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NATURAL OCCURRENCE OF BOTH ENANTIOMERS OF CADINA-3,5-DIENE AND δ-AMORPHENE

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Key Word Index—*Leptospermum scoparium*; Myrtaceae; manuka oil; *Conocephalum conicum*; liverwort; vetiver oil; *Solidago canadensis*; cadina-3,5-diene; δ-amorphene; germacrene D; 2D GC; cyclodextrin derivatives.

Abstract—The labile sesquiterpene hydrocarbon (−)-(1*R*,7*S*,10*R*)-cadina-3,5-diene was isolated from manuka oil (*Leptospermum scoparium*) by preparative gas chromatography, while its enantiomer is present in a chemotype of the liverwort *Conocephalum conicum* collected in southern Germany. The structure and absolute configuration was derived by NMR investigations, enantioselective gas chromatography and by conversion into a series of products of known stereochemistry by acid catalysed rearrangement, e.g. (−)-(7*S*,10*R*)-*trans*-calamenene, (−)-(7*S*,10*R*)-cadina-1(6),4-diene, (−)-(1*R*,7*S*,10*R*)-bicyclosesquiphellandrene and (−)-(1*R*,10*R*)-zonarene. In addition, (+)-δ-amorphene was identified as a constituent of *L. scoparium*, while (−)-δ-amorphene is present in vetiver oil. Both enantiomers of this sesquiterpene, which has not been described as a natural product so far, were prepared by rearrangement of an enantiomeric mixture of germacrene D isolated from *Solidago canadensis*. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Leptospermum scoparium is a tree native to New Zealand. Its essential oil (manuka oil, manex oil) is used in traditional medicine and, more recently, in perfumery and cosmetics. Flynn *et al.* [1] investigated the volatile constituents of the leaves of *Leptospermum* sp. by GC-mass spectrometry and IR spectroscopy and identified several known mono- and sesquiterpenoids. Triterpenoids and flavones were identified by Häberlein and Tschiersch [2] in a dichloromethane extract of *L. scoparium*, while the structures of several β-triketones were identified by Briggs *et al.* [3] and Hellyer [4]. We were mainly interested in the sesquiterpene constituents of manuka oil. The results of these investigations are reported here.

RESULTS AND DISCUSSION

Investigations of manuka oil by GC-mass spectrometry revealed the presence of mainly cadinane type structures with abundant ion intensities at *m/z* 161. As major constituents the structures of a novel sesquiterpene hydrocarbon (−)-cadina-3,5-diene (**1**) and of related cadinanes (−)-bicyclosequiphellandrene (**2**), (−)-cadina-1(6),4-diene (**3**), (−)-zonarene (**4**) and (−)-*trans*-calamenene (**5**) wereassigned. Compounds **2**–**5** and additional known sesquiterpene hydrocarbons (α-cubebene, α-copaene, α-gurjunene, β-caryophyllene, aromadendrene, α-humulene, allo-aromadendrene, β-selinene, α-selinene, δ-cadinene, and cadina-1,4-diene) were identified by comparison of their typical mass spectra and gas chromatographic retention times with authentic reference compounds. Structural assignments of **1**–**5** were confirmed by one- and two-dimensional NMR investigations after micro-preparative GC isolation [5] of highly enriched fractions of the constituents of the essential oil. For the determination of the absolute configuration, capillary GC with cyclodextrin derivatives as chiral stationary phases was applied [6, 7].

In the ^{1}H NMR spectrum of **1** an unresolved doublet for two equivalent methyl groups and an additional doublet of a methyl group typical for cadinane derivatives were present. A broad singlet at δ 1.65 for a methyl group connected to an olefinic carbon and two olefinic protons (a multiplet at δ 5.16 and a singlet at δ 5.55) confirmed structure **1**. The signal assignments were achieved by 2D NMR experiments and are in agreement with the ^{13}C NMR data (see Experimental).

