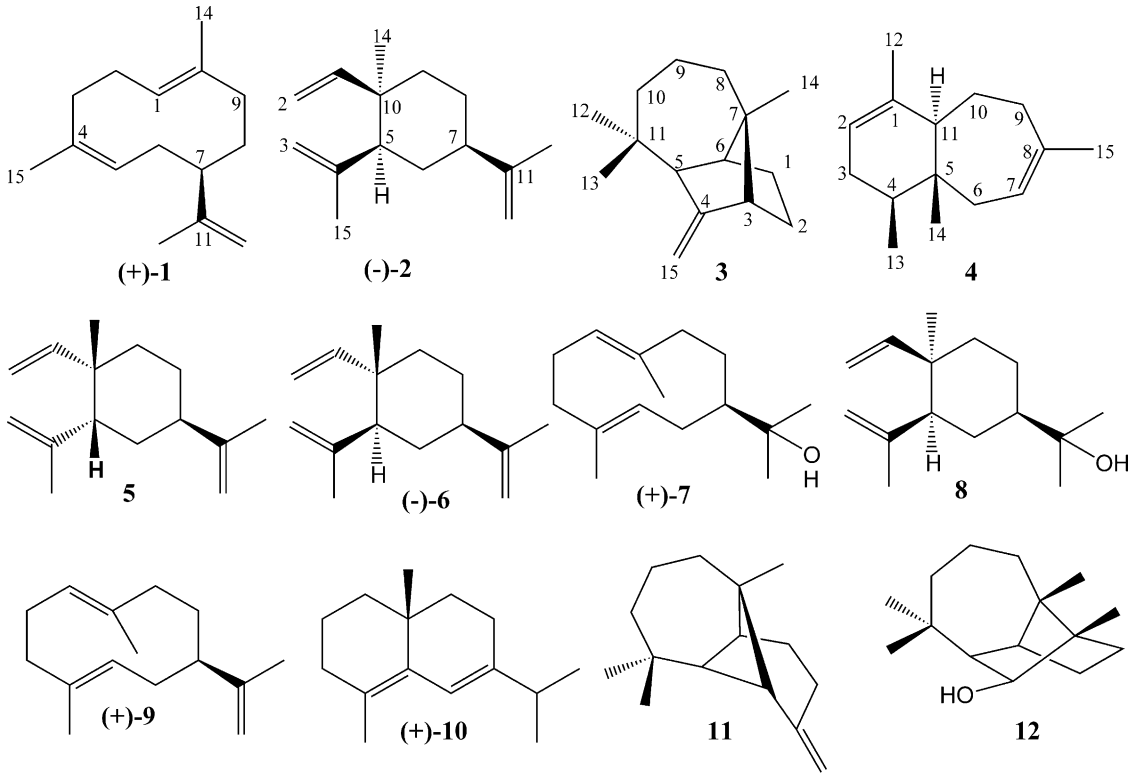


Scheme 1.

Northern Germany, was prepared by hydrodistillation and analysed by GC and GC–MS. In order to exclude artefact formation by hydrodistillation also an ether extract was prepared and investigated by the same methods. All known constituents were identified by comparison of their mass spectra and gas chromatographic retention indices 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 spectroscopic investigation by NMR. The presence of a large amount of longiborneol in the essential oil indicated that the investigated sample belongs to the "longiborneol-chemotype".

The following sesquiterpenes were identified in the order of their elution from a capillary column with polydimethylsiloxane (CPSIL-5) as constituents of the essential oil of *S. undulata* (relative concentrations of major compounds are given in parentheses, those below 1% are only listed): α-longipinene (1.6%), α-ylangene, longicyclene, sativene, β-longipinene (5%), (−)-*cis*-β-elemene (**1**), longifolene (19%), β-maaliene, α-barbatene, *E*-β-caryophyllene, β-ylangene, (+)-β-isolongibornene (**3**, 2.4%), isobazzanene, β-barbatene (1.5%), α-himachalene (4.7%), *ar*-curcumene, isobicyclogermacrene, (+)-α-amorphene, γ-himachalene (2.3%), isolepidozene (Hardt et al., 1995) *Z*-α-bisabolene (1.6%), (+)-α-muurolene, β-himachalene (1.1%), (+)-helminthogermacrene [(+)-1,5-dimethyl-8-(1-methylethenyl)-cyclodeca-1*E*,5*Z*-diene] (**1**, 1%), (+)-α-chamigrene (2.2%), δ-cadinene, *E*-α-bisabolene, (−)-perfora-1,7-diene (**4**, 1.1%) (−)-longipinanol (7.2%, in the ether extract, see below), maaliol, (−)-longiborneol (30.2%), 1-*epi*-cubenol, 2-himachalen-7-ol (2.1%) (Scheme 1). Longipinanol is rather labile, as already indicated by Andersen et al. (1977), and was almost completely degraded during hydrodistillation, but was detected in considerable amounts in the ether extract.

The sense of 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.

