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Absolute configuration of helminthogermacrene

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Abstract—The absolute configuration of the sesquiterpene hydrocarbon helminthogermacrene is established. Helminthogermacrene is an (*E*,*Z*)-configurational isomer of germacrene A and thus undergoes similar transformations forming elemenes via *Cope* rearrangement and yielding bicyclic systems via acid catalyzed reactions. The reaction products are investigated using enantioselective GC and extensive NMR measurements (^{1}H–; ^{1}H^{1}H-COSY; HSQC; HMBC and NOE-experiments). In addition, NMR data of related compounds isolated during the course of this investigation not yet reported in literature are given.

1. Introduction

The sesquiterpene hydrocarbon helminthogermacrene **1** (Fig. 1) known from the fungus *Helminthosporium sativum*[1] and the termite *Amitermes wheeleri*[2] was recently found in the essential oil of the liverwort *Scapania undulata* (Hepaticae).[3] Contrary to the previously assumed configuration[1,3] it was possible to deduce the absolute configuration beyond doubt in this work due to enantioselective GC investigations conducted with authentic standards of (+)-germacrene A **2**, (+)- and (−)-β-elemene **3**, (+)- and (−)-δ-selinene **4**, isolation of known (−)-α-helmiscapene **5**[4] and ^{1}H–, ^{1}H^{1}H-COSY, HSQC, HMBC and NOE-experiments with the respective pure compounds. We also report the NMR data of germacrene B **6**,[5] which was isolated during the course of this work.

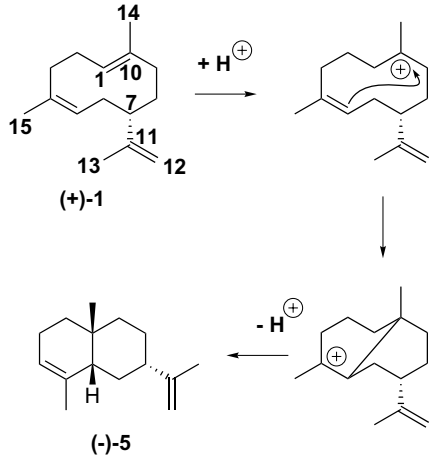

Figure 1. Observed transformations of (+)-helminthogermacrene **1**.

Thermally induced Cope rearrangement has been observed for the members of the germacrene skeleton sesquiterpene family.[6] It is well known that germacrene C **7**[7] yields racemic δ-elemene **8**, germacrene B **6** forms racemic γ-elemene **9** and that (+)-germacrene A **2** is easily converted into (−)-β-elemene **3**.[8–10] It has been observed that helminthogermacrene **1** having 1(10),4-(*E*,*Z*)-configuration is less susceptible to Cope rearrangement than **2** with 1(10),4-(*E*,*E*)- configuration.[3,11] In an earlier communication[3] we have reported that Cope rearrangement of (+)-**1** yielded more than one product, namely (−)-*cis*-β-elemene **10**, another β-elemene diastereoisomer **11** and β-elemene **3** in an 8:1:0.08 ratio as determined by GC.

2. Results and discussion

2.1. Absolute configuration of (+)-helminthogermacrene 1

Compound (+)-**1**, isolated from *S. undulata* undergoes rearrangement when stored in $CDCl_3$ at 4 °C for more than 1 week to form a series of sesquiterpene hydrocarbons with mass 204 as also observed on treatment of

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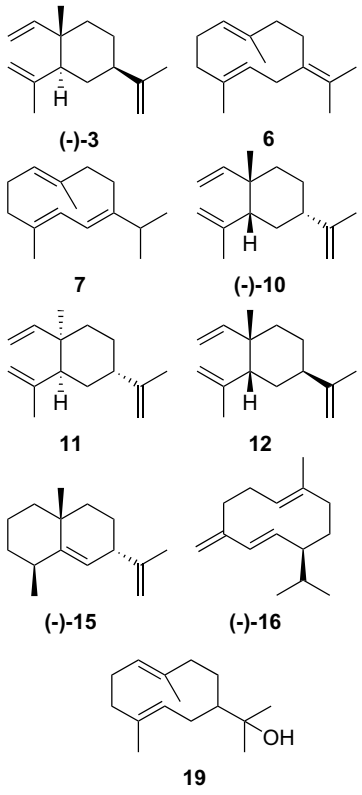

1 with BF_3 in diethyl ether or upon interaction with acidic ion exchange resin amberlyst 15, respectively. The isolation of the major unknown compound common to all three treatments resulted in a product with ^1H NMR, ^{13}C NMR ($CDCl_3$) and MS data totally consistent with α-helmiscapene **5**, earlier isolated from *S. undulata*[4] and synthesized[12–14] with known absolute configuration. The specific rotation of isolated **5** was negative as earlier reported.[4] The MS of **5** is very similar to that of its 10-epimer α-selinene **13**. The formation of **5** from **1** can be rationalized as demonstrated in Figure 1.

