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Sesquiterpenes from the essential oil of the liverwort *Conocephalum conicum*

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Abstract

Three new brasilane type sesquiterpenes related to the known alcohol conocephalenol and the sesquiterpene alcohol presilphiperfolan-1-ol were isolated from a chemotype of the liverwort *Conocephalum conicum* collected in southern Germany and identified by NMR investigations, enantioselective gas chromatography and chemical correlations. In addition *ent*-(−)-dactylol, the enantiomer of a compound so far only found as a constituent of the Caribbean sea hare *Aplysia dactylomela*, was identified. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Conocephalum conicum*; Hepaticae; Sesquiterpenes; Brasiladienes

1. Introduction

Conocephalum conicum is a thallous liverwort widely spread in Europe, North Africa, Asia and North America. The leaves display a pleasant turpentine odor upon squeezing, which is mainly due to the high monoterpene contents. The occurrence of different chemotypes or chemical races of this liverwort has been known for some time. They were differentiated by their flavonoid constituents (Markham, Porter, Mues, Zinsmeister, & Brehm, 1976; Porter, 1981) and by specific volatile components (Toyota, Saito, Matsunami, & Asakawa, 1997). Specimens of the chemotype described in this work were collected near Adelberg (Göppingen), in southern Germany and in Devils Bridge (Wales, UK). It is one out of four distinctly different chemotypes of *C. conicum* investigated in our group.

2. Results and discussion

The structures of the sesquiterpene constituents as obtained by hydrodistillation (Fig. 1) and dichloromethane extraction of the liverwort were almost completely elucidated. Although differing peak areas are observed in the hydrodistillation and solvent extraction gas chromatograms (Fig. 1), all the components, except compound **12**, are present in both samples, which indicates that the sesquiterpene hydrocarbons are genuine constituents in the plant and not artefacts formed under the conditions of sample preparation. One of the major components of the essential oil and of the dichloromethane extract is conocephalenol (**1**), which was first isolated and described by Connolly (1990) in *C. conicum* collected in Scotland and has been synthesized by Tori, Sono, Nakashima, Nakaki, and Asakawa (1991). Somewhat later, it was also isolated from *C. conicum* collected in Germany (Asakawa, 1995). Brasilane type structures have previously only been known from marine organisms (Stallard, Fenical, & Kittredge, 1978; Wright, König, & Sticher, 1991). Most of the volatile constituents as shown in Fig. 1 were isolated by preparative gas chro-

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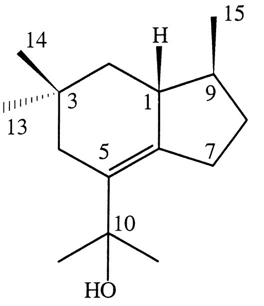

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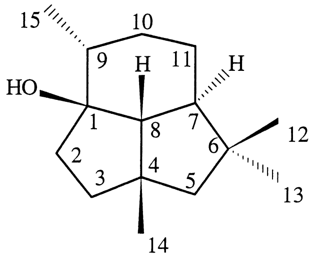

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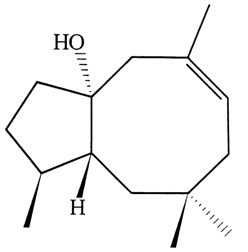

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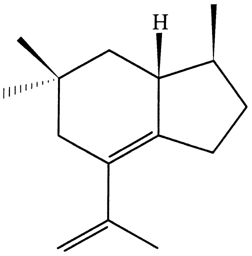

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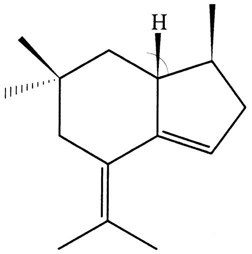

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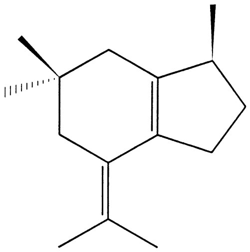

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matography (GC) and investigated by 1- and 2-dimensional NMR spectroscopy.

