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Isogermacrene A, a proposed intermediate in sesquiterpene biosynthesis

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Abstract

In the essential oil of the liverwort *Saccogyna viticulosa*, collected on the island of Madeira, the new sesquiterpene hydrocarbons isogermacrene A (**5**) and its *Cope* rearrangement product iso-β-elemene (**6**) were identified. **5** is proposed to act as the biogenetic precursor of several new sesquiterpenes identified in the volatiles of *S. viticulosa*. These include iso-α-humulene, α-gorgonene, gorgona-1,4(15),11-triene and gorgon-11-en-4-ol. In addition, the *Cope* product of zierene, isozierene, *allo*-aromadendra-4(15),10(14)-diene, aromadendra-4(15),10(14)-dien-1-ol and a prenylguaiane diterpene alcohol, named viticulol, were identified as new natural products.

Keywords: *Saccogyna viticulosa*; Liverwort; Sesquiterpenes; Isogermacrene A; Iso-β-elemene; *Cope* rearrangement; Biosynthesis

1. Introduction

To date more than 300 sesquiterpene skeletons are known, which predominantly originate from enzymatic cyclisations and further transformations of farnesyl diphosphate (FPP) as the common acyclic precursor (Cane, 1999). The germacrenes constitute important intermediates between FPP and many other sesquiterpene structures. Nevertheless, some sesquiterpenes seem to be an exception of the *Isoprene Rule*; examples are the gorgonanes and zieranes which are rarely found in nature (Paknikar and Sood, 1973; Boeckmann and Silver, 1975). Maalioxide (**1**) has been identified from several sources, mainly liverworts (Asakawa, 1995; Narayanan et al., 1964). β-Gorgonene (**2**) (Weinheimer et al., 1968), zierone (Penfold, 1926; Barton and Gupta, 1962) and the recently isolated zierene (**3**), saccogynol (**4**) (Connolly et al., 1994) and argutenol (Fraga, 2000) are more unique members that were obtained from only one or two sources (Fig. 1). These compounds possess an isopropyl group in the unusual C-6 position and apparently contain two tail-to-tail fused isoprene units. Consequently, direct formation from the germacrenes or formation by the usual hydride shift or methyl migration appears highly unlikely. Accordingly, a germacrene like biogenetic intermediate (**5**) has been proposed, which shares the unusual arrangement of isoprene units (Weinheimer et al., 1968). **5** could also be considered as a 7,11-*seco*-bicyclogermacrene, hence, FPP serves as the initial biogenetic precursor. However, this hypothetic intermediate has never been detected since its proposal.

The essential oil of the liverwort *Saccogyna viticulosa* (L.) Dum. (Geocalycaceae), described as a unique source of zierene (**3**) and saccogynol (**4**) (Connolly et al., 1994), was reinvestigated. GC–MS screening revealed the presence of the known β-gorgonene (**2**) and

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maalioxide (**1**), in addition to several unknown compounds, especially in the hydrocarbon fraction. A component (**6**) with a similar mass spectrum to β-elemene (**17**) but a clearly different retention index was detected. Isolation and structure elucidation of **6** revealed a new elemene-like sesquiterpene skeleton with the isopropenyl group in the C-6 position comparable to the gorgonanes and zieranes. This compound was named iso-β-elemene (**6**). Normally, due to their thermal lability, the elemenes are formed by *Cope* rearrangement of the corresponding germacrene precursor. Thus, it was possible to detect the precursor of **6** by variation of the GC injection port temperature in order to observe changes in the ratios of the essential oil constituents. This compound, isogermacrene A (**5**), could be isolated in minor amounts and was shown to be consistent with the proposed (hypothetical) structure by Weinheimer et al. (1968). In addition, a new humulene-like compound related to **5**, named iso-α-humulene (**7**), as well as α-gorgonene (**8**), gorgona-1,4(15),11-triene (**9**) and gorgon-11-en-4-ol (**10**), two aromadendradienes, *allo*-aromadendra-4(15),10(14)-diene (**11**) and aromadendra-4(15),10(14)-dien-1-ol (**12**), the *Cope* rearrangement product of zierene, isozierene (**13**), and a diterpene alcohol, viticulol (**14**), were isolated as new natural compounds (Fig. 1).

2. Results and discussion

The investigation of the essential oil of *S. viticulosa* by GC and GC–MS revealed a complex mixture of monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenoids as well as diterpenes. Several known compounds could be identified by comparison of their mass spectra and retention indices with a spectral library (Joulain and König, 1998; Hochmuth et al., 2002) which

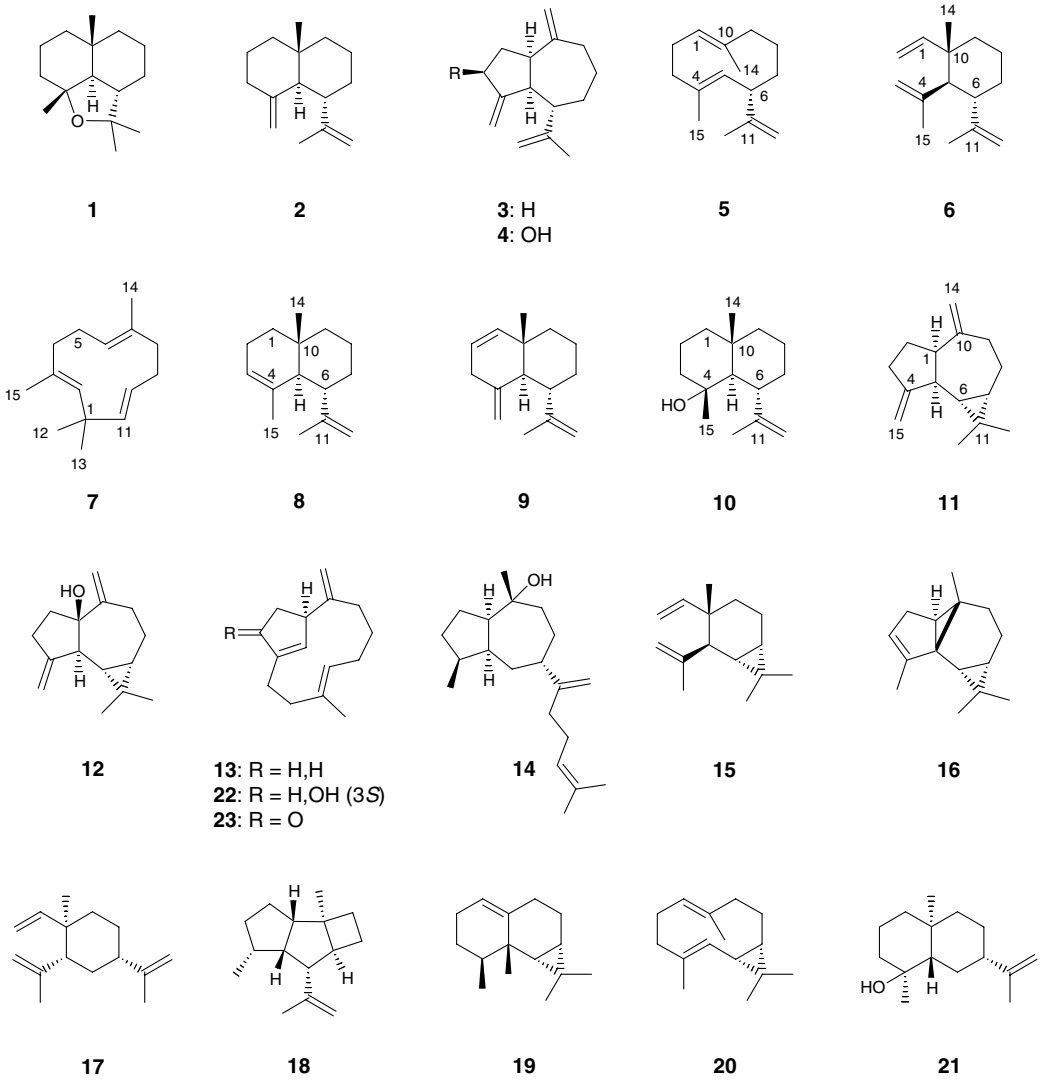


Fig. 1. Constituents of the liverwort *Saccogyna viticulosa*.

was established under identical experimental conditions. The identified components are listed in their order of elution (CPSiL-5 capillary column, relative concentrations of major components [>1%] are given in parentheses): tricyclene, α-pinene, camphene, myrtenylacetate, bicycloelemene (**15**), anastreptene (**16**), β-elemene (**17**), tritomarene (**18**) (Warmers et al., 1998), calarene (**19**), β-gorgonene (**2**, 12%), zierene (**3**, 29%), bicyclogermacrene (**20**, 4%), maalioxide (**1**), eudesm-11-en-4α-ol (**21**, 1%), saccogynol (**4**, 17%), isosaccogynol (**22**), isosaccogynone (**23**, 4%) (Connolly et al., 1994) (Fig. 1). Unknown components were selected for isolation and further chemical investigation. The essential oil was pre-fractionated by column chromatography using deactivated alumina, with respect to the lability of the reported constituents (Connolly et al., 1994). Structural elucidation was performed by one- and two-dimensional NMR experiments as well as chemical correlations. To determine the absolute configurations, chemical conversions were employed and monitored by enantioselective GC using modified cyclodextrin phases (König, 1992).

2.1. (−)-Iso-β-elemene (6)

An early eluting hydrocarbon (**6**) with the molecular formula $C_{15}H_{24}$ (M^+: *m/z* 204) was isolated by a combination of column chromatography and preparative GC. In the ^{1}H NMR spectrum signals corresponding to a tertiary methyl group at δ_H 1.03 (3H, *s*) and two olefinic methyl groups at δ_H 1.64 (3H, *m*) and 1.71 (3H, *m*) were observed. In addition seven olefinic protons at δ_H 4.61 (1H, *m*), 4.73 (1H, *m*), 4.79 (1H, *m*), 4.93 (3H, *m*) and 5.82 (1H, *dd*) corresponded to six olefinic carbons at δ_C 110.1 (*t*), 111.5 (*t*), 114.9 (*t*), 145.2 (*s*), 149.7 (*s*) and 150.8 (*d*). Thus, it was concluded that the compound contains one vinyl and two isopropenyl groups. Considering the four units of unsaturations and three double bonds a monocyclic structure was assumed. Interpretation of the 2D H,H-COSY, HMQC and HMBC spectra led to the new 1-methyl-2,3-di(1-methylvinyl)-1-vinylcyclohexane structure of **6**. The signals at δ_H 1.92 (1H, *d*, J = 12.0 Hz) and 2.33 (1H, *ddd*, J = 3.6, 12.0, 12.0 Hz) were assigned to the two adjacent methine protons H-5 and H-6, with the *J*-coupling constant of 12.0 Hz indicating a *trans*-configuration. Furthermore, a long-range ^4J-W-coupling between the methyl group H-14 and H-5 indicated a 5,10-*trans*-fusion. The relative configuration (5*S**, 6*S**, 10*S**) was confirmed by a NOESY spectrum. A molecular model and important NOEs are shown in Fig. 2.

