PERGAMON

Phytochemistry 59 (2002) 795–803

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Sesquiterpene constituents in *Petasites hybridus*

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Received 10 October 2001; received in revised form 30 November 2001

Abstract

The essential oil of the rhizomes of *Petasites hybridus* (Asteraceae) was investigated by gas chromatography, mass spectrometry, 1- and 2-dimensional NMR techniques and chemical correlations. Two new sesquiterpene hydrocarbons, petasitene and pethybrene, could be identified. Petasitene is the parent sesquiterpene hydrocarbon to the known norsesquiterpene albene. The absolute configuration of petasitene could be assigned by conversion of natural albene to petasitene by partial synthesis. Pethybrene is a tricyclic sesquiterpene hydrocarbon, which rearranges to the structurally related α-isocomene under acidic conditions. Several sesquiterpenes were isolated from the hydrodistillation products of *Petasites hybridus* and investigated by spectroscopic methods and chemical correlations

Keywords: *Petasites hybridus*; Sesquiterpene hydrocarbons; Petasitene; Albene; Pethybrene; Chemical correlation

1. Introduction

The rhizomes of *Petasites hybridus* and the related species *P. albus* are well known since ancient times for their spasmolytic and analgetic effects and extracts are applied for treatment of gastrointestinal disturbances, bronchial asthma and many other diseases (Willuhn, 1997). The pharmacological effects are mainly attributed to functionalized eremophilane type sesquiterpenes, which, among many other compounds, were identified in previous investigations of extracts of *P. hybridus* (Aebi et al., 1955; Stoll et al., 1956; Neuenschwander et al., 1979) and by headspace analysis in flowers of *P. albus* (Brunke et al., 1992). Both *Petasites* species are rich in sesquiterpene hydrocarbons (Fig. 1). In addition, a trinorsesquiterpene, (−)-albene (**3**), was identified as a constituent (Hochmannová et al., 1962). Its absolute configuration was determined by Kreiser et al. (1979) and reconfirmed by Baldwin et al. (1986) and a biogenetic pathway for its biosynthesis was proposed (Kreiser and Janitschke, 1979) with a hypothetic intermediate **12** which is identical with the compound which has now been isolated. The structure determination of this compound, which was named petasitene (**12**), is described in this paper. In addition, a second compound with a mass spectrum with the unusual m/z 149 as its base peak was identified and named pethybrene (**9**).

2. Results and discussion

A hydrodistillate of *P. hybridus* rhizomes was investigated by GC–MS and exhibited a complex sesquiterpene hydrocarbon fraction (Fig. 1). Most of the constituents could be identified by comparing their mass spectra and retention indices with a spectral library which was established under identical experimental conditions (Joulain and König, 1998). Two unknown sesquiterpene hydrocarbons, both with molecular ions at m/z 204 ($C_{15}H_{24}$) were selected for isolation and structural investigations.

2.1. Petasitene (*12*)

The isolation of **12** which is present in only 0.8% of the total volatile constituents was carried out by dry-column silica gel chromatography (Schwanbeck et al., 1982) and repetitive preparative GC using packed columns with different modified cyclodextrins as chiral stationary phases (Hardt and König, 1994) (Fig. 2). The mass spectrum with $M^+ = 204$ suggested the elementary