When **1** was left in chloroform solution at room temperature for three days formation of a series of degradation products was observed (Fig. 1). Products **2**, **3** and **4** were formed by double bond rearrangement,

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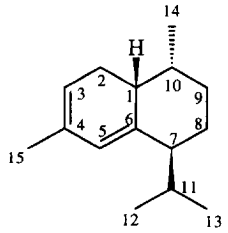

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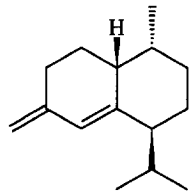

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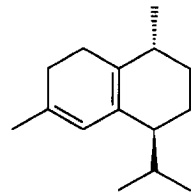

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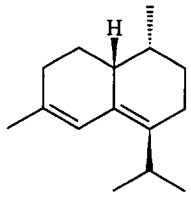

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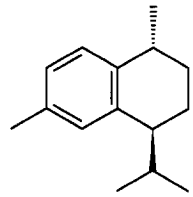


5

while **5** was an oxidation product of **1**. Since both enantiomers of **2**–**5** had been isolated before from different plants {the (−)-enantiomers from *Piper cubeba* oil, and the (+)-enantiomers from the liverwort *Conocephalum conicum* [8]} the configuration of the observed degradation products could be assigned by enantioselective GC. From these results the absolute configuration of **1** was determined to be (1*R*,7*S*,10*R*). Inspection of the GC-mass spectra of a hydrodistillate of the liverwort *C. conicum*, collected in southern Germany, revealed the presence of **1** as a natural constituent in this plant. However, comparison of the samples of **1** from manuka oil and from *C. conicum* by two-dimensional capillary GC with a chiral stationary phase showed that (+)-**1** was present in *C. conicum*. Compound **1** was also detected in the essential oils of *Chromolana odorata* from Cameroon and in *Araucaria heterophylla* (Hamburg).

A minor constituent of manuka oil was identified as δ-amorphene by comparison with a reference sample which was prepared from an enantiomeric mixture of germacrene D (**6**). Although sesquiterpene hydrocarbons from natural sources appear to be homochiral in most cases, enantiomeric mixtures were found occasionally [9]. While the (−)-enantiomer of **6** is very common in higher plants the (+)-isomer was only observed as a constituent of *Dendropanax trifidus* M. [10], of the soft coral *Sinularia mayi* [11] and of the liverwort *Preissia quadrata* [12]. A mixture of both enantiomers of **6** was isolated from *Solidago altissima* [13]. The enantiomeric proportions were calculated from the optical rotation of the isolated material and by comparison with pure (−)-**6**. We have recently isolated both enantiomers of **6** from *Solidago canadensis*, a plant very common in central Europe. Compound **6** is rather labile under acidic conditions and is considered as a key intermediate of cadinenes and bourbonenes by Yoshihara *et al.* [14]. When **6** was treated at 400° in the injector of a preparative gas chromatograph, β-ylangene (**7**) was obtained as the main product (Scheme 1). It was identified by comparison with the spectroscopic data published by Kulkarni *et al.* [15]. *p*-Toluenesulphonic acid catalysed rearrangement of **7** yielded three products in a proportion of 2:3:1—α-ylangene (**8**) [16], α-amorphene (**9**) [17] and δ-amorphene (**10**). The last has already been described as a rearrangement product of **8** by Ohta *et al.* [18], but has never before been described as a natural product. Compound **10** has a conspicuous mass spectrum (with an abundant fragment ion at *m/z* 134) which was practically identical with that of δ-cadinene. However, compound **10** eluted earlier than δ-cadinene on a nonpolar stationary phase, and we have noted its occurrence previously in various essential oils as a then unknown component. In previous rearrangement experiments on **6**, under acidic conditions, **10** was obtained as a minor product which could not be isolated. Since **10** was obtained as a mixture of enantiomers starting from an enantiomeric mixture of **6** [(−)-(+)(3:2)] the absolute stereochemistry could be assigned to the products by GC using cyclodextrin derivatives as chiral stationary phases [7]. All compounds depicted in Scheme 1 are derived from (−)-**6** as a precursor. Again, two-dimensional GC was applied to the investigation of the configuration of **10** in several essential oils. Thus, the (+)-enantiomer is present in manuka oil, while the (−)-enantiomer of **10** was identified in vetiver oil. The latter is known to contain the unusual enantiomers of several other sesquiterpenes, e.g. (+)-**9** (zizanene) [19–21].