2.2. (+)-Isogermacrene A (5)

Isogermacrene A (**5**), the precursor of **6**, exhibited a molecular ion signal at *m/z* 204 ($C_{15}H_{24}$). The retention index on a non-polar stationary phase was identical to germacrene A (**24**) (Fig. 3) but the mass spectrum

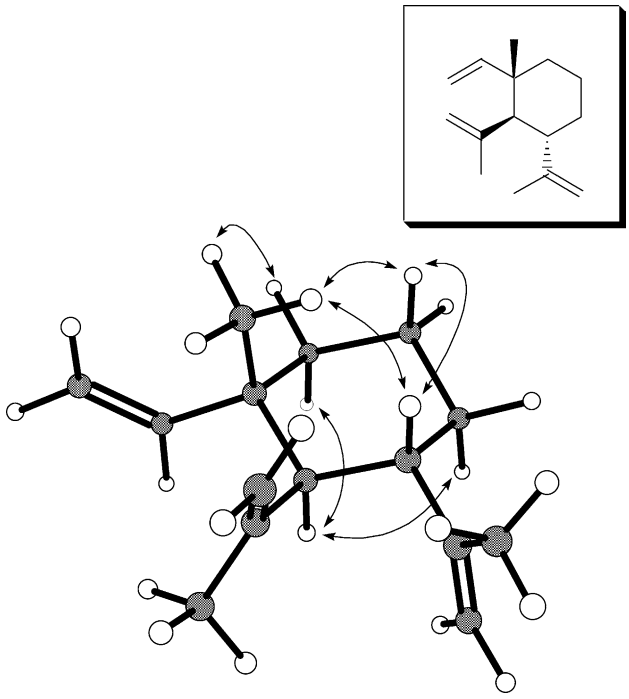


Fig. 2. AM1 MOPAC model of iso-β-elemene (**6**) and important NOEs.

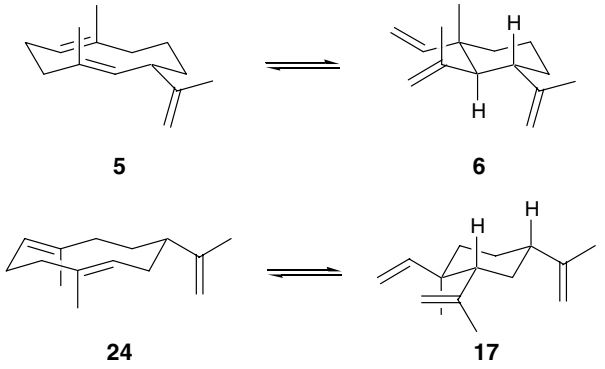


Fig. 3. Comparison of the products obtained by *Cope* rearrangement of isogermacrene A (**5**) and germacrene A (**24**).

showed significant differences. The isolation of **5** was difficult, because it eluted just shortly after zierene (**3**), the major component of the essential oil. Thus, various purification steps were necessary. Due to the lability of **5**, which underwent *Cope* rearrangement under comparable conditions as germacrene A (**24**), only minor amounts could be isolated. Similar to **24** and many other *E*,*E*-configured cyclodecadiene structures the ^{1}H NMR spectrum of **5** exhibited broad signals at room temperature (acetone-d_6), due to a conformational equilibrium in solution (Kulkarni et al., 1964; Weinheimer et al., 1970; Piet et al., 1995). However, reducing the temperature to 10 °C resulted in less broadened, well separated multiplet signals. The observation of one set of signals

indicated the predominance of only one conformation at this temperature. The ^{1}H NMR spectrum showed three signals for olefinic methyl groups at δ_H 1.41 (3H, *br s*), 1.56 (3H, *d*), 1.68 (3H, *s*) and four signals for olefinic protons at δ_H 4.48 (1H, *m*), 4.55 (1H, *br s*), 4.67 (1H, *br s*), 4.81 (1H, *m*). Indicative for the structure of **5** was a signal at 2.69 (1H, *m*), assigned to the methine proton H-6. A correlation signal to the olefinic proton H-5 at δ_H 4.48 in the H,H-COSY spectrum confirmed that the isopropenyl group of compound **5** is adjacent to the C-4/C-5 double bond of the cyclodecadiene ring. Although the complete 2D NMR data of **5** could not be obtained, its structure could be assigned unambiguously from its thermal rearrangement product **6**. *E*-configuration of the double bonds was concluded from the high lability of **5**, because much higher temperatures are necessary for the rearrangement of an *E*,*Z*-configured germacradiene (Adio et al., 2004).

Noteworthy, β-elemene (**17**) and iso-β-elemene (**6**) exhibit a different stereochemistry. While the two isopropenyl groups of **17** are *cis*-configured, *trans*-configuration is found for **6** (Fig. 3). *Cope* rearrangement is a stereospecific reaction that proceeds via a chair like transition state and the configuration of the resulting elemenes depends substantially on the structure and predominant conformation of the corresponding germacrenes (Sutherland, 1974; Houk et al., 1995; de Kraker et al., 1998). Since a chair–chair like conformation is preferred for the *E*,*E*-configured cyclodecadienyl system, *Cope* rearrangement proceeds easily (Takeda, 1974; Terada and Yamamura, 1979, 1982). Even though the cyclodecadienyl system is quite flexible, the conformational equilibrium of the germacrenes is restricted due to the large substituent at C-7. Hence, an equatorial conformation predominates and *Cope* rearrangement leads mainly to a single product (Kodama et al., 1979; Piet et al., 1995; de Kraker et al., 2001). To confirm the stereochemical relationship of **5** and **6**, a semiempirical molecular modelling analysis was performed using CS MOPAC and the potential function AM1. Fig. 4 shows the calculated structure for the most stable conformation of **5**. In agreement with the results obtained for **24** and other germacrenes, the conformation is *syn*, with respect to the two methyl groups, and the endocyclic double bonds are in a crossed arrangement (Takeda, 1974; Terada and Yamamura, 1979, 1982; Piet et al., 1995). The calculated structure of **5** is consistent with the stereochemical requirements for a *Cope* rearrangement to **6** (Fig. 3).

Treatment of **5** with an acidic ion exchange resin (Amberlyst® 15) at room temperature for one hour afforded maalioxide (**1**), β-gorgonene (**2**), and α-gorgonene (**8**) in the ratio 1:5:2, respectively (Fig. 5). Additional minor products were observed but could not be identified. Coinjection of the reaction products with natural **1**, **2** and **8** on a heptakis(6-*O*-*tert*-butyltrimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin column showed no peak separation nor broadening which would be expected in case of different enantiomers. From the known absolute configurations of (−)-**1** and (+)-**2**, *S*-configuration was concluded for (+)-isogermacrene A (**5**) (Paknikar and Sood, 1973; Matsuo et al., 1974). Hence, the absolute configuration (5*S*,6*S*,10*S*) was assigned to (−)-iso-β-elemene (**6**).

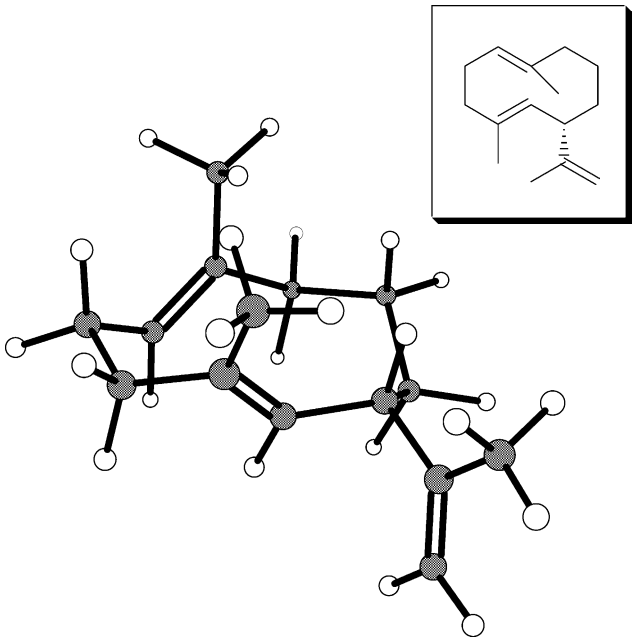


Fig. 4. AM1 MOPAC model of isogermacrene A (**5**).

2.3. Iso-α-humulene (7)

Another unknown hydrocarbon with the molecular formula $C_{15}H_{24}$ (M^+: *m*/*z* 204) exhibited broad signals in the ^{1}H NMR spectrum at room temperature which indicated a medium sized ring system. Nevertheless, the NMR spectroscopic data could be interpreted without decreasing the temperature. A broad singlet at δ_H 1.31 (6H) indicated the presence of two isochoric methyl groups. In addition, two olefinic methyl groups at δ_H 1.38 (3H, *br d*) and 1.48 (3H, *d*) and four olefinic protons at δ_H 4.61 (1H, *br t*), 4.98 (1H, *br s*), 5.02 (1H, *m*) und 5.48 (1H, *d*) were observed. The H,H-COSY spectrum showed two endocyclic double bonds with methyl substitution, hence, an additional endocyclic double bond was deduced. In agreement with three identified double bonds the suspected monocyclic structure of **7** could be confirmed. In the PENDANT spectrum four methyl groups (δ_C 15.4, 16.6 and 28.4/2×C), four methylene groups (δ_C 27.6, 30.3, 41.6 and 42.2), four olefinic methine groups (δ_C 125.0, 128.0, 140.3 and 141.5) and three quaternary carbon atoms (δ_C 38.5, 130.0 and 132.0) were assigned. All methylene protons gave downfield shifted signals between δ_H 1.90 and 2.15 due to