The comparison of hydrogenation products from (−)-α-helmiscapene **5** with the hydrogenated products of compounds of known absolute configuration like (+)-α-selinene **13**, (+)-β-selinene **14** and (−)-selina-5,11-diene **15**, one of the dehydrated products of (−)-maalian-5-ol[15] (^{13}C NMR data are reported for the first time) by enantioselective GC again confirmed the absolute configuration of (−)-**5**, which is characterized by β-orientation of the angular methyl substituent at C-10 and the H-5 and H-7 protons and thus should be (5*R*,7*S*,10*R*). From this it can be concluded that the absolute configuration of the precursor (+)-helminthogermacrene **1** should also have (7*S*)-configuration (β-orientation of the H-7 proton).

In addition, the observation that under BF_3 treatment a ratio of 5:1 in favour of the (+)-enantiomer of δ-selinene **4** is formed[3] underlines the asymmetric induction during the formation of the bicyclic systems. Thus, this may result in the formation of various eudesmanes of all possible conformations by the cyclization of the biogenetic precursor as suggested for termites,[16] which also generate (−)-**1.**

Moreover the co-occurrence of (−)-α-ylangene **8**[4] and (+)-α-amorphene **9** in the essential oil of *S. undulata* is in agreement with the proposed absolute configuration.

2.2. Investigation of Cope rearrangement of helminthogermacrene 1

In addition, the interconversion of (+)-**1** to its Cope products was further investigated. For comparison (+)-germacrene A **2**, isolated from a diethyl ether extract of *Solidago canadensis*, was injected under identical conditions (injection port temperature of 200 °C) as (+)-helminthogermacrene **1**. A minor isomer **12** eluting shortly before the main product (−)-β-elemene **3** from the GC column is a second Cope product of (+)-germacrene A **2** as it increases with increasing amount of rearranged germacrene A and is also an elemene as revealed by GC–MS. Interestingly, diastereomers with a mass spectrum very similar to β-elemene were observed from fresh costus roots[8] and thermal isomerization of (*E*,*E*)-hedycaryol **19**.[17]

The 95:5 mixture of two Cope products from germacrene A was isolated and investigated by ^1H NMR to yield a single set of β-elemene signals probably due to the low concentration of the minor constituent in the mixture. The mixture was hydrogenated to confirm that one more product was generated apart from the pure β-elemene **3**. Coinjection of the two Cope rearrangement products from **1** and **2** on two parallel columns with nonpolar dimethylpolysiloxane CPSil-5 and the more polar CPSil-19 stationary phases, respectively, reveals that **12** is not identical to *cis*-β-elemene **10** but probably is the enantiomer of **11**. Thus, this observation may be similar to the thermal isomerization of bicyclogermacrene[18] and **19**.[17] Coinjections on enantioselective GC show separation of **11** and **12** and hence support the finding that they are of opposite absolute configuration (Fig. 2).

Further investigation of the products obtained from rearrangement of **1** by enantioselective GC on a modified γ-cyclodextrin phase (cf. Fig. 2) suggests that the third product formed is a racemic mixture of β-elemene **3**. The other conceivable possibility that there are epimers formed accidentally coeluting with the respective β-elemenes is highly unlikely as such epimers should be resolved as well.

2.3. Investigation of germacrene A 2 transformations

Diethyl ether extracts of the aerial parts of *S. canadensis* concentrated under reduced pressure below 40 °C were fractionated by silica gel column chromatography without prolonged contact to minimize decomposition of sensitive constituents. The fraction that eluted with 100% *n*-pentane (yellow solution) was repeatedly submitted to preparative TLC using *n*-pentane as the