2.1. (+)-Helminthogermacrene (*1*)

The mass spectrum of the 10-membered monocyclic sesquiterpene hydrocarbon exhibits a molecular ion signal at *m*/*z* 204 and an elemental composition of $C_{15}H_{24}$. The mass spectrum of **1** and the retention index on a non-polar stationary phase are identical to germacrene A (**9**). However a separation from germacrene A is achieved on more polar stationary phases as CPSil 19. Unlike many germacratrienes with *E,E*-configuration of the olefinic double bonds in the cyclodecadiene system which show temperature dependent NMR spectra (multiple sets of signals) indicative of conformational

equilibria in solution (Takeda, 1974; de Kraker et al., 2001), **1** occurs essentially in one preferred conformation even at room temperature. Although the ^{1}H NMR spectrum (C_6D_6) showed broadened signals in the region between δ 1.84–2.24, the three downfield shifted singlets for the methyl groups at δ 1.57 (3H, *s*, CH_3-14), 1.66 (3H, *br.s*, CH_3-12) and 1.70 (3H, *s*, CH_3-15) are well resolved. In the ^{13}C NMR spectrum the olefinic carbon signals at δ 150.6 (*s*) and 109.3 (*t*) suggested an *exo*-methylene double bond, which was confirmed by two signals in the ^{1}H NMR spectrum at δ 4.77 (1H, *br.s*) and 4.85 (1H, *s*). The multiplet signal at δ 5.30-5.44 (2H, *m*) was assigned to the two methine protons H-1 and H-5. As expected, the ^{1}H NMR of **1** recorded in acetone-d_6 at −8 °C and −16 °C showed less broadened and separated multiplet signals and also predominantly one conformation since only one set of signals was observed. All this information from ^{13}C NMR as well as HMBC, HMQC and ^{1}H–^{1}H COSY led to structure **1** for this compound. Its relative configuration resulted from the NOESY spectrum recorded in the region δ 4.4–5.8 in which the methyl group at δ 1.70 (3H, *s*, CH_3-15) spatially interacts with the multiplet signal at δ 5.30–5.44. This interaction suggests that H-15 is in *cis*-configuration with the H-5 methine proton, which is only possible for a *Z*-4-5 double bond. Spatial interaction of H-12 at δ 1.66 (3H, *br.s*) with the methylene protons was also observed (Fig. 1). Considering the minute structural difference between **1** and germacrene A (**9**) the α-orientation of H-7 in **1** was inferred from the known absolute configuration of **9** both having the same positive direction of optical rotation. The ^{1}H NMR spectra recorded in $CDCl_3$ and the ^{13}C NMR recorded in acetone-d_6 were identical with those reported for the levorotatory enantiomer of **1** found in the defensive secretion of the termite *Amitermes wheeleri* (Scheffrahn et al., 1986) and the same enantiomer isolated from the mycelium of the fungus *Helminthosporium sativum* with its structure verified by total synthesis (Winter et al., 1980; McMurry and Kočovský, 1985). The absolute configuration of (−)-helminthogermacrene was not unambiguously established, although the α-orientation of H-7 was concluded from the sesquiterpene congeners (−)-sativene and (−)-longifolene isolated from the same organism (Winter et al., 1980). Since the reacting conformations of **1** can be correlated with the configuration of the products of transannular cyclization reactions, which usually create chiral centers regio- and stereospecifically in reactions starting from trigonal carbon atoms (Bülow and König, 2000), **1** was subjected to acid-catalyzed and thermal (Cope) rearrangement reactions. In contrast to helminthogermacrene, which is thermally more stable, germacrene A undergoes Cope rearrangements at room temperature (Weinheimer et al., 1970). Thermal isomerization of **1** was achieved above 350 °C to afford *cis*-β-elemene (**2**), another β-elemene diastereomer (**5**) and a trace of "normal" (−)-β-elemene (**6**) in the ratio of (8:1:∼0.08), respectively (in this elution order from the dimethylpolysiloxane stationary phase CPSIL-5). This observation is in agreement to investigations of Kodama et al. (1979) who investigated the rearrangement products of all possible geometrical isomers of hedycaryol [germacra-1(10),4-dien-11-ol, **7**]. The (*E*,*Z*)-isomer of hedycaryol is reported to rearrange at 300 °C to the corresponding *cis*-isoelemol (**8**, Scheme 1), but 3 different diastereoisomers, which correspond to **2** (major isomer), **5** and **6**, are formed at 500 °C, apparently by conformational equilibration *before* rearrangement. Similar observations were made by Takeda (1974) for Cope rearrangement reactions with other germacrene type sesquiterpenes, which formed mixtures of diastereoisomers. In other cases, e.g. germacrene A, B and C, only one type of diastereomer is observed upon heating.

(+)-Helminthogermacrene (**1**) was isolated for the first time as a natural product. It should be mentioned that in a recent paper Prosser et al. (2002) claimed the formation of (+)-β-elemene from its precursor (−)-germacrene A from *S. undulata*. It can be speculated that they really obtained (−)-*cis*-β-elemene (**2**) and not (+)-β-elemene as no germacrene A (**9**) and only a minor peak of the (−)-enantiomer of β-elemene (**6**) could be detected in any of the chemotypes of *S. undulata*.