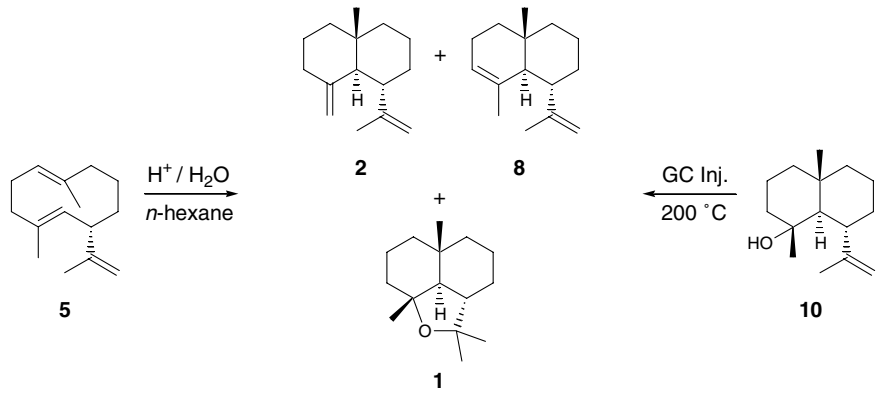


Fig. 5. Acid induced rearrangement of (+)-isogermacrene A (**5**); thermal rearrangement of (−)-gorgon-11-en-4-ol (**10**).

their allylic positions. In the HMBC spectrum a ^3J-H,C-correlation from the isochoric methyl groups at δ_H 1.31 to the methyl carbons at δ_C 28.4 indicated their geminal position. Additional information from the HMBC data led to structure **7**. The *E*-configuration of the C-10/C-11 double bond was concluded from the coupling constant of 15.8 Hz, observed for H-11 at δ_H 5.48. The NOESY spectrum indicated *E*-configuration for the two methyl substituted double bonds, due to the absence of spatial interactions between the olefinic protons and the corresponding methyl group, although this could not be further established. **7** possesses a new skeleton with an eleven-membered monocyclic ring reminiscent to the humulanes (Connolly and Hill, 1991). Since the geminal methyl groups of **7** are relocated from position C-11 to C-1 a structurally related compound to the gorgonanes, zieranes and isogermacrene A (**5**) was concluded (Fig. 6).

2.4. (+)-α-Gorgonene (*8*)

Compound **8** was assigned the molecular formula $C_{15}H_{24}$ (M^+: *m/z* 204). The ^{1}H NMR spectrum exhibited one tertiary methyl group at δ_H 0.88 (3H, *s*) and two olefinic methyl groups at δ_H 1.58 (3H, *s*) and 1.83 (3H, *d*). One exocyclic methylen group at δ_H 4.75 (1H, *m*) and 4.79 (1H, *s*) and one trisubstituted endocyclic double bond at δ_H 5.37 (1H, *br s*) were in agreement with carbon signals at δ_C 110.5 (*t*), 123.6 (*d*), 136.7 (*s*) and 152.7 (*s*). From the H,H-COSY, HMQC and HMBC spectra a gorgonane skeleton was elucidated. The position of the two double bonds were assigned to carbons C-3/C-4 and C-11/C-12 (Fig. 1) and the compound was identified as the endocyclic double bond isomer of β-gorgonene (**2**), called α-gorgonene (**8**). Although the signals for the methine protons H-5 and H-6 coincided at δ_H 2.25 (2H, *br s*) and could not be distinguished, the NOESY spectrum exhibited spatial interactions to protons on both sides of the decaline ring system, which proved a 5,6-*trans*-configuration. A *trans*-configuration for the decaline ring system could be shown by NOEs between the H-14 methyl protons and the axial proton $H_{(Si)}$-2 as well as H-14 and the axial proton $H_{(Si)}$-8. Treatment of (+)-**2** with an acidic ion exchange resin yielded (+)-**8** and (−)-maalioxide (**1**) as major products (Fig. 7). The absolute configuration of natural **8** (5*R*,6*S*,10*S*) could be established by coinjection with the rearrangement product of (+)-**2** using a heptakis(6-*O*-*tert*-butyltrimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin column. Although **8** was isolated for the first time from a natural source, it has previously been described as a synthetic product by treatment of maaliol with a catalytic amount of iodine (Büchi et al., 1959).

2.5. Gorgona-1,4(15),11-triene (*9*)

The early eluting hydrocarbon **9** with a molecular ion signal at *m/z* 202 and the molecular formula $C_{15}H_{22}$ was isolated in minor amounts by a combination

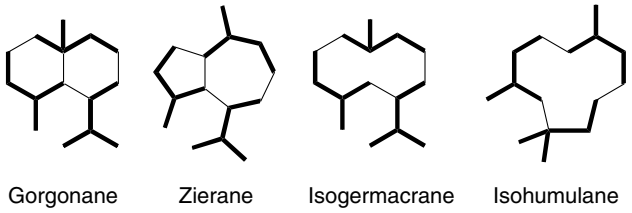


Fig. 6. Rare sesquiterpene skeletons described in this work. The depicted arrangement of isoprene units indicates the structural relationship. Each skeleton apparently contains two tail-tail fused isoprene units.

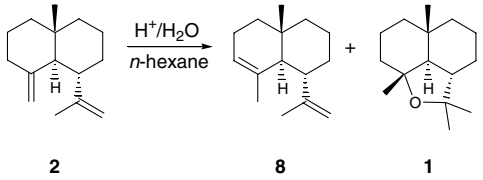


Fig. 7. Acid induced rearrangement of (+)-β-gorgonene (**2**) yielding (+)-α-gorgonene (**8**) and (−)-maalioxide (**1**).

of preparative and semipreparative GC. **9** exhibited similar NMR data as **2** and **8** but contains an additional double bond. In the ^{1}H NMR spectrum one tertiary methyl group at δ_H 0.94 (3H, *s*), one olefinic methyl group at δ_H 1.58 (3H, *s*) and six olefinic protons at δ_H 4.80 (2H, *m*), 4.86 (1H, *d*), 4.95 (1H, *br s*) and 5.44 (2H, *m*) were observed. The olefinic protons were assigned to two exocyclic and one endocyclic double bond, in agreement with carbon signals at δ_C 108.8 (*t*), 111.0 (*t*), 123.5 (*d*), 140.4 (*d*), 145.0 (*s*) and 149.8 (*s*). Interpretation of the 2D NMR data (H,H-COSY, HMQC, HMBC) led to the identification of structure **9**. The relative configuration of **9** was derived from a NOESY spectrum. Hydrogenation of **2** and **9** and coinjection of the reaction products on different chiral columns established the absolute configuration (5*S*,6*S*,10*S*).

2.6. (−)-Gorgon-11-en-4-ol (10)

Compound **10** has the molecular formula $C_{15}H_{26}O$ as shown by HRMS (M^+: *m*/*z* 222.1994). The fragment ion at *m*/*z* 204 $[M-H_2O]^+$ indicated the presence of a hydroxy group. **10** was enriched from the essential oil by column chromatography while further purification by preparative GC could not be achieved due to rearrangement reactions in the injection port. The rearrangement products were identified by GC and GC–MS as **1**, **2** and **8**. Thus, a gorgonane type sesquiterpene alcohol with a hydroxy group at position C-4 or C-11 was proposed. The ^{1}H NMR spectrum indicated the presence of two tertiary methyl groups at δ_H 0.79 (3H, *s*) and 1.22 (3H, *s*) and an olefinic methyl group at δ_H 1.78 (3H, *s*) in addition to two olefinic protons at δ_H 4.63 (1H, *m*) and 4.79 (1H, *d*, $J=2.5$ Hz). The 15 carbon atoms of **10** were assigned by means of a PENDANT spectrum which indicated three methyl groups (δ_C 19.1, 20.3 and 24.1), seven methylene groups (δ_C 20.0, 21.7, 34.2, 42.5, 43.2, 45.5, 112.7), two methine groups (δ_C 44.6, 56.8) and three quaternary carbon atoms (δ_C 35.7, 73.8, 153.7). Although full assignment of all signals could not be achieved due to overlapping of various signals, the 2D NMR data (H,H-COSY, HMQC and HMBC) confirmed the structure of gorgon-11-en-4-ol. The relative configuration of **10** was derived from the NOESY spectrum while the absolute configuration (4*R*,5*R*,6*S*,10*S*) was assigned by enantioselective GC of the reaction products **1**, **2** and **8** obtained by rearrangement and dehydration (Fig. 5).

2.7. (+)-allo-Aromadendra-4(15),10(14)-diene (11)

The mass spectrum of compound **11** was similar to zierene (**3**) with a molecular ion peak at *m*/*z* 202 ($C_{15}H_{22}$). Instead of one olefinic methyl group and three exocyclic methylene groups in the ^{1}H NMR spectrum of **3**, signals for two tertiary methyl groups at δ_H 1.01 (3H, *s*) and 1.05 (3H, *s*) and four olefinic protons at δ_H 4.87 (2H, *m*), 4.94 (1H, *m*) and 5.05 (1H, *m*) were observed. The olefinic protons were assigned to two exocyclic methylene groups in agreement with four olefinic carbons at δ_C 105.7 (*t*), 110.3 (*t*), 150.1 (*s*) and 157.0 (*s*). The observed methine protons at δ_H 0.51 (2H, *m*), corresponding to carbon signals at δ_C 25.1 (*d*) and 29.0 (*d*), suggested a dimethyl substituted cyclopropane unit. One signal at δ_C 17.0 (*s*) was assigned to the quaternary cyclopropane carbon atom. Interpretation of the 2D H,H-COSY-, HMQC- and HMBC spectra confirmed structure **11**. The relative stereochemistry was deduced from the NOESY spectrum. A conformational study applying the AM1 potential function implemented in the CS MOPAC program was utilised for the assignment of NOESY correlation signals. *cis*-Configuration of the azulene ring system was derived from NOE between H-2 and H-6. The *cis*-configured cyclopropane ring was deduced from an interaction of the cyclopropane methyl protons H-12 with H-6 and H-7 and NOE between the methyl protons H-13 and H-5, which also indicated a *trans*-configuration for the methine protons H-5 and H-6. For the assignment of the absolute configuration, samples of (−)-*allo*-aromadendrene (**26**) and (+)-*allo*-aromadendra-4(15),10(14)-diene (**11**) were catalytically hydrogenated (Fig. 8). Two diasteromeric products (**26a** and **26b**) were expected for the hydrogenation of **26** and four diasteromeric products (**11a**–**11d**) for the hydrogenation

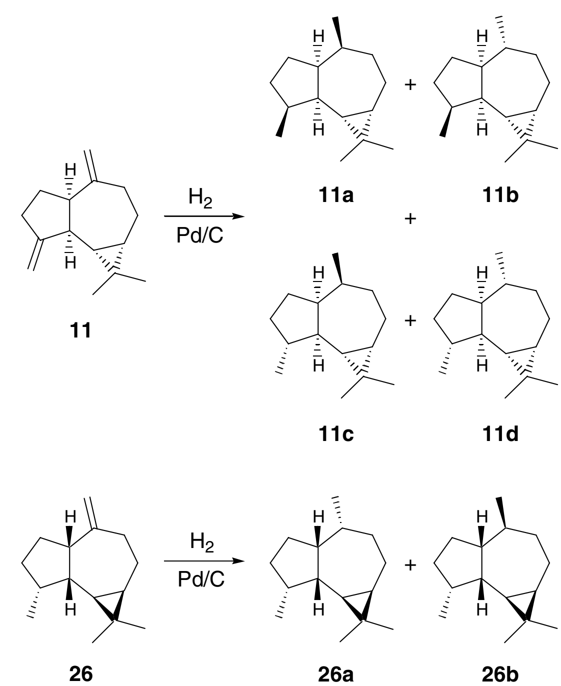


Fig. 8. Products obtained by catalytic hydrogenation of (+)-*allo*-aromadendra-4(15),10(14)-diene (**11**) and (−)-*allo*-aromadendrene (**26**).

of **11**. The expected **11a** and **11b** constitute the optical antipodes of **26a** and **26b**. Even though a complex mixture of products was obtained in both cases, two of the products had identical retention times on non-chiral stationary phases but different retention times on a column with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin. Thus, the absolute configuration (1*R*, 5*S*,6*S*,7*S*) was derived for **11**.