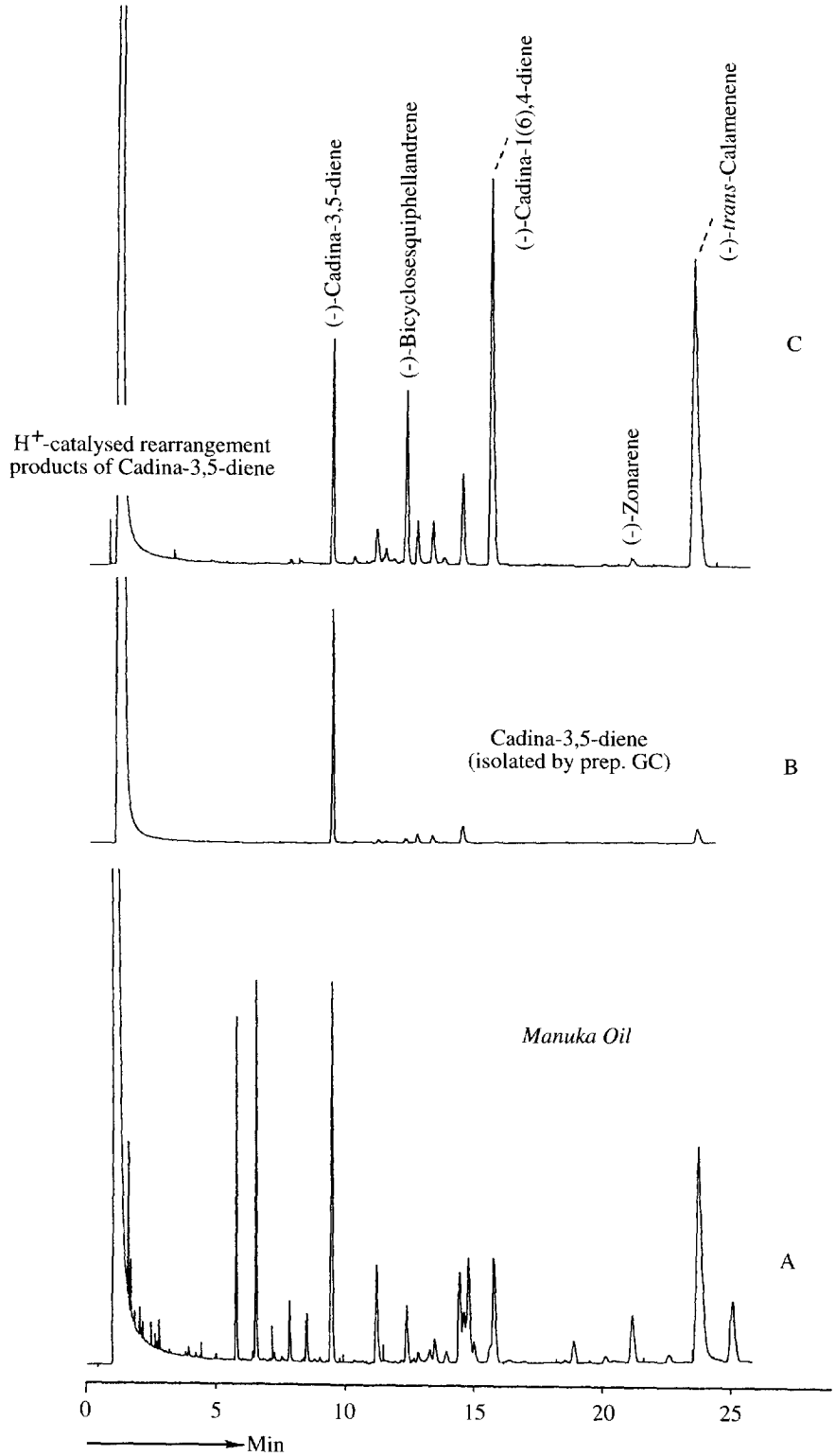
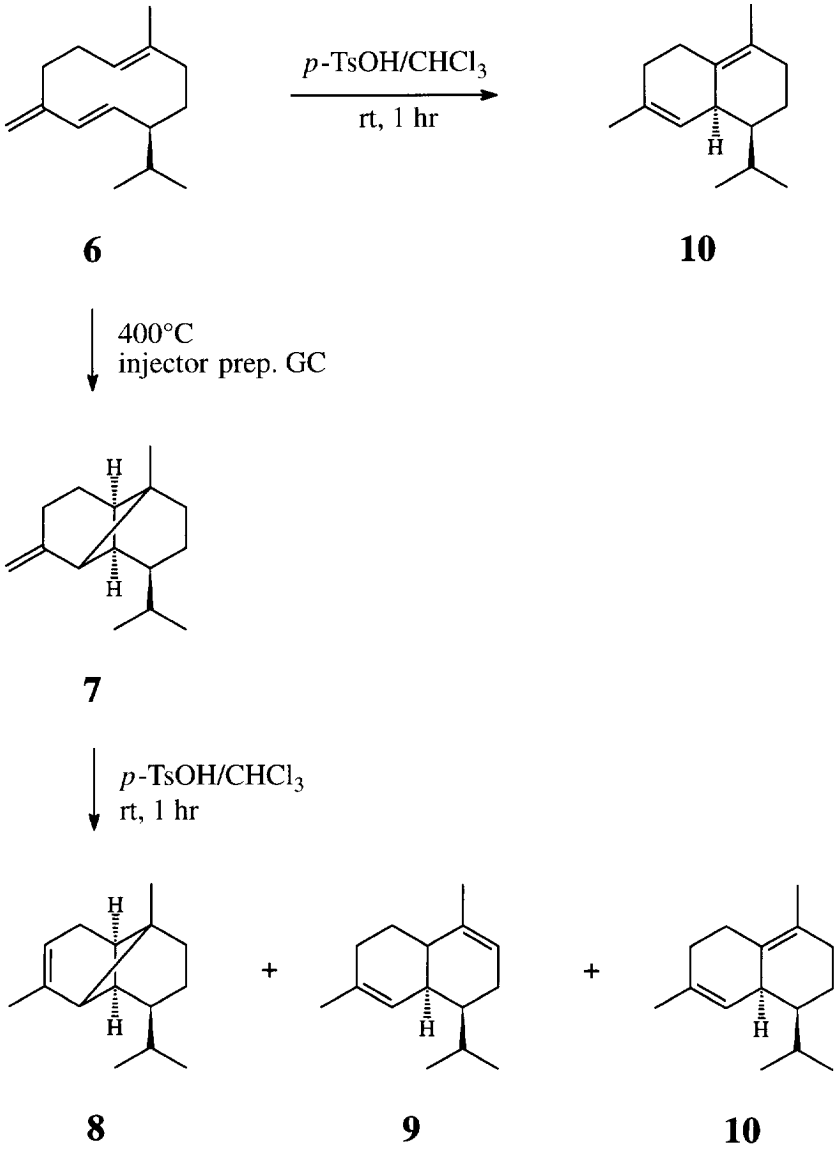