2.2. (−)-*cis*-β-Elemene (**2**)

Compound **2** is present only in trace amounts in the original essential oil of *S. undulata*. As shown above, it can be generated from **1** by Cope rearrangement and isolated by preparative GC by injecting it into the preparative gas chromatograph at an injector temperature of 390 °C. **2** exhibits a mass spectrum undistinguishable from β-elemene with a molecular ion signal at *m*/*z* 204 and an elemental composition of $C_{15}H_{24}$. Its structure was elucidated based on ^{1}H NMR spectra and its relative configuration was established by ^{1}H–^{1}H COSY, HMBC, HSQC and NOESY-NMR in comparison with

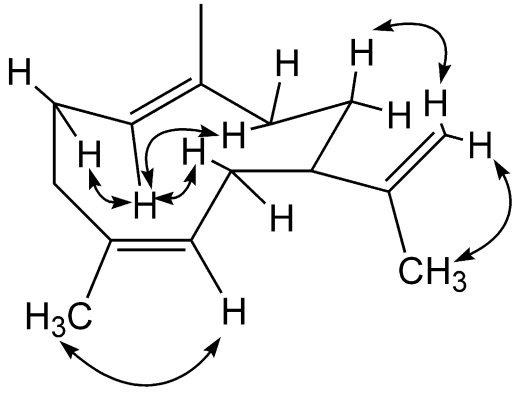


Fig. 1. NOE correlations observed for (+)-**1**.

the spectral data of authentic β-elemene (**6**). The ^{1}H NMR was recorded in both C_6D_6 and $CDCl_3$ to achieve the best resolution over the whole chemical shift range. The ^{1}H NMR (C_6D_6) showed signals of three methyl singlets at δ 1.05 (3H *s*, CH_3-14), 1.66 (3H, *s*, CH_3-15) and 1.67 (3H, *s*, CH_3-12). Interestingly, the CH_3-15 signal in **2** is slightly up-field shifted by 0.04 ppm as compared to β-elemene (**6**). The two methylene protons at δ 5.01 (*ddd*, 2H, H-2, J = 1.26, 11.04, 17.34 Hz) formed an ABX system with a methine proton at δ 6.29 (*dd*, 1H, H-1, J = 11.04, 17.34 Hz). Additional structural information was obtained from the ^{13}C NMR spectrum of **2** which confirmed the presence of three methyl groups at δ 21.38, 22.89 and 27.81, six methylene groups at δ 28.02, 33.76, 42.23, 109.09, 112.90 and 113.35, three methine groups at δ 46.48, 56.24 and 143.27 and three quaternary carbons at δ 39.45, 147.20 and 150.29.

For the relative configuration of **2** a *cis*-relationship at C-5 and C-10 was concluded from a strong NOESY interaction of the H-5 and H-14 protons. Furthermore, interactions between H-9a with both H-5 and H-7 confirmed that the three hydrogens are on the same side of the molecule. Since **2** is formed from **1** with retention of its configuration at C-7, α-orientation for H-7 and consequently for CH_3-14 is suggested (Fig. 2).

The structure of compound **5**, the diastereomer of **2**, was assigned based on identical GC–MS and ^{1}H NMR spectra with **2**. The signals of **5** in the ^{1}H NMR spectrum (C_6D_6) were slightly shifted up-field with the methine proton at δ 6.22 (1H, *dd*, J = 11.03, 17.65 Hz) compared to δ 6.29 in **2**. A slight up-field shift of the methine proton signal to δ 6.23 (*dd*, 1H, J = 11.35, 17.66 Hz) as compared to δ 6.31 (*dd*, 1H, J = 11.03, 17.65 Hz) was also observed when the ^{1}H-NMR of **2** was recorded in $CDCl_3$, while the corresponding proton of authentic β-elemene (**6**) absorbs at δ 5.80 (1H, *dd*, J = 10.7, 17.3 Hz). The C-10 methyl singlet of **5** also appeared slightly shielded at δ 1.04 as compared to 1.05 in **2**.

Treatment of **1** with boron trifluoride etherate in diethyl ether at room temperature for 3 hours gave δ-selinene (**10**) and a few unidentified sesquiterpene hydrocarbons. Interestingly, the comparison of **10** produced by treatment of **1** with BF_3 by enantioselective GC with authentic (+/−)-**10** using a cyclodextrin stationary phase confirmed that a mixture of the (+)- and (−)-enantiomer in the ratio 5 : 1 was formed.