Brasila-5,10-diene (**4**) with a molecular mass of 204 shows ^{1}H NMR signals at δ 4.88 and 5.02 typical for an exocyclic double bond with a coupling correlation to a singlet at δ 1.82 indicationg an isopropenyl residue. Correspondingly, in the ^{13}C NMR spectrum the triplet of an olefinic methylene group is detected at δ 112.6. Three quaternary olefinic carbon signals are indicated by singlets at δ 129.6, 137.9 and 146.4 for carbons C-5, C-6 and C-10. In the ^{1}H NMR spectrum two geminal methyl singlets can be observed at δ 0.94 and 0.99 showing a coupling correlation in the $^{1}H^{1}H$ COSY spectrum. A third methyl doublet signal for C-15 is observed at δ 1.00, which couples with the methine multiplet in the range of δ 1.15–1.30. A methylene signal at unusually high field of δ 0.86 for H-2a couples with H-2b at δ 1.56.

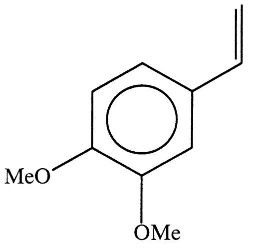
Brasila-5(10),6-diene (**5**), molecular mass 204, was also isolated by preparative GC. In the ^{1}H NMR five methyl signals are present. For two high field singlets at δ 0.91 and 0.97 a coupling correlation can be detected in the $^{1}H^{1}H$ COSY spectrum, indicating two geminal methyl groups C-13 and C-14. Another two signals at lower field (δ 1.69 and 1.9) also couple with each other and can be assigned to methyl groups C-11 and C-12. A methyl doublet at δ 1.08, representing the methyl group C-15, has a coupling correlation to a methine proton at C-9, which again couples with the widely split methylene signal of C-8 at δ 2.10 and 2.53. From this methylene signal a coupling to the only olefinic proton at δ 5.46 (broad singlet) can be observed. Similarly, as in the case of **4**, the C-2 methylene protons absorb at high field (multiplet at δ 1.03 and 2.29).

Brasila-1(6),5(10)-diene (**6**), molecular mass 204, is very labile in the isolated state and usually decomposed in the refrigerator in less than a week, while it appeared to be stable in the mixture of the essential oil. In the ^{1}H NMR spectrum the geminal C-13/C-14 methyl groups resonate at δ 0.96 and 0.98. The signals of the C-11- and C-12 methyl groups next to the olefinic double bond appear at δ 1.15 and 1.90, respectively. A methyl doublet signal representing C-15 is observed at δ 1.01. No olefinic protons were found.

Dehydration of a sample of conocephalenol (**1**) with phosphoryl chloride yielded products **4**–**6** (Fig. 2), giving proof of the assigned structures and stereochemical correlations.

Finally, in addition to the brasiladienes, 3,4-dimethoxystyrene (**7**), a constituent, which also occurs in a northern German chemotype of *C. conicum*, was identified by comparison of its spectroscopic properties with a synthetic reference (Peterson, Russell, & Everson, 1991). Compound **7** was previously identified as a constituent of the ether extract of an unknown Malaysian liverwort of *Asterella* or *Mannia* species (Asakawa, Toyota, Tanaka, Hashimoto, & Joulain, 1995).

The main component of the essential oil of *C. conicum* is (−)-selin-11-en-4-ol (**8**), a compound identified



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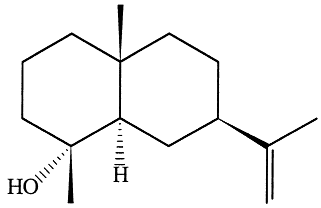

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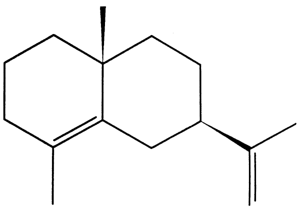

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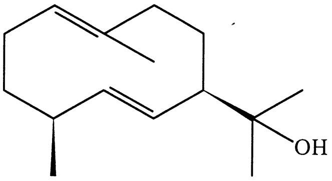