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PII: S0031-9422(01)00489-7

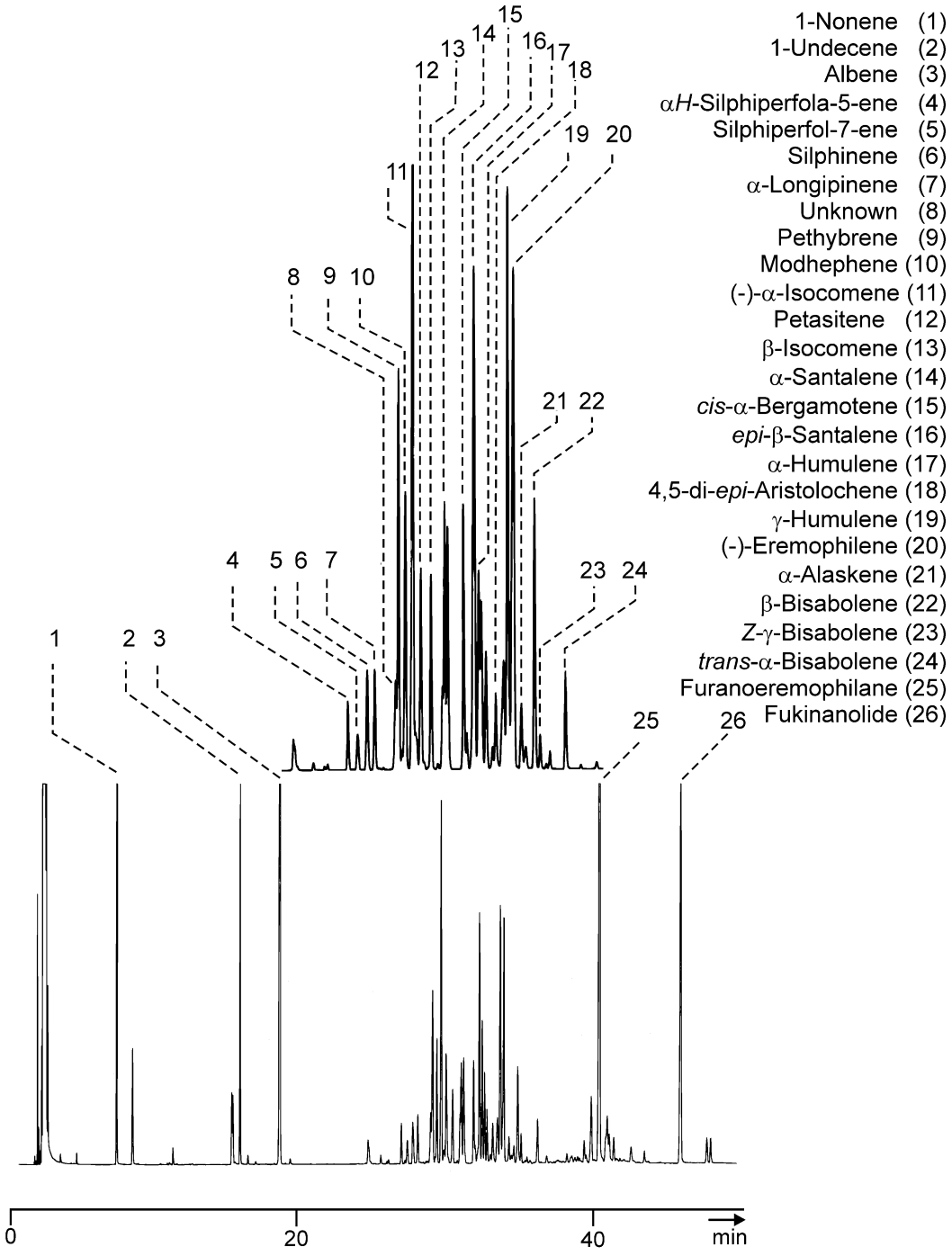

Fig. 1. Gas chromatographic separation of the volatiles of *Petasites hybridus* and peak assignment. 25 m Fused-silica capillary column with CPSil 5, 50 °C, 3 °C/min to 230 °C.

composition $C_{15}H_{24}$. This is supported by 24 proton signals and 15 carbon signals in the ^{1}H and ^{13}C NMR, respectively. The ^{1}H NMR showed one olefinic methine proton (δ 5.38, *t*, 2.6 Hz) and together with the molecular composition a tricyclic system had to be assumed. DEPT experiments indicated 2 tertiary and 2 secondary methyl groups (δ 17.17, 21.14, 25.41, 25.61) which corresponded to proton signals at δ 0.96 (*s*, 6 H), 1.11 (*d*, 3 H) and 1.12 (*d*, 3 H). In addition 4 methylene groups (δ 24.17, 24.40, 34.89 and 50.63), 4 methine groups (δ 27.42, 45.75, 51.39, 121.47) and 3 quaternary carbon signals (δ 47.84, 59.08, 156.91) could be assigned. A detailed investigation of the ^{1}H–^{1}H-COSY and the inspection of long-range coupling correlations in the HMBC- and HMQC spectra allowed the construction of partial structures A, B and C together with 2 methyl groups connected to quaternary carbons (Fig. 3). However, due to overlapping signals and missing correlations