Fig. 1. Gas chromatographic investigation of (A) manuka oil, (B) cadina-3,5-diene purified by preparative GC, and (C) products obtained by keeping sample B in chloroform solution at room temp. A fused silica capillary column (25 m) was used, with heptakis(6-*O*-*t*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (50% in polysiloxane OV-1701, w/w) at 120°; carrier gas: hydrogen at an inlet pressure of 50 kPa.



Scheme 1.

EXPERIMENTAL

Collection of plant material and isolation of essential oils. Manuka oil (New Zealand) was provided by Dr Ute Galle-Hoffmann (Spinnrad GmbH, Gelsenkirchen, Germany). Vetiver oil (*Vetiveria zizanioides*, Reunion) was a gift from K.-D. Protzen, Kaders GmbH (Hamburg, Germany). *Conocephalum conicum* was collected at Uracher Wasserfall, (Urach, Germany; July 1996), while *S. canadensis* was collected at several locations near Hamburg (summer 1995). Dried leaves of *Cromolana odorata* from Cameroon were provided by M. Mekem Sonwa, University of Hamburg, and *A. heterophylla* was collected at the Botanical Gardens of the University of Hamburg (summer 1996). Volatile constituents of the plants were prepared by hydrodistillation (2 hr) of aq. homogenates of fresh or air-dried plant (*C. odorata*) material using *n*-hexane as collection solvent.