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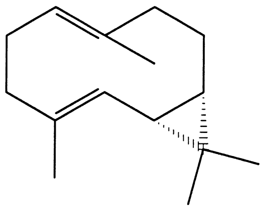

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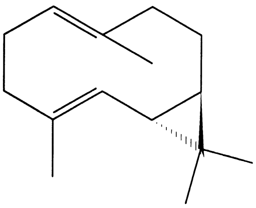

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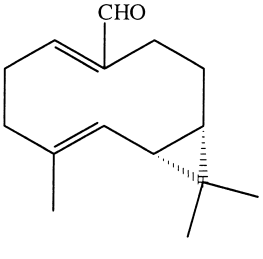

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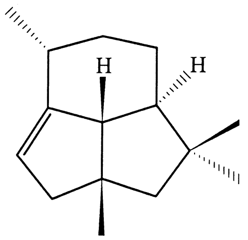

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before as a constituent of *Podocarpus dacrydioides* (San Feliciano, Medarde, Del Rey, Del Corral, & Barrero, 1990) and of the liverwort *Riccardia jackii* (Asakawa, 1995). Compound **8** shows spectroscopic data identical to those published (San Feliciano et al., 1990). (+)-Selina-4,11-diene (**9**) can be considered as a dehydration product of **8** and was identified by comparison of its mass and NMR spectra with published data (Maurer, & Grieder, 1977). Dehydration of **8** gave (+)-**9**, (−)-α-selinene (**10**) and (+)-β-selinene (**11**) (Fig. 3). The absolute configuration of all products was determined by enantioselective GC with heptakis(6-O-t.butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin as stationary phases.

Peak 12 (Fig. 1) could be assigned to (−)-germacradien-11-ol (**12**), a component also found in the liverwort *Dumortiera hirsuta* (Toyota, Yoshida, Matsunami, & Asakawa, 1997). The configuration is identical to a sample of **12**, which was isolated from the liverwort *Porella obtusata* (C. Fricke and W.A. König, unpublished results) and the spectroscopic data correspond to those published (Ganßer, Pollak, & Berger, 1995). Known compounds are also (−)-bicyclogermacrene (**13**), (−)-isolepidozene (**14**) (König et al., 1996) and bicyclogermacren-14-al (**15**). The spectroscopic data of the latter compound were identical with those published by Connolly (1990). The NMR spectral data of compound **3** matched with those published for (+)-dactylol, a sesquiterpene alcohol isolated from the Caribbean sea hare *Aplysia dactylomela* (Schmitz, Hollenbeak, & Vanderah, 1978). However, we measured a negative optical rotation and the presence of the *ent*-(−)-dactylol can be concluded.

The NMR spectroscopic data of **2** (presilphiperfolan-1-ol) indicated a tricyclic sesquiterpene alcohol ($C_{15}H_{26}O$) without an olefinic double bond. Four methyl groups (three singlets and one doublet) could be assigned: δ 1.32 corresponding to CH_3-14, δ 0.80 and 0.94, which showed a coupling correlation in the $^1H^1H$ COSY spectrum, corresponding to CH_3-12 and