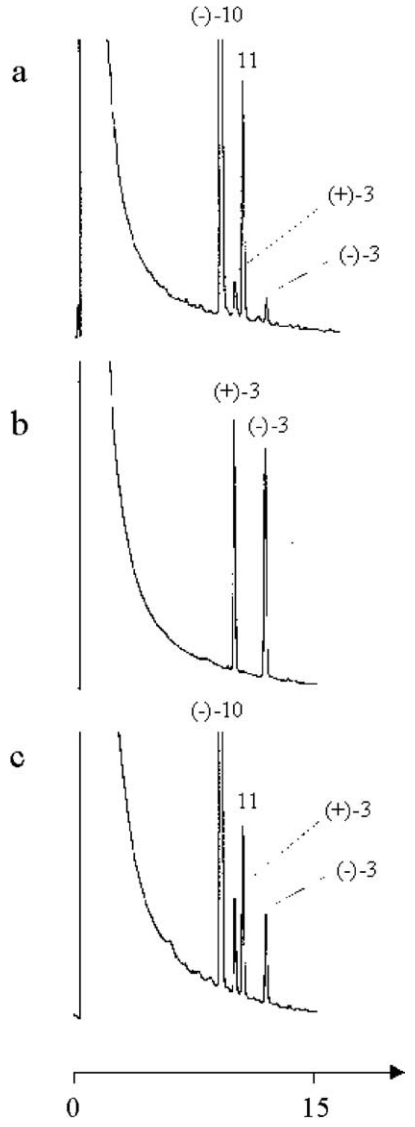

Figure 2. Comparison of the elemenes from (+)-**1** (a) (±)-(**3**) (b) and co-injection (c) on cyclodextrin chiral phase 2,6-Me-3-Pe-γ-CD at 100 °C isothermal.

developing solvent at −25 °C to give (+)-germacrene A **2** (R_f 0.40), germacrene B **6** (R_f 0.45), both enantiomers of germacrene D **16**[19] (R_f 0.61), and a single band [containing (−)-α-selinene (41%) **13**, (+)-β-selinene **14** (45%) and (+)-selina-4,11-diene **17** (12%)] (R_f 0.74) and other known constituents in trace amounts adding up to 4% (Fig. 3).

Interestingly, the germacrene A **2** analyzed by GC at 120 °C injection port temperature resulted in only a very small amount of 'normal' (−)-β-elemene **3** (ca. 5%), a broadened germacrene A **2** peak (ca. 81%), which was preceded by a 'hump' in the baseline containing **13** (0.2%), **14** (0.3%) and **17** (0.08%).

When the diethyl ether extract of *S. canadensis* was exposed to silica gel at room temperature for 24 h it formed a well known compound (−)-selin-11-en-4-ol **18** (23%), (**18** had been reported as a major constituent of the essential oil of *Conocephalum conicum*)[20] in addition to reported compounds **13**, **14** and **17**,[21] indicating that germacrene A **2** rearranges to these bicyclic systems in an analogous fashion as helminthogermacrene **1** does. The proposed biosynthetic pathway for the formation of **18** is shown in Figure 3. This observation has never been reported before for compound **2**, even though it has been reported that germacrenes B **6** and C **7** gave similar oxygenated products when subjected to acid rearrangement reactions.[22–24] Moreover, similar acid induced rearrangement reaction products of germacrene B **6** were obtained, which include selina-4(15),7(11)-diene, selina-3,7(11)-diene and eudesm-7(11)-en-4α-ol as reported,[22–24] but we noticed that this occurred rather slowly with very low yield as compared to **2** under the same conditions. This suggests that **6** is much more stable than **2** when treated with silica gel under the same conditions. Even though **2** proved to be much more stable than reported in literature with silica gel if the experiment is run within a very short period of time.

2.4. Investigation of germacrene B 6

The NMR data of the known naturally occurring **6** were recorded in C_6D_6 in which all the methyl and the methine signals are well resolved. The ^{1}H NMR spectrum showed signals of four methyl singlets at δ 1.47 (3H, br s, H-14), 1.48 (3H, br s, H-15), 1.63 (3H, s, H-12/13) and 1.67 (3H, s, H-12/13). The vinylic protons at δ 4.76 (1H, br d, $J = 12$ Hz) and 4.65–4.72 (1H, br s) are assigned to H-1 and H-5, respectively. Only the ^{1}H NMR in $CDCl_3$ of **6**[5] and the ^{1}H NMR and ^{13}C NMR of the (*E*,*Z*)-isomer of **6** have been reported before.[25]

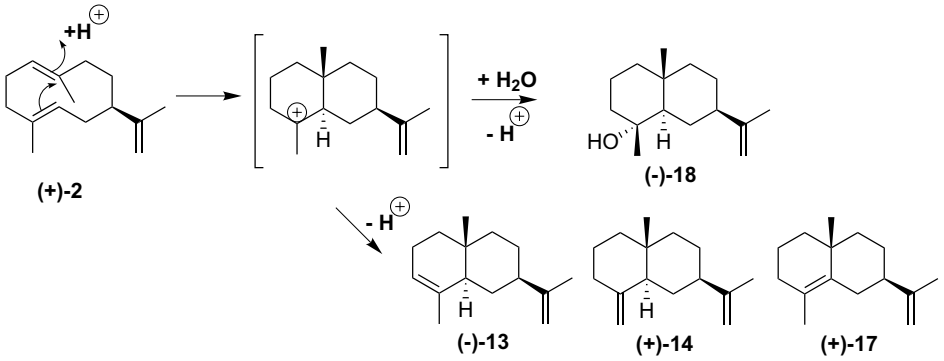

Figure 3. Observed transformations of (+)-germacrene A **2**.