The formation of both enantiomers of **10** and the co-ocurrence of a small amount of diastereomers of **2** suggests that more than one conformation might be present during the rearrangement process, although ^{1}H NMR recordings at ambient temperature, −8 °C and −16 °C did not reflect any significant doubling of signals. Thus, the conformational equilibrium in solution predominantly favoured the (+)-(*E*,*Z*)-**1** configurational isomer (Fig. 3). The existence of a trace [~0.08% = (−) > (+)] of β-elemene (**6**), corresponding to the (*E*,*E*)-isomer (**9**), was confirmed by enantioselective GC.

It is also interesting to note that compound **1** did not isomerise when treated with silica gel for 3 h, which proves its stability in comparison to (*E*,*E*)-germacrene A (**9**).

All the information from spectral data, transannular cyclization reactions in addition to the biosynthetic relationship suggested by Winter et al. (1980) for the identified sesquiterpenes from *S. undulata* (Scheme 1) is in agreement with the 1(10)*E*,4*Z*-configuration for the cyclodecadiene system of compound **1** and the *cis*-configuration of compound **2** which was unambiguously assigned based on the NOESY correlation of H-5 and H-14, while the absolute configuration is still tentative and mainly based on the assumptions of Winter et al. (1980).

It is also noteworthy that a series of *cis*-fused selinenes was found in the *S. undulata* sample investigated by Andersen et al. (1977), as well as in the volatiles of *Helminthosporium sativum* (Dorn, 1975) and *Amitermes excellens* (termite), (Naya et al., 1982) which are more closely allied to the germacrene-sativene group of sesquiterpenes than to the more common *trans*-fused selinenes.

2.3. (+)-β-Isolongibornene (**3**)

In the mass spectrum of **3** a molecular ion signal at m/z = 204, corresponding to the elemental composition $C_{15}H_{24}$, is observed. In the ^{1}H NMR spectrum one exocyclic double bond can be assigned to resonances at δ 4.91 (*s*, 1 H, H-15a) and 4.96 (*br.s.*, 1 H, H-15b). In addition, three methyl singlets at δ 0.83 (CH_3-14), 0.95 (CH_3-12) and 1.12 (CH_3-13) are present. In the ^{13}C NMR spectrum signals of five methylene groups appear at δ 22.6 (C-9), 30.5 (C-2), 31.0 (C-1), 40.5 (C-8), 41.4 (C-10) and 104.5 (C-15). Furthermore, the signals of three methine groups at δ 46.3 (C-6), 57.0 (C-3) and 60.7

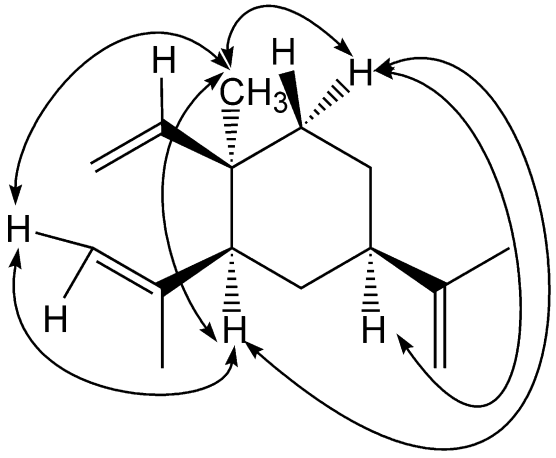


Fig. 2. NOE correlations for (−)-**2**.

(C-5) and 3 quaternary carbon signals at δ 36.2 (C-11), 48.6 (C-7) and 161.6 (C-4) can be detected. The assignment of these resonances to the corresponding carbon atoms was achieved by HMBC and HSQC measurements and is supported by the ^1H–^1H COSY NMR data. Particularly the series of connectivities from C-14 to C-7 and further to C-8, C-9, C-10, C-11, C-12 and C-13 can be established from the HMBC measurements (Fig. 4) as well as the position of the geminal methyl groups to C-11. Also from the HMBC experiment the position of C-5 next to C-11, the position of C-2 next to C-3 (^2J coupling), the position of the methylene groups CH_2-1 and CH_2-2 next to methines CH-3 and CH-6 can be concluded. The spatial relationship of the methylene groups CH_2-1 and CH_2-2 and CH_2-8, CH_2-9 and CH_2-10 and the spatial relationship of CH_2-1 and CH-6 is also confirmed by the NOESY experiment.

The structure of **3** is closely related (but with a rearranged carbon skeleton) to the known β-longipinene (**11**) and longiborneol (**12**), both major constituents of *S. undulata*. The assigned name for **3** is intended to reflect this relationship.