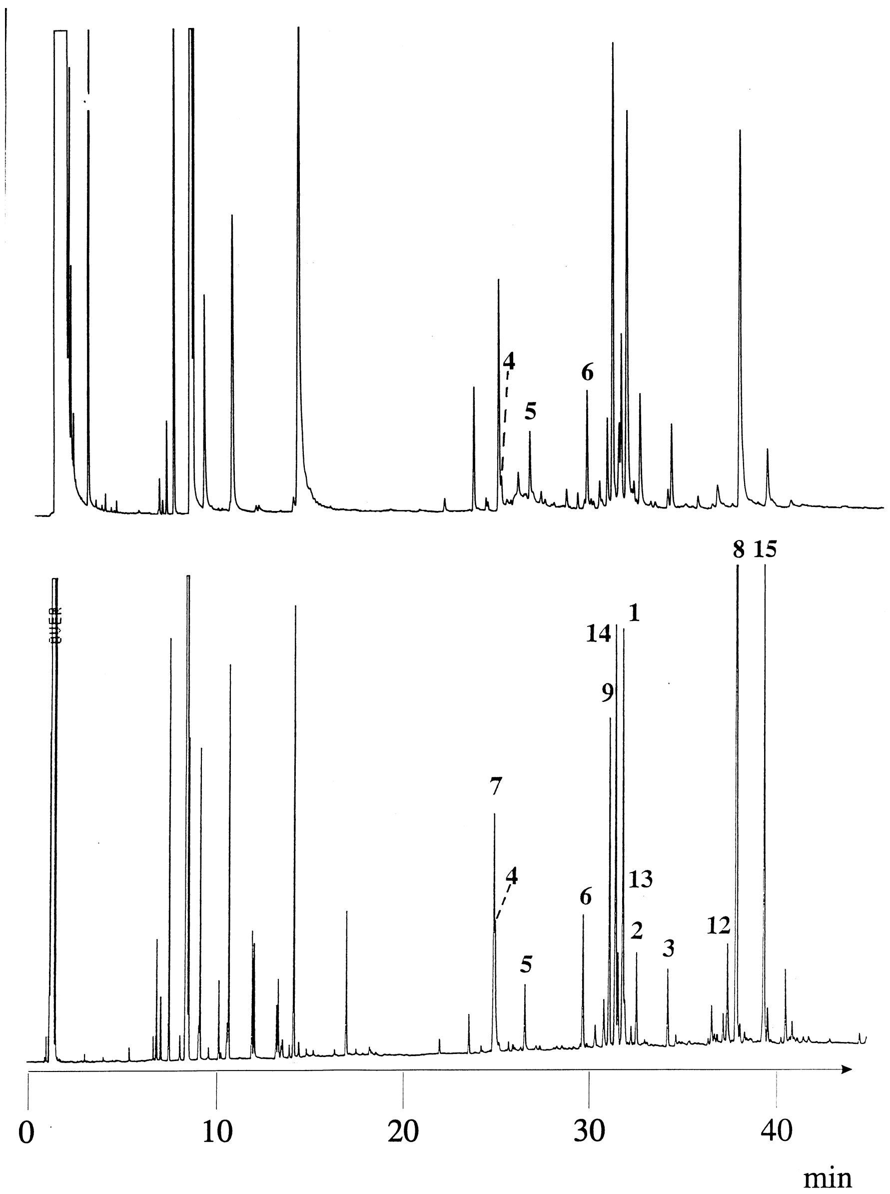
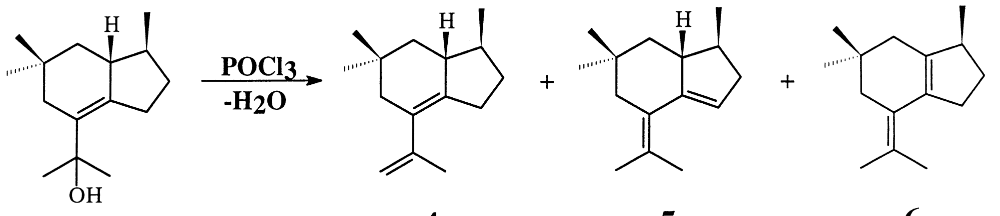


Fig. 1. Gas chromatographic separation of the dichloromethane extract (top) and the hydrodistillate (bottom) of *C. conicum*. 25 m CP Sil 5; column temperature 50°C, 3°C min^{-1} to 230°C.

CH_3-13, and a doublet at δ 0.89, corresponding to CH_3-15. The latter couples to a multiplet in the absorption range of δ 1.52–1.65. A methylene singlet at δ 1.50 shows a coupling correlation to one of the high-field methyl singlets in the $^1H^1H$ COSY spectrum. In the HMBC spectrum coupling correlations to



1 **4** **5** **6**

3 : 1 : 2

Fig. 2. Dehydration of conocephalenol (**1**) with phosphoryl chloride.