2.8. (+)-Aromadendra-4(15),10(14)-dien-1-ol (12)

The molecular formula of **12** was assigned as $C_{15}H_{22}O$ by HRMS (M^+: *m/z* 218.1662). Although only a small amount of compound **12** was isolated it could be identified by interpretation of the ^1H NMR and H,H-COSY spectra. The ^1H NMR spectrum exhibited two tertiary methyl groups at δ_H 0.98 (3H, *s*) and 1.06 (3H, *s*), four olefinic protons at δ_H 4.81 (1H, *t*), 4.84 (1H, *m*), 4.89 (1H, *br s*) and 5.06 (1H, *m*) as well as two cyclopropyl protons at δ_H 0.36 (1H, *dd*) and 0.50 (1H, *m*), in agreement with the structure of the aromadendrane alcohol **12**. The position of the hydroxy group was concluded from the absence of the H-1 proton. In the NOESY spectrum NOEs between the H-12 methyl protons and H-6 as well as H-7 indicated a *cis*-fused cyclopropane ring. In addition, NOE between the H-13 methyl protons and H-5 confirmed a *trans*-configuration for H-5 and H-6. Although the relative configuration at C-1 could not, be derived unambiguously, missing spatial interactions between the cyclopentane unit and the C-6 methine proton a *trans*-fused azulene ring system (Fig. 9).

2.9. (+)-Isozierene (13)

During the GC investigation of the essential oil with different injection port temperatures for monitoring variations in peak areas, the assigned peak of **13** was detected to change its ratio in parallel with zierene (**3**). As *Cope* rearrangement has been described for saccogynol (**4**) upon the attempt of its oxidation, compound **13** was suspected to be the corresponding thermal rearrangement product of **3** (Connolly et al., 1994). **13** exhibited a molecular ion signal at *m/z* 202 ($C_{15}H_{22}$). The ^1H NMR spectrum indicated signals corresponding to one olefinic methyl group at δ_H 1.45 (3H, *d*) and four olefinic protons at δ_H 4.66 (1H, *dd*), 4.85 (1H, *d*), 5.05 (1H, *s*) and 5.34 (1H, *dd*). The PENDANT spectrum revealed one methyl group (δ_C 14.9), eight methylene groups (δ_C 26.8, 28.5, 29.3, 30.5, 34.1, 34.8, 37.1 and 109.8), three methine groups (δ_C 55.6, 127.2 and 130.0) and three quaternary carbon atoms (δ_C 134.3, 143.2 and 155.8), in agreement with structure **13**. The formation of isozierene (**13**) was established by injection of a pure sample of **3** at varying injection port temperatures.

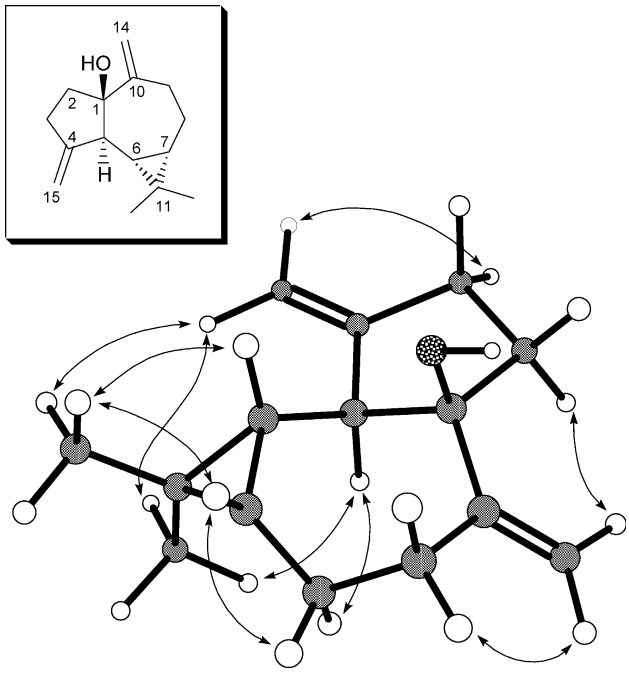


Fig. 9. Proposed relative configuration of aromadendra-4(10),10(14)-dien-1-ol (**12**) (AM1 MOPAC model) and the observed NOEs.

2.10. (+)-Viticulol (14)

Compound **14**, a diterpene alcohol of the molecular formula $C_{20}H_{34}O$, exhibited a molecular ion peak of low intensity at *m/z* 290 (<1%), but a relative intense $[M-H_2O]^+$ fragment at *m/z* 272 (36%). In the ^1H NMR spectrum a secondary methyl group at δ_H 0.96 (3H, *d*), a tertiary methyl group at δ_H 1.13 (3H, *s*), two olefinic methyl groups at δ_H 1.58 (3H, *s*) and 1.68 (3H, *s*) and three olefinic protons at δ_H 4.90 (1H, *d*), 5.02 (1H, *s*) and 5.24–5.30 (1H, *m*) were detected. A quaternary carbon atom at δ_C 73.92 suggested the presence of a tertiary hydroxy group. The molecule contains four units of unsaturation and two double bonds (δ_C 107.5, 125.0, 132.2, 155.5), hence, a bicyclic structure was assumed. Analysis of the H,H-COSY, HMQC and HMBC spectra revealed a prenylguaiane skeleton with the two double bonds located in the C-11/C-19 and C-14/C-15 position of the side chain. The carbon atom containing the hydroxy group was assigned to C-10. The relative configuration was determined by inspection of the NOESY spectrum as (1*R**,4*S**,5*S**,7*S**,10*S**). To our knowledge viticulol (**14**) is the first diterpene with the prenylguaiane skeleton isolated from liverworts. Prenylguaianes are mainly found in algae of the genus *Dictyota* and *Aplysia* and less frequently in corals of the species *Xenia* (Breitmaier, 1999). Examples are dictyol E (Danise et al., 1977), pachydictyol A (Hirschfeld et al., 1973) or acutilol B (Hardt et al., 1996).

3. Conclusions

The essential oil of *S. viticulosa* is composed of unique sesquiterpenes, especially of the rare gorgonane and zierane type. The new sesquiterpene isogermacrene A (**5**) and its *Cope* rearrangement product iso-β-elemene (**6**) were isolated and identified. The novel diterpene viticulol (**14**) could be considered as another example of a phylogenetic relationship between bryophytes and algae. Functionalised diterpenes with prenylsesquiterpene structures, such as prenylguaianes, prenylaromadendranes, prenylanastreptanes or prenylbourbonanes, with diverse biological activities such as antibacterial, antifungal, feeding-deterrent, antifoulant or antitumoral properties have been described (Kurata et al., 1990; Schmitt et al., 1998; Hardt et al., 1996). So far, the biological activity of **14** has not been investigated. The authenticity of all isolated compounds as plant constituents was confirmed by the praparation of a *n*-pentane extract.

From a biogenetic point of view, **5** is proposed as the missing link between FPP and the gorgonanes and zieranes. Nevertheless, direct formation of **5** in a single cyclisation step seems improbable, because the compound apparently contains two tail-to-tail fused isoprene units. A possible biosynthetic pathway for the formation of **5** would be preceded by the formation of (−)-bicyclogermacrene (**20**) with subsequent enzymatically induced ring opening of the cyclopropane unit and the deprotonation of the resulting *E*,*E*-isogermacra-1(10),4-dienyl cation (**27**) (Fig. 10). This proposal is supported by the identification of (−)-**20** as an essential oil constituent of *S. viticulosa*. To support the hypothesis of involvement of an enzyme in the ring opening reaction, the proton induced rearrangement of (+)-**20**, obtained from a higher plant, was investigated by GC–MS. Exposure