Preparative GC [5]. Isolation of **1**–**10** was performed by prep. GC on a Varian 1 400 instrument, equipped with stainless steel columns (Silcosteel, Amchro). Compounds **1** and **6**–**10** were fractionated using a column of 10% polysiloxane SE-30 on Chromosorb W-HP (1.85 m × 4.3 mm). Compound **1** was further purified on a column of 2.5% octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin in OV-1701 (w/w) on Chromosorb G-HP (2.00 m × 5.3 mm; 2,6-Me-3-Pe-γ-CD [22]). Compound **2** was also isolated using 2,6-Me-3-Pe-γ-CD, while **3** was isolated using a column with 6% octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin in polysiloxane PS-086 (w/w) on Chro-

mosorb W-HP (2.05 m × 5.1 mm; 6-Me-2,3-Pe-γ-CD [23]). Compound **4** was first chromatographed on 6-Me-2,3-Pe-γ-CD and then on 2,6-Me-3-Pe-γ-CD. Compound **5** was isolated using a column of 6-Me-2,3-Pe-γ-CD; helium was used as carrier gas (240 ml min^{-1}).

Two-dimensional gas chromatography [9]. Essential oil samples were injected onto a 25-m (0.25 mm i.d.) capillary column with dimethylpolysiloxane CpSil 5 (Chrompack) in a Siemens Sichromat 2 gas chromatograph at 50° and programmed at 3° min^{-1} to 200°. Sample transfer was performed after 34.89 min (R_t of **1**) and after 38.56 min (R_t of **9**) to a 25-m capillary column containing heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (50% in polysiloxane OV-1701, w/w), which was kept isothermally at 100° or at 110° (**9**). Chromatograms from both columns were recorded with a 2-channel Merck-Hitachi model 2 500 integrator. H_2, at an entrance pressure of 80 kPa for the Cpsil 5 capillary and 65 kPa for the cyclodextrin capillary, was used as carrier gas.

NMR spectroscopy. NMR measurements were performed with the instruments WM 250 (250 MHz) and WM 400 (400 MHz) (Bruker) using TMS as int. standard.

GC-MS. Electron impact (70 eV) GC-MS measurements were carried out on a Hewlett Packard HP 5 890 gas chromatograph coupled with a VG Analytical VG 70-250S mass spectrometer.

Polarimetry. Optical rotation measurements were performed with a Perkin Elmer 241 polarimeter.

Preparation of β-ylangene (**7**). An enantiomeric mixt. (5 mg) of **6** [(−)-(+)(3:2)] in 80 μl *n*-hexane was injected into prep. GC column at 400° and the fr. containing **7** (main fr.) was collected.

Acidic rearrangement of β-ylangene (**7**). An enantiomeric mixt. (2 mg) of **7** [(+)-(−)(3:2)] in a solution (1 ml) of 3 mg *p*-toluenesulphonic acid in $CHCl_3$ was stirred for 1 hr at room temp. A satd aq. soln (1 ml) of $NaHCO_3$ was added and the mixt. was vigorously shaken. The organic layer was removed and used for fractionation by prep. GC. The frs containing **8–10** were collected.

(−)-*Cadina*-3,5-*diene* (**1**). ^{1}H NMR (400 MHz, C_6D_6): δ 5.55 (1H, *s*, H-5), 5.16 (1H, *m*, H-3), 1.65 (3H, *br s*, H-15), 1.08 (3H, *d*, H-14, J = 7 Hz), 0.89 (6H, *d*, H-12, H-13, J = 6.6 Hz). ^{13}C NMR (100 MHz, C_6D_6): δ 141.93, 129.40 (C-4, C-6), 122.93, 117.28 (C-3, C-5), 52.71, 39.56, 35.26, 29.68, 28.69, 26.25, 22.08, 21.92, 21.41, 12.78. EIMS (70 eV), *m/z* (rel. int.): 204 (24), 161 (100), 146 (3), 133 (6), 119 (48), 105 (74), 91 (24), 81 (30), 77 (12), 69 (10), 55 (14), 41 (28). $[\alpha]_D^{22} = -216.8°$ (c = 0.14, $CDCl_3$).