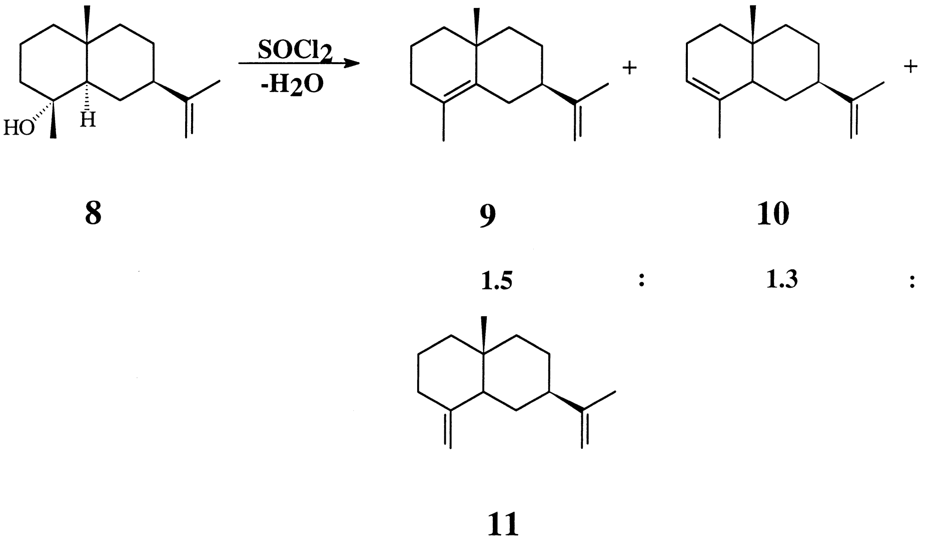


Fig. 3. Dehydration of (−)-selin-11-en-4-ol (**8**) with thionyl chloride.

methyl groups 12, 13 and 14, to the tertiary carbon atoms C-8 and C-7, the quarternary carbons CA and C-6 and to the secondary carbon atom C-3 can be observed (Fig. 4).

The relative configuration of **2** was elucidated by NOESY NMR measurements and confirmed by the chemical shift differences of the methyl protons. The C-14 methyl group (δ 1.32) should be on the same side as the hydroxy group, while the C-15 methyl group (δ 0.89) is opposite to the hydroxy group. Clear NOE signals are found between the CH-8 signal CH_3-12 and CH_3-14 (Fig. 5). The presilphiperfolanes are sesquiterpenes which were initially isolated by Bohlmann, Zdero, Jakupovic, Robinson, and King (1981) from *Eriophyllum staechadifolium*. They are considered as biogenetic precursors of the silphinanes, silphiperfolanes and terrecyclanes (Weyerstahl, Marschall, Schulze, & Schwope, 1996). Compound **2** was previously obtained as a rearrangement product from isocaryophyllene by treatment with HSO_3F/SO_2FCl at 120°C and its structure was derived from X-ray crystallographic analysis (Khomenko et al., 1985).

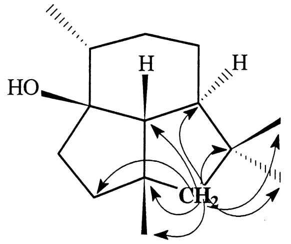


Fig. 4. HMBC-couplings from hydrogen atoms CH_2-5 of **2** to further carbon atoms.

Compound **2** is easily dehydrated under slightly acidic conditions to presilphiperfol-1-ene (**16**), which was not detected as a natural compound yet. In the ^{1}H NMR spectrum four methyl groups are observed. Two of them absorb as singlets at δ 0.90 and 0.94, which show a coupling correlation in the ^{1}H, ^{1}H COSY spectrum. They are assigned to the geminal C-12- and C-13 methyl groups. Furthermore, a doublet at δ 1.09 (C-15) and a singlet at δ 1.09 (C-14) are observed. The C-15 doublet is coupled to a multiplet at δ 2.73, which is assigned to CH-9. A singlet of two protons at δ 2.30 of CH_2-3 couples with the only olefinic proton at δ 4.83. The signal of CH-7 at δ 0.90–0.93 appears at very high field.

3. Experimental

3.1. Plant material

C. conicum was collected in July 1997 at Zachersmühle (Adelberg, near Göppingen, southern Germany). A specimen of the plant is deposited in the herbarium of the Institut für Allgemeine Botanik, University of Hamburg.