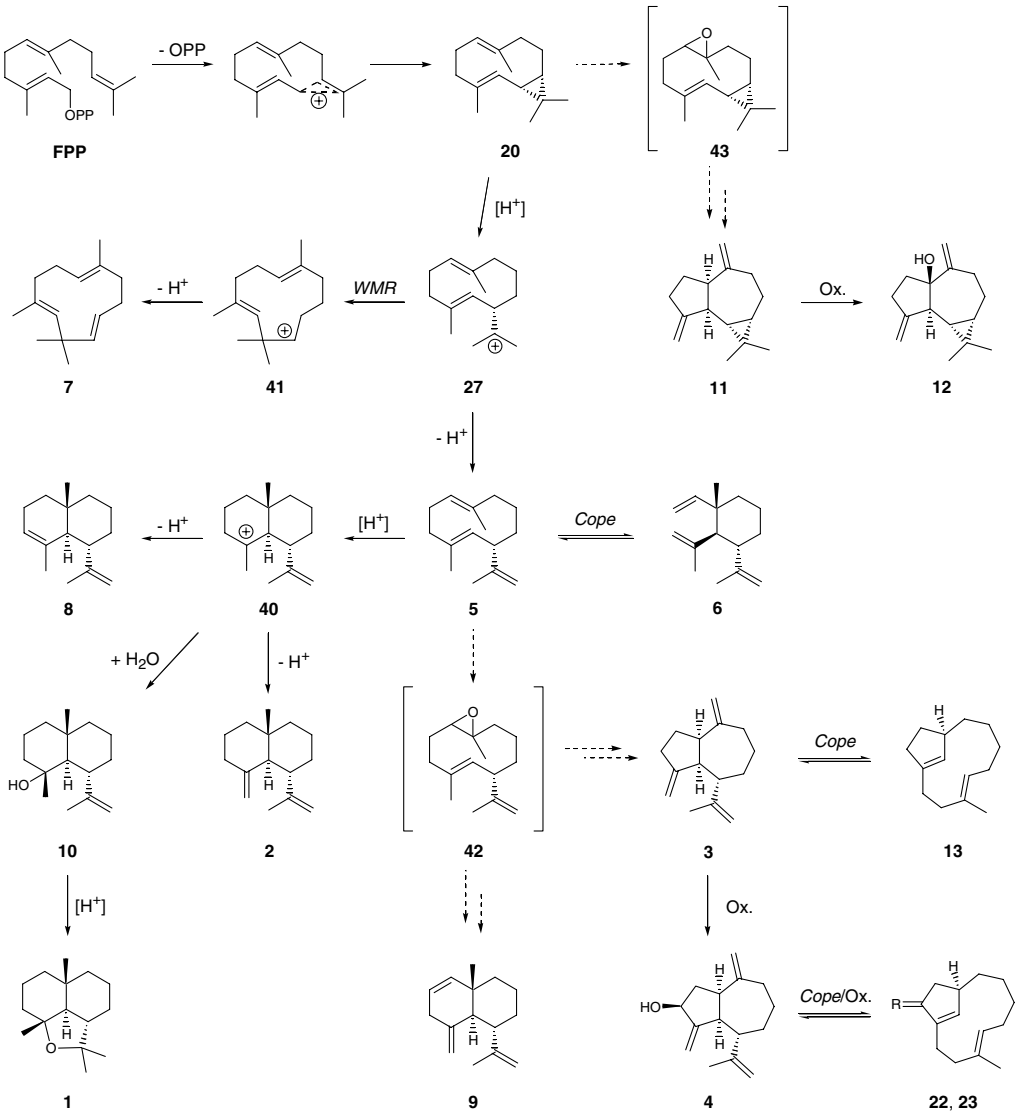


Fig. 10. Proposed biogenetic pathways for compounds **1**–**13**, **22** and **23** from *Saccogyna viticulosa* (*WMR* = *Wagner–Meerwein* rearrangement).

of (+)-**20** for one day to acidic ion exchange resin in *n*-hexane yielded a complex mixture of hydrocarbons and oxygenated sesquiterpenoids. Various unidentified compounds were formed, in addition to isoledene (**28**), β-elemene (**17**), β-maaliene (**29**), γ-maaliene (**30**), α-maaliene (**31**), aromadenrene (**32**), selina-4(15),7-diene (**33**), *allo*-aromadendrene (**34**), δ-selinene (**35**), δ-cadinene (**36**), palustrol (**37**), rosifoliol (**38**), 5-guaiene-11-ol (**39**) (the compounds are listed in order of their elution from a CPSiL5 capillary column) (Fig. 11). None of the gorgonane or zierane type compounds described in this work were detected. The identification of **17** indicated the formation of germacrene A (**24**) during the rearrangement process. In conclusion, under non-enzymatic conditions C-6/C-11 cleavage of the cyclopropane system is favoured.

A biogenetic scheme for the new sesquiterpenes (**5**–**13**), β-gorgonene (**2**), zierene (**3**) and saccogynol (**4**) and a new biogenetic pathway for the formation of maalioxide (**1**) is proposed (Fig. 10). Similar to the formation of the eudesmane-type sesquiterpenes from "normal" germacrenes, protonation of **5** followed by cyclisation would lead to the gorgonenyl cation **40** and subsequent deprotonation would afford either **2** or **8** (Cane, 1999; Dewick, 2002). Addition of a water molecule to **40** would result in the formation of gorgon-11-en-4-ol (**10**). Finally, protonation of **10** should give rise to **1**. These biogenetic correlations between **1**, **2**, **5**, **8** and **10** are in agreement with the rearrangement reactions described for **5** and **10** (Fig. 5).

Fig. 6 demonstrates the strucutural relationship between iso-α-humulen (**7**) and **5** which both contain tail-to-tail fused isoprene units. Therefore, **7** can not originate from FPP directly and a *Wagner–Meerwein* rearrangement from the *E*,*E*-isogermacra-1(10),4-dienyl cation (**27**) to an *E*,*E*-isohumula-1,5-dienyl cation (**41**) is proposed for its biogenesis.

The formation of the sesquiterpene hydrocarbons zierene (**3**), gorgona-1,4(15),11-triene (**9**) and *allo*-aromadendra-4(15),10(11)-diene (**11**) suggested an additional step, due to the reduced molecular mass of 202. Epoxidation of **5** could lead to intermediate **42** and subsequent cyclisation followed by deprotonation and dehydration would give **3** or **9**, respectively. A corresponding intermediate **43** may be predicted in the formation of **11**. Oxidation of **3** and **11** would result in the sequiterpene alcohols saccogynol (**4**) and aromadendra-4(15),10(14)-dien-1-ol (**12**).

It should be mentioned that another class of sesquiterpenes, the nardosinanes (**44**), are structurally related to the compounds presented in this work (Fig. 12) (Vidari et al., 1998; Melching, 1999). Although no nardosinane type sesquiterpenes could be detected in *S. viticulosa*, a biosynthetic analogue to the formation of eremophilanes (Cane, 1999; Calvert et al., 2002) starting from isogermacrene A (**5**) may be proposed. In some cases the formation of gorgonanes, zieranes or nardosinanes through an enzymatic cyclopropane cleavage of the maalianes, aromadendranes or aristolanes has been predicted (Barton and Gupta, 1962; Paknikar and Sood,

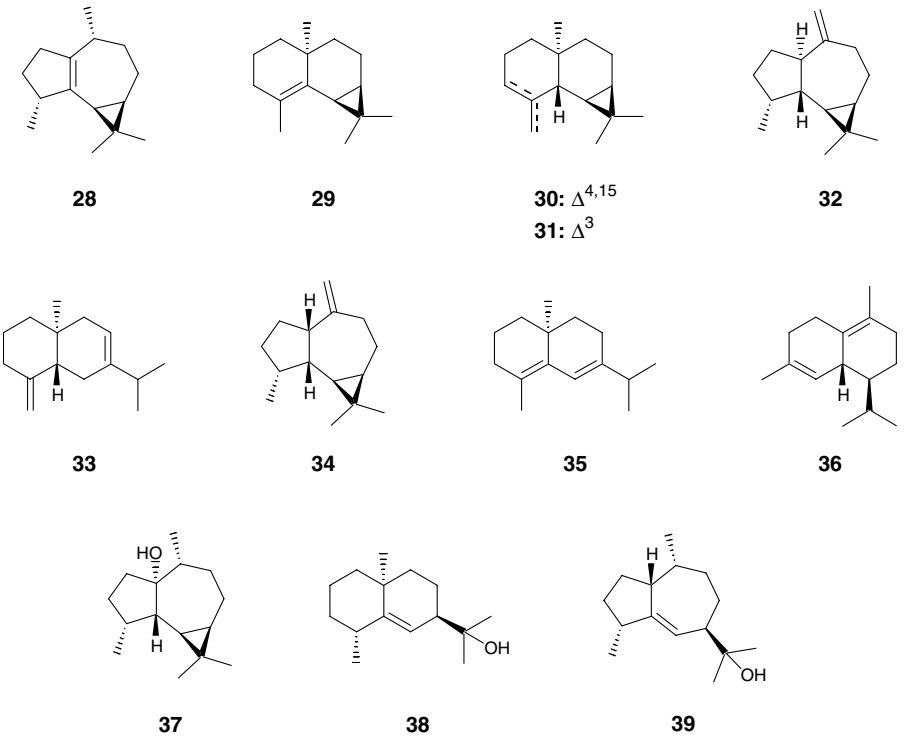
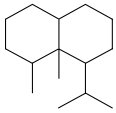


Fig. 11. Some selected products obtained by proton induced rearrangement of (+)-bicyclogermacrene (**20**).



44

Fig. 12. Nardosinane skeleton.

1973; Rücker, 1975). This concept is similar to the proposed biosynthesis of **5** and may not be excluded in particular cases. During a GC–MS screening, maalioxide (**1**) and iso-β-elemene (**6**) have been detected in the essential oil of the liverwort *Lophozia ventricosa*, thus, it could be expected that **5** is also a constituent of this plant (Lu et al., 2004).

4. Experimental

4.1. General experimental procedures

4.1.1. Gas chromatography

Carlo Erba Mega 5300 or GC 8000 double column instruments equipped with 25 m silica capillaries with polysiloxane CPSil-5 and polisiloxane CPSil-19 (Chromopack); 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 ca. 1:30; FID; carrier gas 0.5 bar H_2; injector and detector temperatures were 200 and 250 °C, respectively.

4.1.2. GC–MS and GC–HRMS

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

4.1.3. Preparative GC

Modified Varian 1400 instrument, 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 OV1701 (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; He as carrier gas at a flow rate of 120 ml min^{-1}; injector and detector temperatures were 200 and 250 °C, respectively; eluting fractions were trapped in teflon tubes cooled with liquid nitrogen (Hardt and König, 1994).

4.1.4. Semipreparative GC

HP 6890 gas chromatograph equipped with an autosampler and a 30 m megabore thickfilm capillary column (i.d. 0.53 mm, film thickness 5 μm) with polysiloxane DB-1701 or a 25 m megabore thickfilm capillary column (i.d. 0.53 mm) with heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in OV1701 (50%, w/w); He was used as carrier gas; injector and detector (FID) temperatures were 200 and 250 °C, respectively; eluting fractions were trapped at −20 °C in glass tubes using the automatic fraction collector PFC 1 from Gerstel, Mülheim, Germany, and a cryostat.

4.1.5. NMR spectroscopy

NMR measurements were carried out with a Bruker WM 400 (400 MHz), a Bruker DRX 500 (500 MHz) or a Bruker DRX 700 (700 MHz) instrument in C_6D_6 or acetone-d_6 using TMS as internal standard.

4.1.6. Polarimetry

Measurements were performed with a polarimeter 341 Perkin–Elmer at 589 nm at 20 °C in *n*-hexane, C_6D_6 or acetone-d_6. To avoid inaccuracies only the sense of optical rotation was determined due to the small quantity of the isolated compounds.