(−)-*Bicyclosesquiphellandrene* (**2**). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.01 (1H, *br s*, H-5), 4.68, 4.63 (each 1H, *br s*, H-15), 0.93 (3H, *d*, H-14, J = 6 Hz), 0.87, 0.76 (each 3H, *d*, H-12, H-13, each J = 7 Hz). EIMS (70 eV), *m/z* (rel. int.): 204 (20), 189 (4), 161 (100), 147 (6), 133 (16), 119 (36), 105 (50), 91 (40), 81 (28), 77 (18), 67 (20), 55 (20), 41 (38).

(−)-*Cadina*-1(6),4-*diene* (**3**). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.61 (1H, *br s*, H-5), 1.79 (3H, *s*, H-15), 0.95 (3H, *d*, H-14, J = 6.6 Hz), 0.90 0.69 (each 3H, *d*, H-12, H-13, each J = 6.6 Hz). EIMS (70 eV), *m/z* (rel. int.): 204 $[M]^+$ (28), 189 (14), 175 (2), 161 (100), 147 (10), 133 (20), 119 (36), 105 (46), 93 (36), 81 (36), 77 (16), 59 (14), 55 (20), 53 (10), 41 (44).

(−)-*Zonarene* (**4**). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.27 (1H, *br s*, H-5), 3.05 (1H, *sept*, H-11), 1.79 (3H, *s*, H-15), 0.99, 0.98 (each 3H, *d*, H-12, H-13, each J = 4 Hz), 0.82 (3H, *d*, H-14, J = 7 Hz). ^{13}C NMR (60 MHz, $CDCl_3$): δ 135.62, 134.04, 125.84 (C-4, C-6, C-7), 120.53 (C-5), 38.74, 31.17, 30.50, 29.61, 28.04, 27.72, 24.34, 21.02, 20.60 (C-12, C-13), 19.28, 12.47. EIMS (70 eV), *m/z* (rel. int.): 204 (58), 189 (40), 161 (100), 147 (18), 133 (18), 119 (42), 105 (46), 91 (36), 81 (66), 77 (22), 69 (8), 65 (8), 55 (20), 41 (42).

(−)-trans-*Calamenene* (**5**). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12 (1H, *d*, H-2(3), J = 8 Hz), 7.01 (1H, *br s*, H-5), 6.94 (1H, *br d*, H-3(2), J = 8 Hz), 2.76 (1H, *m*), 2.69 (1H, *m*), 2.29 (3H, *s*, H-15), 2.23 (1H, *m*), 1.26 (3H, *d*, H-14, J = 7 Hz), 1.00, 0.71 (each 3H, *d*, H-12, H-13, each J = 7 Hz). EIMS (70 eV), *m/z* (rel. int.): 202 (7), 159 (100), 131 (26), 129 (22), 128 (18), 91 (11), 41 (42).

α-*Ylangene* (**8**). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.27 (1H, *m*, H-3), 2.19 (2H, *m*, H-2), 2.00 (1H, *dd*, J = 1.5, 6.6 Hz), 1.66 (3H, *m*, H-15), 1.45–1.75 (8H, *m*), 0.86 (3H, *d*, H-12, J = 6.6 Hz), 0.84 (3H, *d*, H-13, J = 6.6 Hz), 0.76 (3H, *s*, H-14).

δ-*Amorphene* (**10**). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38 (1H, *br s*, H-5), 2.86 (1H, *m*), 2.70 (1H, *m*), 1.65, 1.63 (each 3H, *br s*, H-14, H-15), 0.95, 0.93 (each 3H, *d*, H-12, H-13, each J = 6.6 Hz). EIMS (70 eV), *m/z* (rel. int.): 204 (43), 189 (17), 162 (20), 161 (100), 134 (58), 133 (14), 119 (48), 105 (48), 93 (15), 91 (31), 81 (24), 79 (11), 77 (15), 69 (9), 55 (14), 41 (29).

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