3. Experimental

3.1. General experimental procedures

3.1.1. 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. All compounds were identified by comparison of their mass spectra and gas chromatographic retention indices with a spectral library established under identical experimental conditions.[26,27]

3.1.2. 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 using TMS as internal standard if not otherwise stated.

3.1.3. 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,[28] heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin[28] or heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin[29] in OV 1701 (50%, w/w), split injection; split ratio approx. 1:30; FID; carrier gas 0.5 bar H_2; injector and detector temperatures were 200, and 250 °C, respectively.

3.1.4. 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[28] 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[29] 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.[30]

3.1.5. Polarimetry. Measurements were performed with a polarimeter 341 (Perkin–Elmer) at 589 nm at 20 °C. Due to very small amounts of isolated compounds only the sign of optical rotation is given to avoid inaccuracies.

3.1.6. Reactions. Hydrogenation reactions were performed by bubbling hydrogen gas through a stirred solution of ca. 1 mg of sample in 1 mL *n*-hexane and 0.5 mg Pd/C at room temp for 1 h. The reaction mixture was filtered and the reaction products were analyzed by GC–MS and by GC on several capillary columns with cyclodextrin derivatives.

3.1.7. Thin layer chromatography. Thin layer chromatography was effected using aluminium supported plates of silica 60 F_{254} (Merck). An ethanolic solution of molybdatophosphoric acid (20% w/v) reagent was used as spray reagent. The TLC plate was developed twice in *n*-pentane at −25 °C and immediately extracted with diethyl ether.

3.2. Isolation of reference compounds

3.2.1. Isolation of (−)-α-helmiscapene 5.

(a) From the reaction mixture: A solution of ca. 1 mg of (+)-**1** in *n*-hexane (1.5 mL) was mixed with acidic ion exchange resin Amberlyst 15 and kept at room temperature for 2 h. After (+)-**1** had completely disappeared (GC control) the solution was filtered and the catalyst was washed with *n*-hexane. The solution was shaken with a saturated aqueous solution of $NaHCO_3$ (3 mL). The organic layer was partitioned and dried over Na_2SO_4, the solvent was evaporated and **5** was isolated using preparative GC.

(b) From *Radula perrottettii* (liverwort): An independent isolation and structure elucidation of (−)-**5** recently isolated from the essential oil obtained by hydrodistillation of the liverwort *R. perrottettii* collected in Tokushima, Japan, in October 2003,[31] and spectral comparisons confirm complete identity.

3.2.2. Isolation of (+)-germacrene A 2. The macerated air-dried aerial parts of *S. canadensis*, collected in Hamburg, Germany in September 2003, was extracted for 3 days at room temperature with diethyl ether. The concentrated crude extract obtained below 40 °C by evaporation of the solvent was fractionated using flash silica gel column chromatography. The 100% *n*-pentane fraction was then subjected to repeated TLC at −25 °C, and *n*-pentane as the developing solvent.

3.2.3. Isolation of germacrene B 6. Compound **6** was isolated simultaneously with **2** by using the same method since both are present in the same fraction.

3.2.4. Characterization of (−)-α-helmiscapene 5. Colourless oil; $RI_{CPSIL5} = 1451$; sense of optical rotation ($CDCl_3$): (−); ^1H NMR (500 MHz, $CDCl_3$): 0.87 (3H, s), 1.36–1.42 (2H, m), 1.66 (3H, br s), 1.71 (3H, s), 1.81–1.90 (4H, m), 2.00–2.09 (2H, m), 4.66 (1H, s), 4.68 (1H, s), 5.24 (1H, br s); ^{13}C NMR (125.7 MHz, C_6D_6): 21.2, 23.1, 23.7, 26.7, 27.4, 27.8, 29.3, 35.3, 41.4, 46.4, 48.0, 109.0, 119.8, 137.1, 150.7; MS (EI, 70 eV), *m*/*z* (rel int.): 204 [M^+] (38), 189 (59), 175 (9), 161 (32), 147 (19), 133 (28), 121 (27), 119 (30), 107 (75), 105 (56), 93 (82), 91 (68), 81 (48), 79 (47), 77 (45), 67 (38), 55 (47), 53 (48), 41 (100).