4.1.7. Molecular modelling

Molecular models were calculated with the program CSChem 3D Pro® (Version 5.0) from *Cambridge Soft*. The start geometries were calculated by the molecule mechanical MM2 force field method (Allinger, 1977). Geometries were optimised by the semiempirical SCF method of the implemented program CS MOPAC Pro® (Version MOPAC 97) from *Fujitsu*, using the potential function AM1 (Dewar et al., 1985). All calculations were executed on a standard PC equipped with an AMD 830 MHz processor and 256 MB RAM.

4.2. Plant material, essential oil and extract

S. viticulosa was collected in April 2003 at Ribero Frio/Levada de Furado, Madeira. Aqueous homogenates of the fresh plant material were submitted to hydrodistillation (2 h) to yield the essential oil, which was collected in 1 ml of *n*-hexane and not further quantified. Alternatively, air dried plant material was powdered

under liquid nitrogen and extracted with *n*-pentane at 7 °C. Because of the greatly differing weight the plant material was not weighed.

4.3. Isolation of single constituents of the essential oil

The essential oil from *S. viticulosa* was fractionated by column chromatography over deactivated alumina with increasing proportions of Et_2O in *n*-pentane. Three hydrocarbon fractions and five fractions containing oxygenated sesquiterpenes and diterpenes were obtained. Each fraction was investigated by GC and GC–MS and submitted to further isolation procedures.

4.3.1. (+)-(5S,6S,10S)-β-Gorgonene (2)

^{1}H NMR (500 MHz, C_6D_6): δ 0.82 (3H, *s*, H-14), 1.09 (1H, *ddd*, J = 4.1, 4.1, 13.2 Hz, $H_{(Re)}$-9), 1.18 (1H, *ddd*, J = 4.4, 4.4, 13.2 Hz, $H_{(Si)}$-1), 1.26 (1H, *m*, $H_{(Si)}$-7), 1.31–1.38 (2H, *m*, $H_{(Re)}$-1, $H_{(Si)}$-9), 1.40–1.44 (1H, *m*, $H_{(Re)}$-8), 1.47–1.60 (3H, *m*, $H_{(Re)}$-2, $H_{(Si)}$-2, $H_{(Si)}$-8), 1.61 (3H, *s*, H-13), 1.66–1.71 (1H, *m*, $H_{(Re)}$-7), 1.76 (1H, *d*, J = 11.7 Hz, H-5), 1.90 (1H, *ddd*, J = 5.4, 5.4, 12.9 Hz, $H_{(Re)}$–3), 2.21 (1H, *m*, $H_{(Si)}$-3), 2.32 (1H, *m*, H-6), 4.77–4.80 (2H, *m*, $H_{(Z)}$-12, $H_{(Z)}$-15), 4.81-4.82 (1H, *m*, $H_{(E)}$-12), 4.88 (1H, *br s*, $H_{(E)}$-15); ^{13}C NMR (100.6 MHz, C_6D_6): δ 17.5 (*q*, C-14), 19.2 (*q*, C-13), 21.9 (*t*, C-8), 24.7 (*t*, C-2), 35.1 (*t*, C-7), 36.8 (*s*, C-10), 38.5 (*t*, C-3), 41.8 (*t*, C-9), 42.9 (*d*, C-6), 43.1 (*t*, C-1), 52.6 (*d*, C-5), 108.4 (*t*, C-15), 110.9 (*t*, C-12), 148.3 (*s*, C-4), 150.3 (*s*, C-11); MS (EI, 70 eV) *m*/*z* (rel. int.): 204 [M^+] (5), 189 (100), 175 (3), 161 (38), 147 (42), 133 (42), 119 (30), 105 (48), 91 (55), 79 (51), 67 (41), 55 (40), 41 (58).

4.3.2. (+)-(1R*,5R*,6S*)-Zierene (3)

^{1}H NMR (500 MHz, C_6D_6): δ 1.29–1.38 (1H, *m*, $H_{(Si)}$-8), 1.40–1.49 (1H, *m*, $H_{(Si)}$-7), 1.56–1.63 (1H, *m*, $H_{(Re)}$-7), 1.64 (3H, *s*, H-13), 1.69–1.85 (3H, *m*, $H_{(Re)}$-2, $H_{(Si)}$-2, $H_{(Re)}$-8), 1.91 (1H, *ddd*, J = 4.6, 10.7, 12.5 Hz, $H_{(Re)}$-9), 2.08 (1H, *ddd*, J = 1.3, 9.7, 9.7 Hz, H-6), 2.20–2.31 (2H, *m*, $H_{(Re)}$-3, $H_{(Si)}$-9), 2.35–2.43 (1H, *m*, $H_{(Si)}$-3), 2.51–2.56 (1H, *m*, H-5), 2.72 (1H, *ddd*, J = 7.4, 7.4, 7.4 Hz, H-1), 4.72–4.75 (3H, *m*, H-12, $H_{(Z)}$-14), 4.84–4.85 (1H, *m*, $H_{(E)}$-14), 4.90–4.92 (1H, *m*, $H_{(E)}$-15), 4.96–4.98 (1H, *m*, $H_{(Z)}$-15); ^{13}C NMR (100.61 MHz, C_6D_6): δ 20.4 (*q*, C-13), 29.4 (*t*, C-2), 30.8 (*t*, C-8), 31.8 (*t*, C-3), 35.2 (*t*, C-7), 37.5 (*t*, C-9), 48.8 (*d*, C-6), 49.6 (*d*, C-1), 52.7 (*d*, C-5), 107.5 (*t*, C-15), 110.2 (*t*, C-12), 110.8 (*t*, C-14), 150.4 (*s*, C-11), 153.2 (*s*, C-10), 154.6 (*s*, C-4); MS (EI, 70 eV) *m*/*z* (rel. int.): 202 [M^+] (27), 187 (92), 173 (53), 159 (59), 145 (60), 131 (65), 117 (44), 105 (75), 91 (100), 79 (83), 67 (38), 53 (44), 41 (97).

4.3.3. (+)-(1R*,3S*,5R*,6S*)-Saccogynol (4)

^{1}H NMR (500 MHz, C_6D_6): δ 1.31–1.44 (2H, *m*, $H_{(Si)}$-7, $H_{(Si)}$-8), 1.54–1.58 (1H, *m*, $H_{(Re)}$-7), 1.60 (3H, *s*, H-13), 1.63–1.69 (1H, *m*, $H_{(Re)}$-8), 1.71 (1H, *ddd*, J = 6.6, 7.9, 13.6 Hz, $H_{(Si)}$-2), 1.87 (1H, *ddd*, J = 5.4, 9.8, 12.9 Hz, $H_{(Re)}$-9), 2.14 (1H, *m*, $H_{(Re)}$-2), 2.21 (1H, *ddd*, J = 1.0, 9.5, 9.5 Hz, H-6), 2.28 (1H, *ddd*, J = 5.4, 5.4, 12.9 Hz, $H_{(Si)}$-9), 2.46 (*1H*, *dddd*, J = 1.6, 1.6, 7.9, 7.9 Hz, H-5), 2.56 (1H, *ddd*, J = 7.9, 7.9, 7.9 Hz, H-1), 4.28 (1H, *dddd*, J = 1.9, 1.9, 6.6, 6.6 Hz, H-3), 4.72 (2H, *m*, H-12), 4.83 (1H, *m*, $H_{(E)}$-14), 4.86 (1H, *m*, $H_{(Z)}$-14), 5.03 (1H, *m*, $H_{(E)}$-15), 5.13 (1H, *m*, $H_{(Z)}$-15). OH missing; ^{13}C NMR (100.6 MHz, C_6D_6): δ 20.6 (*q*, C-13), 30.3 (*t*, C-8), 34.8 (*t*, C-7), 36.9 (*t*, C-9), 39.7 (*t*, C-2), 46.9 (*d*, C-1), 48.7 (*d*, C-6), 49.9 (*d*, C-5), 74.0 (*d*, C-3), 109.2 (*t*, C-15), 110.7 (*t*, C-12), 111.9 (*t*, C-14), 150.6 (*s*, C-11), 151.9 (*s*, C-10), 156.7 (*s*, C-4); MS (EI, 70 eV) *m*/*z* (rel. int.): 218 [M^+] (6), 203 (38), 185 (43), 175 (31), 157 (52), 143 (34), 131 (32), 117 (37), 105 (49), 91 (79), 79 (68), 67 (46), 55 (43), 41 (100).

4.3.4. Isolation of (+)-(S)-isogermacrene A (5)

(+)-(*S*)-1,5-Dimethyl-7-(1-methylethenyl)-cyclodeca-1*E*,5*E*-diene. **5** was enriched by preparative GC at an injector temperature of 140 °C using a heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin column (110 °C isothermal, 1.8 bar He) and later purified by semipreparative GC applying a DB-1701 column (130 °C isothermal) and with temperatures for injector, transferline and switching device of 150 °C. Colourless oil (ca. 0.2 mg); RI_{CPSiL5} = 1501; sense of optical rotation (acetone-d_6): (+); ^{1}H NMR (700 MHz, acetone-d_6, 283 K): δ 1.41 (3H, *br s*), 1.56 (3H, *d*), 1.68 (3H, *s*), 2.68–2.70 (1H, *m*), 4.48 (1H, *m*), 4.55 (1H, *br s*), 4.67 (1H, *br s*), 4.80–4.81 (1H, *m*); ^{13}C NMR (data from HMBC spectrum, 700 MHz, acetone-d_6, 283 K): δ 16.3, 20.4, 40.0, 42.4, 48.1, 107.7, 126.5, 129.2, 135.3, 138.1, 151.2, 4 C missing; MS (EI, 70 eV), *m*/*z* (rel. int.): 204 [M^+] (8), 189 (36), 175 (7), 161 (38), 147 (22), 133 (30), 119 (41), 107 (73), 93 (100), 81 (72), 79 (68), 67 (47), 55 (35), 41 (49).