2.4. (−)-Perfora-1,7-diene (4)

Pefora-1,7-diene (**4**), a new bicyclic sesquiterpene with perforane skeleton exhibited a molecular ion signal at *m/z* 204 and an elemental composition of $C_{15}H_{24}$. The ^1H NMR spectrum (C_6D_6) showed signals of a doublet and three singlets for methyl groups at δ 0.82 (3 H, *d*, H-13, $J = 6.6$ Hz), 0.74 (3 H, *s*, H-14), 1.62 (3 H, *br.s*, H-12) and 1.74 (3 H, *s*, H-15). The downfield shifted signals at δ 5.40 (*br.s*, 1 H) and 5.45 (*br.s*, 1 H) were assigned to the olefinic methine protons H-2 and H-7, respectively. Additional structural information was obtained from the ^{13}C NMR and by the ^1H–^1H COSY spectrum. The connectivity derived from the HMBC spectrum (Fig. 5) confirmed a perforane skeleton. The proposed relative configuration at C-4, C-5 and C-11 was established by the NOESY spectrum (Fig. 6). The compound exhibits identical ^1H NMR and MS data as the unknown compound "undulatene" earlier reported from the same species by Andersen et al. (1977). Scheme 2 shows the proposed biogenetic pathway for the perforane skeleton from *cis-trans*-farnesyldiphosphate. Ketohalides and hydroxyl derivatives with perforane backbone have earlier been isolated from the marine alga *Laurencia perforata* and their structures were confirmed by total synthesis (Gonzalez et al., 1975, 1978). Howard

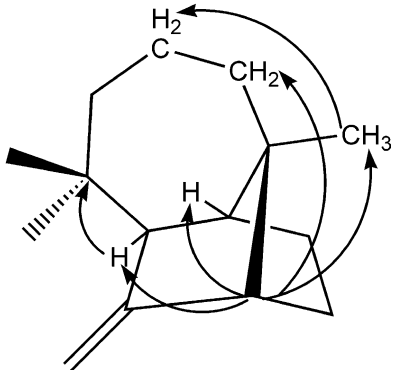


Fig. 4. Long-range correlations in the HMBC spectrum of (+)-β-isolongibornene (**3**).

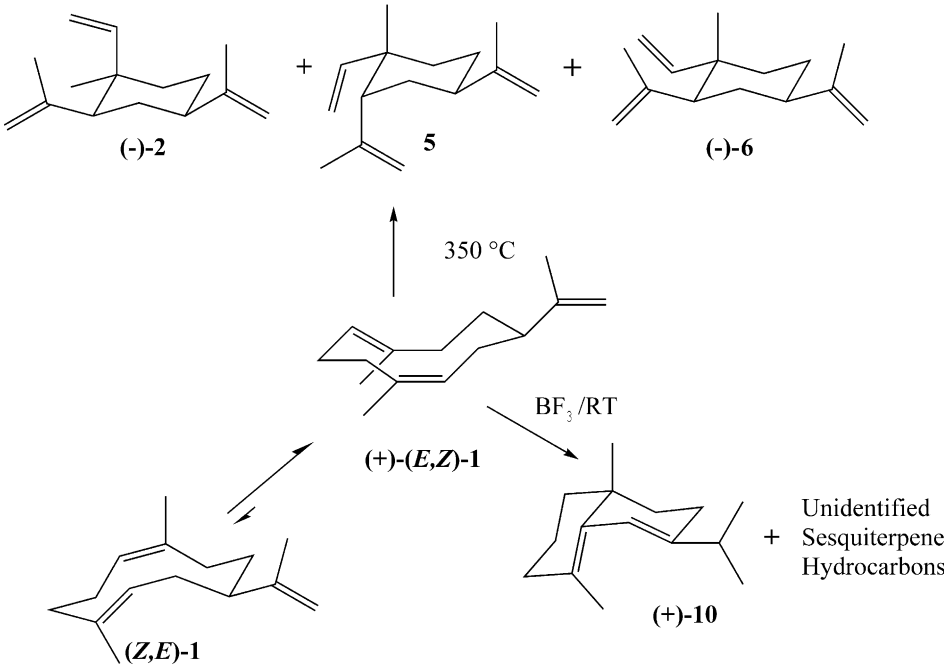


Fig. 3. Rearrangement reactions with (+)-helminthogermacrene (**1**).

and Fenical (1979) reported some additional hydroxy and acetoxy derivatives with perforane skeleton from a related *Laurencia* species.

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 °C and 250 °C, respectively.

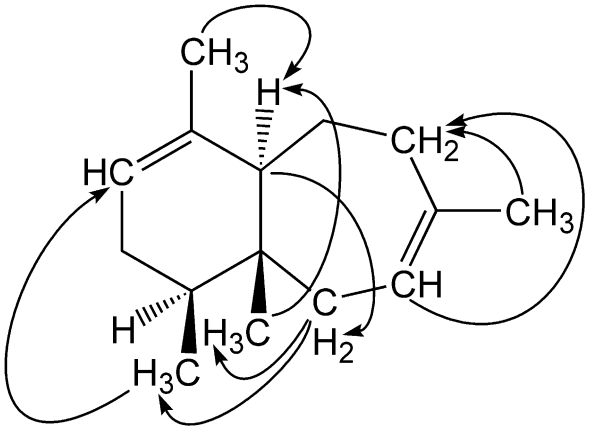


Fig. 5. Long-range correlations in the HMBC spectrum of (−)-perfora-1,7-diene (**4**).