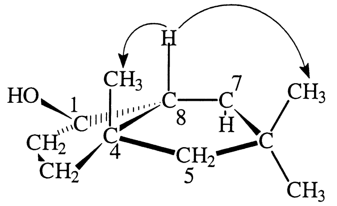


Fig. 5. NOE-couplings from the hydrogen atom CH-8 of **2** (partial structure).

3.2. Hydrodistillation

The essential oil was prepared by hydrodistillation (2 h) of homogenized fresh plant material using *n*-hexane as collection solvent. Because of the greatly differing weight the fresh material was not weighed.

3.3. Solvent extraction

Fresh plant material was crushed after freezing in liquid nitrogen and extracted with CH_2Cl_2 overnight.

3.4. Liquid chromatography

Silica column chromatography (20 cm × 8 mm, 15 cm × 8 mm, respectively) with a gradient of petrol (bp 40–60°C) and CH_2Cl_2 as eluent.

3.5. Dehydration reactions

Ca. 1 mg of **1** or **8**, respectively, was taken up in 0.5 ml pyridine and 1 drop of phosphoryl chloride (or thionyl chloride) was added under ice cooling. After 1 h (or 5 min, respectively) of stirring at room temp, the reaction was quenched by adding a few drops of water and the mixture was extracted three times with *n*-hexane. The organic phase was washed several times with water and dried over Na_2SO_4.

3.6. Preparative GC

Compounds **1**–**9**, **15** and **16** were isolated by prep. GC using a Varian 1400 instrument equipped with a stainless steel column (1.8 m × 4.3 mm) with 10% SE 30 on Chromosorb W-HP (for compounds **2**, **3** and **16**) and with a stainless steel column (2 m × 5.3 mm; Silcosteel, Amchro) packed with 3% heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin/OV 1701 (1:1, w:w) on Chromosorb W-HP (for compounds **1**, **4**–**7** and **15**). Compounds **8** and **9** were isolated using a stainless steel column (2.05 m × 5.1 mm; Silcosteel, Amchro) with 6% octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin/polysiloxane PS 086 (1:1, w:w) on Chromosorb W-HP. He was used as carrier gas at a flow rate of 240 ml min^{-1}.

3.7. NMR spectroscopy

Bruker WM 400 (400 MHz) and DRX 500 (500 MHz) using TMS as internal standard.

3.8. GC–MS

EI (70 eV) GC–MS measurements were carried out on a Hewlett Packard HP 5890 gas chromatograph equipped with a 25-m fused silica capillary column with CpSil 5 (Chrompack) coupled to a VG Analytical 70-250S mass spectrometer.

3.9. Polarimetry

Measurements of the optical rotation were performed with a Perkin Elmer 341 polarimeter. Because of the small sample amounts only the sense of rotation and not the specific optical rotation was determined.

3.10. Brasila-5,10-diene (**4**)

^{1}H NMR (400 MHz, C_6D_6): δ 0.86 (1 H, t, $J = 12$ Hz, H-2a), 0.94 (3 H, s, Me), 0.99 (3 H, s, Me), 1.00 (3 H, d, $J = 7$ Hz, Me), 1.07–1.31 (2 H, m, H-8a and H-9), 1.56 (1 H, ddd, $J = 12$ Hz, $J = 6$ Hz, $J = 2$ Hz, H-2b), 1.82 (3 H, s, Me-11), 2.07 (1 H, in, H-4a), 2.31–2.5 (2 H, m, H_2-7), 4.91 (1 H, bs), 5.03 (1 H, bs), ^{13}C NMR (100.6 MHz, C_6D_6): δ 18.29 (q), 22.30 (q), 26.11 (q), 28.74 (t, C-7), 30.17 (s), 32.38 (q), 33.38 (t, C-8), 40.81 (t, C-2), 41.23 (d, C-9), 43.37 (t, C-4), 47.02 (d, C-1), 112.61 (t, C-12), 129.64 (s), 137.94 (s), 146.42 (s). EIMS (70 eV), *m*/*z* (rel. int.): 204 (67), 189 (70), 175 (44), 161 (33), 147 (33), 133 (100), 119 (48), 105 (78), 91 (72), 77 (35), 55 (43), 41 (78).