4.3.5. Isolation of (−)-(5S,6S,10S)-iso-β-elemene (6)

(−)-(1*S*,2*S*,3*S*)-1-Ethenyl-1-methyl-2,3-di(1-methylethenyl)-cyclohexane. **6** was isolated by preparative GC using a heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin column (120 °C isothermal, 1.8 bar He). Colourless oil (ca. 2 mg); RI_{CPSiL5} = 1364; sense of optical rotation (C_6D_6): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 1.03 (3H, *s*, H-14), 1.17–1.27 (1H, *m*, $H_{(Si)}$-7), 1.30–1.39 (2H, *m*, $H_{(Re)}$-8, $H_{(Si)}$-8), 1.41–1.47 (2H, *m*, $H_{(Re)}$-9, $H_{(Si)}$-9), 1.63–1.65 (3H, *m*, H-13), 1.65–1.70 (1H, *m*, $H_{(Re)}$-7), 1.71 (3H, *m*, H-15), 1.92 (1H, *d*, J = 12.0 Hz, H-5), 2.33 (1H, *ddd*, J = 3.6, 12.0, 12.0 Hz, H-6), 4.60–4.61 (1H, *m*, $H_{(Z)}$-3), 4.73 (1H, *m*, $H_{(Z)}$-12), 4.78–4.79 (1H, *m*, $H_{(E)}$-12), 4.90–4.96 (3H, *m*, H-2, $H_{(E)}$-3), 5.82 (1H, *dd*, J = 10.4, 17.8 Hz, H-1); ^{13}C NMR (100.6 MHz, C_6D_6): δ 18.4 (*q*, C-14), 20.0 (*q*, C-13), 22.3 (*t*, C-8), 24.3 (*q*, C-15), 33.9 (*t*, C-7), 40.3 (*t*, C-9), 40.7 (*s*, C-10), 45.3 (*d*, C-6), 56.3 (*d*, C-5), 110.1

(*t*, C-2), 111.5 (*t*, C-12), 114.9 (*t*, C-3), 145.2 (*s*, C-4), 149.7 (*s*, C-11), 150.8 (*d*, C-1); MS (EI, 70 eV), *m*/*z* (rel. int.): 204 [M^+] (2), 189 (17), 175 (6), 161 (19), 147 (16), 135 (52), 122 (55), 107 (85), 93 (100), 81 (84), 79 (85), 67 (59), 55 (45), 41 (68).

*4.3.6. Isolation of iso-α-humulene (**7**)*

1,3,3,8-Tetramethylcycloundeca-1,4,8-triene. **7** was enriched by preparative GC using a heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin column (110 °C, isothermal, 1.8 bar He) and purified by repeating this separation step twice. Colourless oil (ca. 1 mg); RI_{CPSiL5} = 1471; ^{1}H NMR (500 MHz, C_6D_6): δ 1.31 (6H, *s*, H-12, H-13), 1.38 (3H, *s*, H-14), 1.48 (3H, *d*, *J* = 1.3 Hz, H-15), 1.93-1.97 (6H, *m*, H-3, H-7, H-8), 2.10 (2H, *br s*, H-4), 4.61 (1H, *br t*, H-5), 4.98 (1H, *br s*, H-1), 4.99-5.05 (1H, *m*, H-9), 5.48 (1H, *d*, *J* = 15.8 Hz, H-10); ^{13}C NMR (100.6 MHz, C_6D_6): δ 15.4 (*q*, C-14), 16.6 (*q*, C-15), 27.6 (*t*, C-4), 28.4 (*q*, 2×C, C-12, C-13), 30.3 (*t*, C-8), 38.5 (*s*, C-11), 41.6 (*t*, C-7), 42.2 (*t*, C-3), 125.0 (*d*, C-9), 128.0 (*d*, C-5), 130.0 (*s*, C-2), 132.0 (*s*, C-6), 140.3 (*d*, C-1), 141.5 (*d*, C-10); MS (EI, 70 eV), *m*/*z* (rel. int.): 204 [M^+] (16), 189 (5), 161 (5), 147 (4), 136 (10), 121 (100), 107 (34), 93 (94), 79 (33), 67 (24), 53 (27), 41 (126).

*4.3.7. Isolation of (+)-(5R,6S,10S)-α-gorgonene (**8**)*

(+)-(1*S*,4a*S*,8a*R*)-4a,8-Dimethyl-1-(1-methylvinyl)-1,2,3,4,4a,5,6,8a-octahydro-naphthalene. **8** was purified by preparative GC with a column with heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (110 °C isothermal, 1.8 bar He). Colourless oil (ca. 2 mg); RI_{CPSil5} = 1488; sense of optical rotation (C_6D_6): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.88 (3H, *s*, H-14), 0.98–1.09 (1H, *m*, $H_{(Si)}$-9), 1.19–1.25 (1H, *m*, $H_{(Re)}$-7), 1.27–1.31 (1H, *m*, $H_{(Si)}$-1), 1.34–1.43 (3H, *m*, $H_{(Re)}$-1, $H_{(Re)}$-8, $H_{(Re)}$-9), 1.44–1.60 (1H, *m*, $H_{(Si)}$-8), 1.64 (3H, *s*, H-13), 1.64–1.72 (1H, *m*, $H_{(Si)}$-7), 1.83 (3H, *d*, *J* = 1.5 Hz, H-15), 1.98–2.10 (2H, *m*, $H_{(Re)}$-2, $H_{(Si)}$-2), 2.15 (2H, *br s*, H-5, H-6), 4.75 (1H, *m*, $H_{(Z)}$-12), 4.79 (1H, *s*, $H_{(E)}$-12), 5.37 (1H, *br s*, H-3); ^{13}C NMR (100.6 MHz, C_6D_6): δ 16.8 (*q*, C-14), 20.4 (*q*, C-13), 21.7 (*t*, C-8), 23.5 (*t*, C-2), 24.6 (*q*, C-15), 34.0 (*s*, C-10), 36.8 (*t*, C-7), 39.3 (*t*, C-1), 41.3 (*t*, C-9), 44.0 (*d*, C-5), 48.4 (*d*, C-6), 110.5 (*t*, C-12), 123.6 (*d*, C-3), 136.7 (*s*, C-4), 152.7 (*s*, C-11); MS (eI, 70 eV), *m*/*z* (rel. int.): 204 [M^+] (7), 189 (82), 175 (3), 161 (19), 147 (17), 133 (32), 119 (38), 107 (100), 93 (65), 79 (46), 67 (27), 55 (32), 41 (49).

*4.3.8. Isolation of (5S,6S,10S)-gorgona-1,4(15),11-triene (**9**)*

(4*S*,4a*S*,8a*S*)-8a-Methyl-5-methylene-4-(1-methylvinyl)-1,2,3,4,4a,5,6,8a-octahydro-naphthalene. **9** was enriched by preparative GC using a heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin column (110 °C isothermal, 1.8 bar He) and later purified by semipreparative GC applying a heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin thick-film column (100–150 °C, 1 °C/min, 150 °C isothermal). Colourless oil (ca. 0.6 mg); RI_{CPSil5} = 1426; ^{1}H NMR (500 MHz, C_6D_6): δ 0.94 (3H, *s*, H-14), 1.22 (1H, *dd*, *J* = 4.7, 12.3 Hz, $H_{(Si)}$-7), 1.31 (1H, *dd*, *J* = 4.4, 13.2 Hz, $H_{(Re)}$-9), 1.41–1.51 (3H, *m*, $H_{(Si)}$-9, $H_{(Re)}$-8, $H_{(Si)}$-8), 1.58 (3H, *s*, H-13), 1.65–1.69 (1H, *m*, $H_{(Re)}$-7), 2.11 (1H, *d*, *J* = 11.7 Hz, H-5), 2.41 (1H, *ddd*, *J* = 3.8, 11.7, 11.7 Hz, H-6), 2.51–2.55 (1H, *m*, H-3), 2.76–2.81 (1H, *m*, H-3), 4.79–4.81 (2H, *m*, H-12), 4.86 (1H, *d*, *J* = 1.9 Hz, $H_{(Z)}$-15), 4.95 (1H, *br s*, $H_{(E)}$-15), 5.40–5.47 (2H, *m*, H-1, H-2); ^{13}C NMR (data from HMQC/HMBC spectra, 500 MHz, C_6D_6): δ 19.1 (*q*, C-12), 20.0 (*q*, C-14), 22.0 (*t*, C-8), 34.6 (*t*, C-7), 37.3 (*t*, C-3), 38.4 (*s*, C-10), 39.4 (*t*, C-9), 42.4 (*d*, C-6), 50.8 (*d*, C-5), 108.8 (*t*, C-15), 111.0 (*t*, C-12), 123.5 (*d*, C-2), 140.4 (*d*, C-1), 145.0 (*s*, C-4), 149.8 (*s*, C-11); MS (EI, 70 eV) *m*/*z* (rel. int.): 202 (6), 187 (100), 173 (4), 159 (18), 145 (42), 131 (59), 117 (45), 105 (71), 91 (75), 79 (55), 67 (22), 55 (24), 41 (44).

*4.3.9. Isolation of (−)-(4R,5R,6S,10S)-gorgon-11-en-4-ol (**10**)*

(−)-(1*R*,4a*S*,8*S*,9*R*)-1,4a-Dimethyl-8-(1-methylethenyl)-decahydro-naphthalen-1-ol. **10** was obtained by column chromatography in a separate fraction. Colourless oil (ca. 2 mg); RI_{CPSiL5} = 1607; sense of optical rotation (C_6D_6): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.79 (3H, *s*, H-14), 0.99–1.09 (2H, *m*, $H_{(Si)}$-1, $H_{(Re)}$-9), 1.16–1.20 (2H, *m*, $H_{(Re)}$-1, $H_{(Si)}$-9), 1.22 (3H, *m*, H-15), 1.32–1.46 (6H, *m*, $H_{(Re)}$-2, $H_{(Si)}$-2, $H_{(Re)}$-7, $H_{(Si)}$-7, $H_{(Re)}$-8, $H_{(Si)}$-8), 1.49 (1H, *d*, *J* = 11.3 Hz, H-5), 1.59–1.65 (1H, *m*, H-3), 1.74–1.76 (1H, *m*, H-3), 1.78 (3H, *d*, H-13), 2.31–2.36 (1H, *m*, H-6), 4.63 (1H, *m*, H-12), 4.79 (1H, *d*, *J* = 2.5 Hz, H-12); ^{13}C NMR (100.6 MHz, C_6D_6): δ 19.1 (*q*, C-13), 20.3 (*q*, C-14), 24.1 (*q*, C-15), 20.0, 21.7, 34.2 (3×*t*, C-2, C-7, C-8), 35.7 (*s*, C-10), 43.2 (*t*, C-3), 44.6 (*d*, C-6), 42.5, 45.5 (2×*t*, C-1, C-9), 56.8 (*d*, C-5), 73.8 (*s*, C-4), 112.7 (*t*, C-12), 153.7 (*s*, C-11); MS (eI, 70 eV), *m*/*z* (rel. int.): 222 [M^+] (2), 207 (14), 204 (9), 189 (100), 179 (13), 163 (23), 149 (28), 137 (29), 121 (25), 109 (47), 95 (35), 81 (43), 67 (28), 55 (29), 43 (58). HRMS: *m*/*z* = 222.1994 [M^+] (calc. for $C_{15}H_{26}O$: 222.1984).