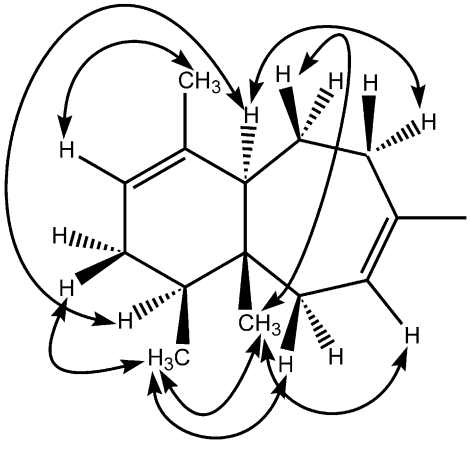


Fig. 6. NOESY correlations for (−)-perfora-1,7-diene (**4**).

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

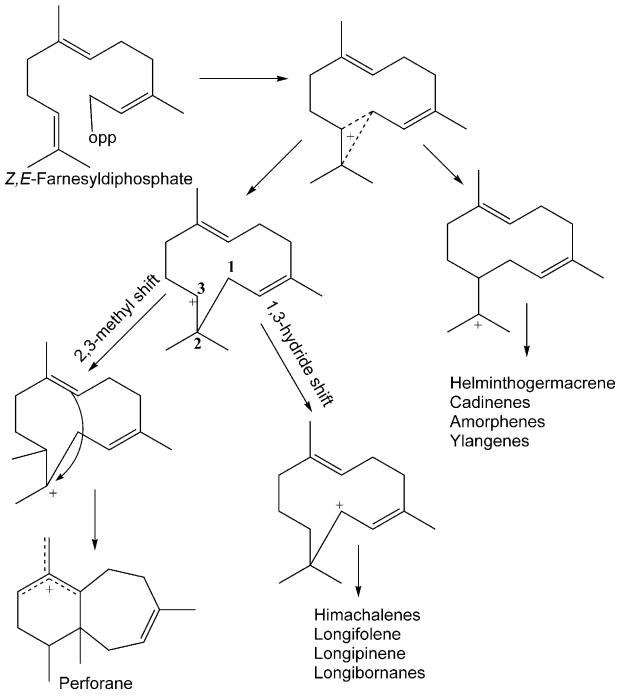
Electron impact (70 eV) GC–MS was carried out with a Hewlett Packard HP 5890 gas chromatograph coupled to a VG Analytical 70-250S mass spectrometer.

3.1.4. NMR-spectroscopy

NMR measurements were carried out with a Bruker WM 400 (400 MHz) or a Bruker WM 500 (500 MHz) 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 sense of optical rotation is given to avoid inaccurracies.



Scheme 2. Proposed biogenetic pathway to perfora-1,7-diene from *Z*,*E*-farnesyldiphosphate.

3.1.6. Acidic rearrangement of 1

To a solution of **1** in diethyl ether (5 ml) BF_3 Et_2O (3 ml) was added at room temp. The solution was stirred for 5 min and allowed to stand for 3 h. Then ice cooled aqueous 0.5 M KOH (20 ml) was added. The organic layer was washed several times with aqueous 0.5 M KOH, then with saturated aqueous NaCl solution and dried over $MgSO_4$; the solvent was concentrated and analysed with GC and GC–MS using achiral polysiloxane and chiral cyclodextrin derived GC phases.

3.1.7. Thermal isomerization of 1

Compund **1** undergoes thermal isomerization at an injector port temperature of 390 °C of the preparative GC instrument. The isomerized products **2**, **5** and **6** were isolated using an SE 30 column.

3.2. Plant material and essential oils

S. undulata was collected from several sites from rocks in small rivers or submersed. Aqueous homogenates of the fresh plant material was submitted to hydrodistillation (2 h) using *n*-hexane as collection solvent. Alternatively diethyl ether extracts were prepared from the air-dried plant material at room temp. (24 h). Because of the greatly differing weight the fresh material was not weighed.

3.3. Isolation of single constituents of the essential oils

The isolation of **1** was carried out using preparative GC at an injector temperature of 120–140 °C. The essential oil of *S. undulata* was frationated using an SE-30 column from 90 to 150 °C with a heating rate of 2 °C/min. The fraction with high concentration in **1** was further purified using prep. GC columns with heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cylodextrin (120 °C, isothermal) and octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin (125 °C, isothermal) consecutively. The last stage of purification was achieved using SE-52 to remove a co-eluting impurity of α-chamigrene of about 1%.