3.2.5. Characterization of (+)-germacrene A 2. Isolated from *S. canadensis* as colourless oil; $RI_{CPSIL5} = 1501$; $R_{f(pentane)} = 0.40$; sense of optical rotation (benzene): (+); ^1H NMR (500 MHz, C_6D_6): δ 1.31 (3H, br s, H-14), 1.44 (3H, br s, H-15), 1.48–1.55 (1H, m, H-8a), 1.67 (3H, s, H-12), 1.67–1.73 (1H, m, H-8b), 1.76–2.36 (9H, br m, H-2ab, 3ab, 6ab, 7, 9ab), 4.68 (1H, s, H-13a), 4.51 (1H, br

d, 10.2 Hz, H-5), 4.79 (1H, s, H-13b), 4.71–4.75 (1H, m, H-1); ^{13}C NMR (125.7 MHz, C_6D_6): δ 16.6 (q, C-14), 17.0 (q, C-15), 20.6 (q, C-12), 26.8 (t, C-2 or C-3), 34.3 (t, C-8), 35.1 (t, C-6), 40.2 (t, C-3 or C-2), 42.3 (t, C-9), 52.1 (d, C-7), 108.1 (t, C-13), 126.9 (d, C-1), 128.8 (s, C-4), 131.9 (d, C-5), 137.7 (s, C-10), 153.1 (s, C-11); MS (EI, 70 eV), m/z (rel int.): 204 [M^+] (10), 189 (25), 175 (8), 161 (28), 147 (32), 133 (25), 121 (42), 107 (59), 93 (84), 81 (82), 68 (100), 53 (57), 41 (82).

3.2.6. Characterization of germacrene B 6. Colourless oil; $RI_{CPSIL5} = 1555$; $R_{f(pentane)} = 0.45$; ^{1}H NMR (500 MHz, C_6D_6): δ 1.46 (3H, s, H-14/15), 1.48 (3H, s, H-15/14), 1.63 (3H, s, H-12/13), 1.67 (3H, s, H-13/12), 1.93–2.27 (9H, m, H-2ab, 3ab, 9ab, 8ab, 6a), 2.53 (1H, br s, H-6b), 4.67 (1H, br s, H-1/5), 4.76 (1H, br d, $J = 12.0$ Hz, H-5/1); ^{13}C NMR (125.7 MHz, C_6D_6): 16.7 (q, C-14/15), 16.7 (q, C-15/14), 20.6 (q, C-12/13), 20.7 (q, C-13/12), 26.5 (t, C-2), 32.8 (t, C-6), 39.3 (t, C-3/C-8), 39.5 (t, C-3/C-8), 41.3 (t, C-9), 126.3 (s, C-7/11), 126.9 (d, C-5/1), 128.3 (d, C-1/5), 131.6 (s, C-10/4), 133.9 (s, C-11/7), 136.8 (s, C-4/10); MS (EI, 70 eV), m/z (rel int.): 204 [M^+] (28), 189 (20), 175 (5), 161 (31), 147 (19), 133 (27), 121 (100), 107 (53), 105 (51), 93 (69), 81 (47), 67 (55), 53 (48), 41 (85).

3.2.7. (−)-Selina-5,11-diene 15. Colourless oil; $RI_{CPSIL5} = 1447$; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 1.14 (3H, s), 1.14–1.21 (2H, m), 1.17 (3H, d, $J = 7.6$ Hz), 1.32–1.38 (1H, m), 1.45–1.55 (5H, m), 1.70 (3H, s), 1.71–1.80 (1H, m), 1.81–1.88 (1H, m), 2.45–2.51 (1H, m), 2.60 (1H, t, $J = 5.4$ Hz), 4.88 (1H, br s), 4.94 (1H, br s), 5.34 (1H, d, $J = 4.1$ Hz); ^{13}C NMR (125.7 MHz, C_6D_6): δ 18.2 (t), 22.5 (q), 23.1 (q), 23.2 (t), 27.2 (q), 34.2 (t), 35.1 (s), 38.0 (t), 39.1 (d), 42.4 (d), 42.6 (t), 112.0 (t), 124.1 (d), 148.1 (s), 148.5 (s); MS (EI, 70 eV), m/z (rel int.): 204 [M^+] (40), 189 (42), 161 (37), 147 (39), 133 (46), 121 (38), 108 (100), 107 (62), 105 (52), 93 (62), 81 (48), 67 (22), 55 (38), 41 (49).

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