3.11. Brasila-5(10),6-diene (**5**)

^{1}H NMR (400 MHz, C_6D_6): δ 0.91 (3 H, s), 0.97 (3 H, s), 1.03 (1 H, t, $J = 12$ Hz), 1.08 (3 H, d, $J = 7$ Hz), 1.69 (3 H, s), 1.83–1.89 (1 H, 1.90 (3 H, d, $J = 2$ Hz), 2. 10 (1 H, m, $J = 16$ Hz, $J = 9$ Hz), 2.24–2.3 3 (1 H, m), 2.39 (1 H, dd, $J = 13$ Hz, $J = 2$ Hz), 2.53 (1 H, tdd, $J = 16$ Hz, $J = 9$ Hz, $J = 3$ Hz), 5.46 (1 H, m). EIMS (70 eV), *m*/*z* (rel. int.): 204 (79), 189 (67), 175 (31), 161 (39), 148 (26), 147 (26), 133 (100), 119 (43), 105 (61), 91 (54), 77 (31), 55 (26), 41 (50).

3.12. Brasila-1(6),5(10)-diene (**6**)

^{1}H NMR (400 MHz, C_6D_6): δ 0.96 (3 H, s), 0.98 (3 H, s), 1.01 (3 H, d), 1.33–1.40 (1 H, m), 1.76 (3 H, s), 1.90 (3 H, s), 2.43–2.53 (2 H, m), 2.59–2.69 (1 H, m), 2.70–2.80 (1 H, m). EIMS (70 eV), *m*/*z* (rel. int.): 204 (30), 189 (100), 133 (18), 105 (22), 41 (22).

3.13. Presilphiperfolan-1-ol (**2**)

^{1}H NMR (500 MHz, C_6D_6): δ 0.80 (3 H, s, Me), 0.89 (3 H, d, $J = 7$ Hz, Me-15), 0.94 (3 H, d, $J = 7$ Hz, Me), 1.08–1.17 (2 H, m, H-11a and H-7), 1.32 (3 H, s, Me-14), 1.20–1.33 (2 H, m, H-11b and H-10a), 1.39–1.45 (1 H, m, H-10b), 1.50 (2 H, s, H_2-5), 1.53–1.62 (3 H, m, H-8 and H-3a and H-9), 1.62–1.72 (1 H, m, H-2a), 1.77–1.92 (2 H, m, H-2b and H-3b), ^{13}C NMR (125.8 MHz, C_6D_6): δ 15.69 (q, Me-15), 20.26 (t, C-

11), 22.31 (q), 28.73 (q), 29.91 (t, C-10), 31.35 (q, Me-14), 36.87 (d, C-9), 39.71 (t, C-2), 41 (s, C-6), 42.64 (t, C-3), 46.21 (s, C-4), 51.69 (d, C-7), 59.43 (t, C-5), 63.90 (d, C-8), 89 (q, C-1). EIMS (70 eV), *m*/*z* (rel. int.): 222 (13), 207 (28), 204 (11), 189 (17), 166 (78), 165 (57), 151 (50), 133 (24), 123 (100), 111 (37), 110 (31), 105 (24), 97 (56), 91 (31), 81 (25), 55 (37), 41 (65).

3.14. Presilphiperfol-1-ene (**16**)

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (3 H, s), 0.90–0.93 (1 H, m), 0.94 (3 H, s), 1.09 (3 H, d, $J = 7$ Hz), 1.09 (3 H, s), 1.26–1.40 (3 H, m), 1.67–1.73 (1 H, m), 2.17–2.22 (1 H, m), 2.30 (2 H, m), 2.73 (1 H, m), 4.83 (1 H, bs). EIMS (70 eV), *m*/*z* (rel. int.): 204 (46), 189 (65), 162 (15), 161 (15), 148 (72), 147 (65), 135 (52), 133 (100), 119 (44), 107 (37), 106 (67), 105 (76), 91 (89), 77 (33), 55 (33), 41 (70).

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