3.4. (+)-Helminthogermacrene (1)

Colourless oil; $RI_{CPSIL5} = 1503$; sense of optical rotation ($CDCl_3$): (+); ^{1}H NMR (500 MHz, C_6D_6, 20 °C): $\delta = 1.35$–1.45 (*br.s*, 2H), 1.57 (*s*, 3H), 1.66 (*br.s*, 3H), 1.70 (*s*, 3H), 1.85–2.01 (*m*, 6H), 2.05–2.15 (*m*, 2H), 2.16–2.25 (*m*, 1H), 4.77 (*br.s*, 1H), 4.85 (*s*, 1H), 5.30–5.44 (*m*, 2H); ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C): 1.40–1.53 (*m*, 2H), 1.67 (*br.s*, 3H), 1.71(*s*, 6H), 1.80–2.05 (*m*, 6H), 2.18–2.45 (*m*, 3H), 4.66 (*br.s*, 1H), 4.70 (*br.s*, 1H), 5.19–5.24 (*m*, 1H), 5.28–5.34 (*m*, 1H); ^{1}H NMR (500 MHz, acetone-d_6, −16 °C): 1.25–1.40 (*m*, 2H), 1.45–1.58 (*m*, 2H), 1.68 (*br.s*, 3H), 1.70 (*br.s*, 3H), 1.72 (*br.s*, 3H), 1.93–2.01 (*m*, 3H), 2.13–2.19 (*m*, 2H), 2.29–2.36 (*br.s*, 1H), 2.48–2.54 (*m*, 1H), 4.60 (*br.s*, 1H), 4.67 (*br.s*, 1H), 5.21 (*br.d*, 1H, $J = 10.7$ Hz), 5.33 (*br.s*, 1H); ^{13}C NMR [data taken from HSQC/HMBC, one carbon missing (125.7 MHz, C_6D_6, 20 °C)]: $\delta = 16.32$, 24.27, 25.30, 29.52, 31.09, 33.15, 41.01, 49.49, 109.31, 124.68, 126.59, 132.02, 134.42, 150.59; ^{13}C NMR [data taken from HSQC/HMBC (125.7 MHz, acetone-d_6, −8 °C)]: 16.06, 19.21, 24.19, 25.53, 29.97, 30.91, 33.08, 41.27, 49.99, 109.27, 124.96, 126.57, 132.43, 134.84, 151.19; MS (EI, 70 eV), *m*/*z* (rel. int.): 204 [M^+] (17), 189 (19), 175 (5), 161 (34), 147 (36), 133 (18), 121 (42), 107 (43), 93 (67), 81 (58), 68 (100), 67 (52), 53 (44), 41 (68).

3.5. (−)-cis-β-Elemene (2)

Colourless oil; $RI_{CPSIL5} = 1382$; sense of optical rotation (C_6D_6): (–); ^{1}H NMR (500 MHz, C_6D_6): $\delta = 1.05$ (3H, *s*, CH_3-14), 1.27 (*dt*, 1H, H-9a, $J = 5.04$, 12.93 Hz), 1.49–1.54 (*m*, 2H, 8-H_2), 1.58–1.64 (*m*, 3H, 6-H_2, H-9b), 1.66 (*s*, 3H, CH_3-15), 1.67 (*s*, 3H, CH_3-12), 1.87-1.92 (*m*, 1H, H-7), 1.94 (*dd*, 1H, H-5, $J = 3.46$, 11.98 Hz), 4.75 (*s*, 1H, H-3a), 4.82 (*d*, 2H, 13-H_2, $J = 13.24$ Hz), 4.87 (*s*, 1H, H-3b), 5.01 (*ddd*, 2H, 2-H_2, $J = 1.26$, 5.99, 11.03 Hz), 6.29 (*dd*, 1H, H-1, $J = 11.04$, 17.34 Hz); ^{1}H NMR (500 MHz, $CDCl_3$): 1.03 (*s*, 3H), 1.42 (*dt*, 1H, $J = 5.04$, 12.92 Hz), 1.43–1.58 (*m*, 2H), 1.59–1.67 (*m*, 3H), 1.68 (*s*, 3H), 1.72 (*d*, 1H, $J = 14.5$ Hz), 1.75 (*s*, 3H), 1.97–2.01 (*m*, 1H), 2.03 (*dd*,1H, $J = 3.47$, 12.3 Hz), 4.65 (*s*, 1H), 4.71 (*d*, 1H, $J = 5.99$), 4.78 (*s*, 1H), 5.01 (*ddd*, 2H, $J = 1.58$, 11.04, 18.9 Hz), 6.31 (*dd*, 1H, $J = 11.03$, 17.65 Hz); ^{13}C NMR [data taken from HSQC/ HMBC (125.7 MHz, C_6D_6)]: $\delta = 21.38$ (*q*, C-12), 22.89 (*q*, C-15), 27.81 (*q*, C-14), 28.02 (*t*, C-8), 33.76 (*t*, C-6), 39.45 (*s*, C-10), 42.23 (*t*, C-9), 46.48 (*d*, C-7), 56.24 (*d*, C-5), 109.09 (*t*, C-13), 112.90 (*t*, C-2), 113.35 (*t*, C-3), 143.27 (*d*, C-1), 147.20 (*s*, C-4), 150.29 (*s*, C-11); MS (EI, 70 eV), *m*/*z* (rel. int.): 204 [M^+] (15), 189 (40), 175 (10), 162 (11), 161 (44), 147 (31), 133 (25), 121 (41), 119 (37), 107 (66), 105 (51), 95 (31), 93 (100), 91 (42), 81 (98), 79 (59), 77 (30), 68 (79), 67 (65), 55 (44), 53 (37), 41 (65).