*4.3.10. Isolation of (+)-(1R,5S,6S,7S)-allo-aromadendra-4(15),10(14)-diene (**11**)*

(+)-(1*S*,4a*R*,7a*S*,8*S*)-1,1-Dimethyl-4,7-dimethylene-decahydro-cyclopropa[e]azulene. The same isolation procedure as for isogermacrene A (**5**) was applied to obtain **11**. Colourless oil (ca. 1.5 mg); RI_{CPSiL5} = 1457; sense of optical rotation (C_6D_6): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.48–0.54 (2H, *m*, H-6, H-7), 1.01 (3H, *s*, H-12), 1.05 (3H, *s*, H-13), 1.08–1.14 (1H, *m*, $H_{(Si)}$-8),

1.63–1.69 (1H, *m*, $H_{(Re)}$-8), 1.71–1.77 (2H, *m*, $H_{(Si)}$-2, $H_{(Re)}$2), 2.14–2.21 (1H, *m*, $H_{(Re)}$-3), 2.21–2.29 (1H, *m*, $H_{(Si)}$-9), 2.30–2.40 (3H, *m*, $H_{(Si)}$-3, H-5, $H_{(Re)}$-9), 2.46 (1H, *m*, H-1), 4.86-4.87 (2H, *m*, $H_{(Z)}$-14, $H_{(Z)}$-15), 4.94 (1H, *m*, $H_{(E)}$-14), 5.04–5.05 (1H, *m*, $H_{(E)}$-15); ^{13}C NMR (100.6 MHz, C_6D_6): δ 16.0 (*q*, $C_{(Re)}$-12), 17.1 (*s*, C-11), 21.7 (*t*, C-8), 25.1 (*d*, C-7), 29.0 (*q*, $C_{(Si)}$-13), 29.5 (*d*, C-6), 30.0 (*t*, C-2), 34.0 (*t*, C-3), 37.2 (*t*, C-9), 41.4 (*d*, C-5), 48.3 (*d*, C-1), 105.7 (*t*, C-15), 110.3 (*t*, C-14), 150.1 (*s*, C-10), 158.0 (*s*, C-4); MS (eI, 70 eV), *m*/*z* (rel. int.): 202 [M^+] (15), 187(25), 174 (13), 159 (88), 145 (51), 131 (70), 117 (54), 105 (67), 91 (100), 79 (64), 67 (33), 55 (24), 41 (70).

4.3.11. Isolation of (+)-aromadendra-4(15),10(14)-dien-1-ol (*12*)

(+)-1,1-Dimethyl-4,7-dimethylene-decahydro-cyclopropa[e]azulen-4a-ol. Enrichment of **12** was achieved by preparative GC using a heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin column (150 to 180 °C, 1 °C min^{-1}, 1.7 bar He), following further purification by using a semipreparative DB-1701 thickfilm column (150 °C isothermal). Colourless oil (0.3 mg); RI_{CPSiL5} = 1579; sense of optical rotation (C_6D_6): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.36 (1H, *dd*, *J* = 9.1, 10.7 Hz, H-6), 0.47–0.52 (1H, *m*, H-7), 0.58 (1H, *s*, OH), 0.98 (3H, *s*, H-12), 1.06 (3H, *s*, H-13), 1.35–1.44 (2H, *m*, H-2, H-8), 1.65–1.71 (1H, *m*, H-8'), 1.97 (1H, *ddd*, *J* = 7.25, 12.93, 12.93 Hz, H-2'), 2.18–2.28 (2H, *m*, H-3, H-9), 2.39 (1H, *d*, *J* = 10.7 Hz, H-5), 2.66–2.78 (2H, *m*, H-3', H-9'), 4.81 (1H, *t*, *J* = 1.6 Hz, $H_{(E)}$-14), 4.84 (1H, *m*, $H_{(Z)}$-14), 4.89 (1H, *br s*, $H_{(Z)}$-15), 5.06 (1H, *m*, $H_{(E)}$-15); MS (eI, 70 eV), *m*/*z* (rel. int.): 218 [M^+] (7), 203 (16), 200 (17), 185 (23), 175 (49), 157 (50), 147 (29), 129 (43), 119 (43), 105 (67), 91 (100), 79 (61), 67 (39), 55 (51), 41 (92). HRMS: *m*/*z* = 218.1662 [M^+] (calc. for $C_{15}H_{22}O$: 218.1671).

4.3.12. Isolation of (+)-isozierene (*13*)

(+)-4-Methyl-9-methylene-bicyclo[8.2.1]trideca-1(13),4-diene. **13** was obtained during the isolation of zierene (**3**) by preparative GC applying a 6-*O*-*tert*-butyldimethylsilyl-2,3-*O*-dimethyl-β-cyclodextrin column (120 °C isotherm, 1.8 bar He) and an injector temperature of 200 °C. Colourless oil (ca. 1.5 mg); RI_{CPSiL5} = 1553; sense of optical rotation (C_6D_6): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 1.08–1.18 (1H, *m*), 1.45 (3H, *d*, *J* = 1.0 Hz), 1.57–1.75 (4H, *m*), 1.86–2.01 (5H, *m*), 2.09–2.19 (2H, *m*), 2.26 (1H, *ddd*, *J* = 5.3, 11.8, 11.8 Hz), 2.32–2.39 (1H, *m*), 3.48–3.51 (1H, *m*), 4.66 (1H, *dd*, *J* = 1.0, 2.6 Hz), 4.85 (1H, *d*, *J* = 2.0 Hz), 5.05 (1H, *s*), 5.34 (1H, *dd*, *J* = 7.8, 7.8 Hz); ^{13}C NMR (100.6 MHz, C_6D_6): δ14.9 (*q*), 26.8 (*t*), 28.5 (*t*), 29.3 (*t*), 30.5 (*t*), 34.1 (*t*), 34.8 (*t*), 37.1 (*t*), 55.6 (*d*), 109.8 (*t*), 127.2 (*d*), 130.0 (*d*), 134.3 (*s*), 143.2 (*s*), 155.8 (*s*); MS (eI, 70 eV), *m*/*z* (rel. int.): 202 [M^+] (83), 187 (64), 173 (33), 159 (43), 145 (50), 131 (57), 117 (36), 105 (61), 91 (86), 79 (100), 67 (37), 53 (42), 41 (79).

4.3.13. Isolation of (+)-(1*R**,4*S**,5*S**,7*S**,10*S**)-viticulol (*14*)

(+)-(1*S**,3a*R**,4*S**,7*S**,8a*S**)-1,4-Dimethyl-7-(5-methyl-1-methylene-hex-4-enyl)-decahydroazulen-4-ol. In order to isolate compound **14** preparative GC on a heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin column (150 °C, 1 °C/min, 180 °C, 1.7 bar He) was applied. Colourless oil (ca. 1.5 mg); $RI_{CP\text{-}SiL5}$ = 2103; ^{1}H NMR (500 MHz, C_6D_6): δ 0.96 (3H, *d*, *J* = 6.9 Hz, H-17), 1.03 (3H, *s*, H-18), 1.11–1.19 (1H, *m*, H-3), 1.26–1.33 (1H, *m*, H-2), 1.35–1.41 (1H, *m*, H-9), 1.58 (3H, *s*, H-20), 1.46–1.66 (6H, *m*, H-2′, H-3′, H-6, H-6′, H-8, H-9′), 1.68 (3H, *s*, H-16) 1.81–1.96 (2H, *m*, H-1, H-4), 1.97–2.05 (1H, *m*, H-8'), 2.06–2.11 (1H, *m*, H-5), 2.13–2.21 (3H, *m*, H-7, H-12), 2.22–2.30 (2H, *m*, H-13), 4.90 (1H, *d*, *J* = 1.3 Hz, $H_{(E)}$-19), 5.02 (1H, *s*, $H_{(Z)}$-19), 5.24–5.30 (1H, *m*, H-14); ^{13}C NMR: (100.6 MHz, C_6D_6) δ 17.8 (*q*, C-18), 25.9 (*q*, C-16), 26.7 (*t*, C-2), 27.3 (*t*, C-6), 27.7 (*t*, C-13), 28.0 (*t*, C-8), 31.1 (*t*, C-3), 32.2 (*q*, C-17), 35.7 (*q*, C-20), 35.8 (*t*, C-12), 35.9 (*t*, C-9), 40.0 (*d*, C-4), 40.2 (*d*, C-5), 42.5 (*d*, C-7), 56.2 (*d*, C-1), 73.9 (*s*, C-10), 107.5 (*t*, C-19), 125.0 (*d*, C-14), 132.2 (*s*, C-15), 155.5 (*s*, C-11); MS (eI, 70 eV), *m*/*z* (rel. int.): 272 [$M - H_2O$]$^+$ (36), 257 (9), 243 (3), 229 (14), 215 (6), 201 (16), 190 (24), 173 (10), 161 (59), 147 (31), 133 (27), 119 (37), 107 (56), 93 (50), 81 (44), 69 (85), 55 (36), 41 (100). HRMS: *m*/*z* = 272.2501 [$M - H_2O$] (calc. for $C_{20}H_{32}$: 272.2504).

4.4. Derivatization reactions

4.4.1. Acid induced rearrangement of compounds *2*, *5* and *20*

A solution of compound **2**, **5** or **20** in 0.5 ml *n*-hexane was treated with 0.4 mg of the acidic ion exchange resin Amberlyst® 15 and stirred at room temperature for a varying reaction time (1 h for **5**, 24 h for **20** and 48 h for **2**). Subsequently the solutions were filtered and investigated by GC–MS.

4.4.2. Hydrogenation of compounds *11* and *26*

To 0.5 mg (+)-*allo*-aromadendra-4(15),10(14)-diene (**11**) in 1 ml *n*-hexane 0.5 mg of Pd-C (15%) were added and H_2 was passed through the solution for 3 min. After stirring for 3 h at room temperature the suspension was filtered and the resulting solution concentrated. (−)-*allo*-Aromadendrene (**26**) was treated accordingly and the products of both reactions were compared by GC and GC–MS. Two products were identified that had identical retention times on non-chiral stationary phases but different retention times on a column with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin.

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