3.6. (+)-β-Isolongibornene (3)

Colourless oil; $RI_{CPSIL5} = 1440$; ^{1}H NMR (500 MHz, C_6D_6): $\delta = 0.83$ (*s*, 3H, CH_3-7), 0.95 (*s*, 3H, CH_3-12/13), 1.12 (*s*, 3H, CH_3-12/13), 1.13-1.19 (*m*, 1H, H-1a), 1.19-1.25 (*m*, 1H, H-10a), 1.16-1.49 (*m*, 4H, H-2a, H-8a, H-9a, H-10b), 1.62-1.78 (*m*, 3H, H-1b, H-8b, H-9b), 1.80-1.88 (*m*, 3H, H-2b, H-5, H-6), 2.03 (*d*, 1H, H-3, $J = 3.8$ Hz), 4.91 (*s*, 1H, H-15a), 4.96 (*br.s*, 1H, H-15b). ^{13}C NMR (100.6 MHz, C_6D_6): $\delta = 22.6$ (*t*, C-9), 23.7 (*q*, C-14), 30.0 (*q*, C-13), 30.5 (*t*, C-2), 31.0 (*t*, C-1), 32.0 (*q*, C-12), 36.2 (*s*, C-11), 40.5 (*t*, C-8), 41.4 (*t*, C-10), 46.3 (*d*, C-6), 48.6 (*s*, C-7), 57.0 (*d*, C-3), 60.7 (*d*, C-5), 104.5 (*t*, C-15), 161.6 (*s*, C-4); MS (EI, 70 eV), *m*/*z* (rel. int.): 204

[M^+] (33), 189 (26), 175 (11), 161 (44), 147 (22), 133 (51), 119 (85), 105 (62), 93 (88), 79 (55), 69 (51), 63 (4), 55 (59), 41 (100).

3.7. (−)-Perfora-1,7-diene (4)

Colourless oil; RI_{CPSIL5} = 1543 ; sense of optical rotation (C_6D_6): (−); ^{1}H NMR (500 MHz, C_6D_6): δ = 0.74 (*s*, 3H, H-14), 0.82 (*d*, 3H, H-13, *J* = 6.6 Hz), 1.18 (*dq*, 1H, H-10a, *J* = 1.6, 12.3 Hz), 1.47–1.54 (*m*, 1H, H-4), 1.62 (*br.s*, 3H, H-12), 1.74 (*s*, 3H, H-15), 1.71–1.80 (*m*, 3H, H-10b, H-3a, H-6a), 1.86–1.94 (*m*, 2H, H-3b, H-9a), 2.00 (*br.d*, 1H, H-11, *J* = 12.3 Hz), 2.16 (*br.t*, 1H, H-9b, *J* = 13.2 Hz), 2.25 (*dd*, 1H, H-6b, *J* = 9.2, 14.5 Hz), 5.40 (*br.s*, 1H, H-2), 5.45 (*br.s*, 1H, H-7). ^{13}C NMR (125.7 MHz, C_6D_6): δ = 10.9 (*q*, CH_3-14), 16.6 (*q*, CH_3-13), 23.1 (*q*, CH_3-12), 24.3 (*t*, C-10), 25.9 (*q*, CH_3-15), 33.7 (*t*, C-3), 35.0 (*t*, C-9), 37.2 (*s*, C-5), 37.8 (*t*, C-6), 38.9 (*d*, C-4), 56.2 (*d*, C-11), 122.9 (*d*, C-2), 123.9 (*d*, C-7), 136.3 (*s*, C-1), 140.4 (*s*, C-8); MS (EI, 70eV), *m*/*z* (rel. int.) : 204 [M^+] (12), 189 (16), 176 (30), 161(13), 147 (7), 136 (55), 135 (17), 134 (10), 133 (18), 121 (100), 119 (21), 107 (37), 93 (23), 91 (25), 79 (21), 67 (18), 55 (24), 41 (49).

3.8. −cis-β-Elemene diastereomer (5)

Colourless oil; RI_{CPSIL5} = 1387; ^{1}H NMR (500 MHz, C_6D_6): δ = 1.04 (*s,* 3H), 1.65 (*s,* 3H), 6.22 (*dd,* 1H, *J* = 11.03, 17.65 Hz); ^{1}H NMR (500 MHz, $CDCl_3$): 1.02 (*s,* 3H,), 6.23 (*dd,* 1H, *J* = 11.35, 17.66 Hz); MS (EI, 70 eV), *m*/*z* (rel. int.): 204 [M^+] (5), 189 (32), 175 (9), 162 (8), 161 (33), 147 (28), 133 (31), 121 (40), 119 (38), 107 (67), 105 (46), 93 (100), 91 (47), 81 (76), 79 (59), 68 (55), 67 (56), 55 (37), 53 (32), 41 (49).

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