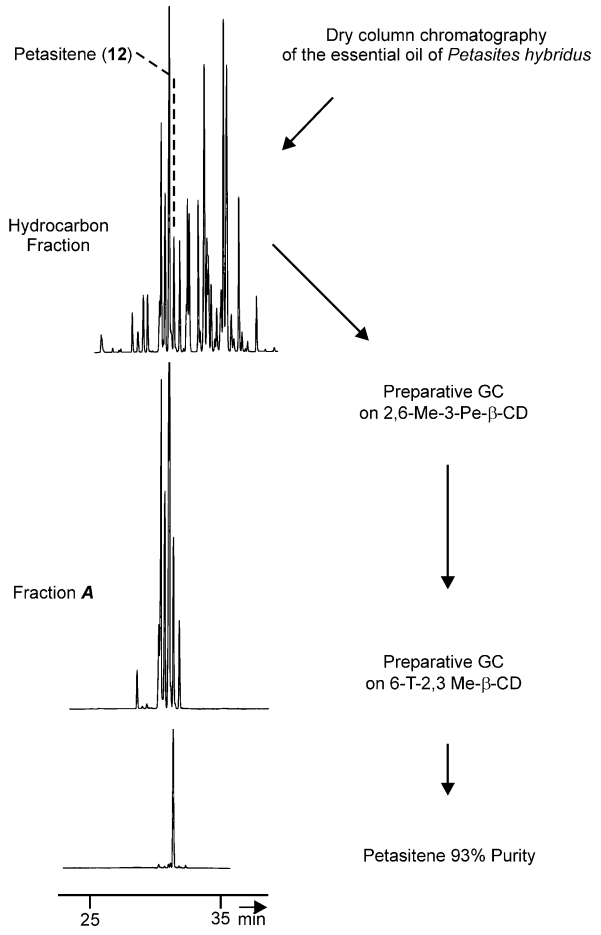

Fig. 2. Gas chromatographic control of the isolation procedure for petasitene (**12**) from the hydrodistillation product of *P. hybridus*. 25 m Fused-silica capillary column with CPSil 5, 50 °C, 3 °C/min to 230 °C.

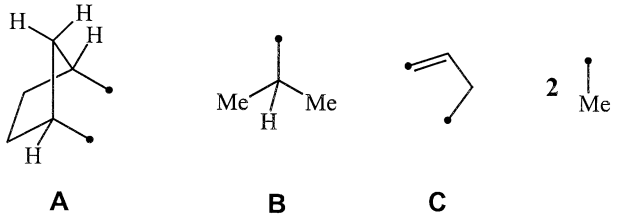

Fig. 3. Partial structures of petasitene (**12**) derived by NMR investigations.

of the tertiary methyl groups the assignment of an unambiguous total structure was still not possible. Therefore, a small amount of **12** was converted to epoxide **27** by treatment with *m*-chloroperbenzoic acid. In this derivative the tertiary methyl signals could now clearly be distinguished (δ 0.83 (*s*, 3 H) and 20.98, C-16; 0.99 (*s*, 3 H) and 13.89, C-15) and from the long-range coupling correlations in the HMBC spectrum (Fig. 4) the structure of **27**, and subsequently also of **12**, could be assigned.

While the relative configuration of **12** was derived from a NOESY spectrum (Fig. 5), the absolute configuration was determined by a chemical correlation (Fig. 6) with natural albene (**3**), which is present in the hydrodistillation products of *P. hybridus* and could be isolated in sufficient amounts. As shown in Fig. 6, (−)-albene (**3**) was converted to albenone (**28**). After hydrogenation of the olefinic double bond to albanone (**29**) nucleophilic addition of isopropyl-lithium yielded the tertiary alcohol **30**, which lost a water molecule by treatment with acidic ion exchange resin Amberlyst®15. Both natural and synthetic **12** showed a negative optical rotation and displayed completely identical NMR and mass spectra as well as identical GC retention times on chiral and non-chiral stationary phases.

2.2. Pethybrene (*9*)

The isolation of this new sesquiterpene hydrocarbon was complicated as it is only a minor constituent with 0.9% of the total volatiles eluting between two larger peaks from the GC column. A combination of preparative GC and TLC with $AgNO_3$-precoated plates finally resulted in the isolation of compound **9** in 95% purity (Fig. 7). From the mass spectrum and $M^+ = 204$ the molecular composition $C_{15}H_{24}$ was derived. From the ^1H and ^{13}C NMR spectra proton signals for an exocyclic double bond (δ 4.80, *s*, and 5.04, *s*), and one secondary and two tertiary methyl groups (δ 1.09, *d*, 1.26, *s*, 1.31, *s*) could be derived, but no other spin systems could be recognized because of largely overlapping signals. In the DEPT NMR spectra 3 methyl groups (δ 17.90, 19.53 and 24.89), 7 methylene groups (δ 21.26, 27.59, 30.31, 40.14, 44.00, 48.66, and 99.17), 1 methine group (δ 37.03) and 4 quaternary carbon signals (δ 45.60, 48.95, 62.32 and 162.37) could be detected. From these data together with the ^1H–^1H-COSY and HMQC coupling correlations 6 partial structures could be derived (Fig. 8), which could be connected to a tricyclic sesquiterpene skeleton by a careful interpretation of the HMBC data (Fig. 9). The NOESY spectrum of **9** afforded the relative configuration (Fig. 10).

In cases when the direct determination of the absolute configuration is not possible, a chemical correlation by acid catalyzed rearrangement can be conclusive if a product of known absolute configuration is formed. By treating **9** with acidic ion exchange resin for 1 hour at room temperature only one product was obtained almost quantitatively which turned out to be the known (−)-α-isocomene (**11**). This sesquiterpene hydrocarbon which is also present in *P. hybridus* has been isolated before from *Isocoma wrightii* (Zalkov et al., 1977) and *Berkheya radula* (Bohlmann et al., 1977) and is also a constituent of *Silphium perfoliatum* (Bohlmann and Jakupovic, 1980). A tentative rearrangement mechanism from (−)-pethybrene (**9**) to (−)-α-isocomene (**11**) is depicted in Fig. 11.

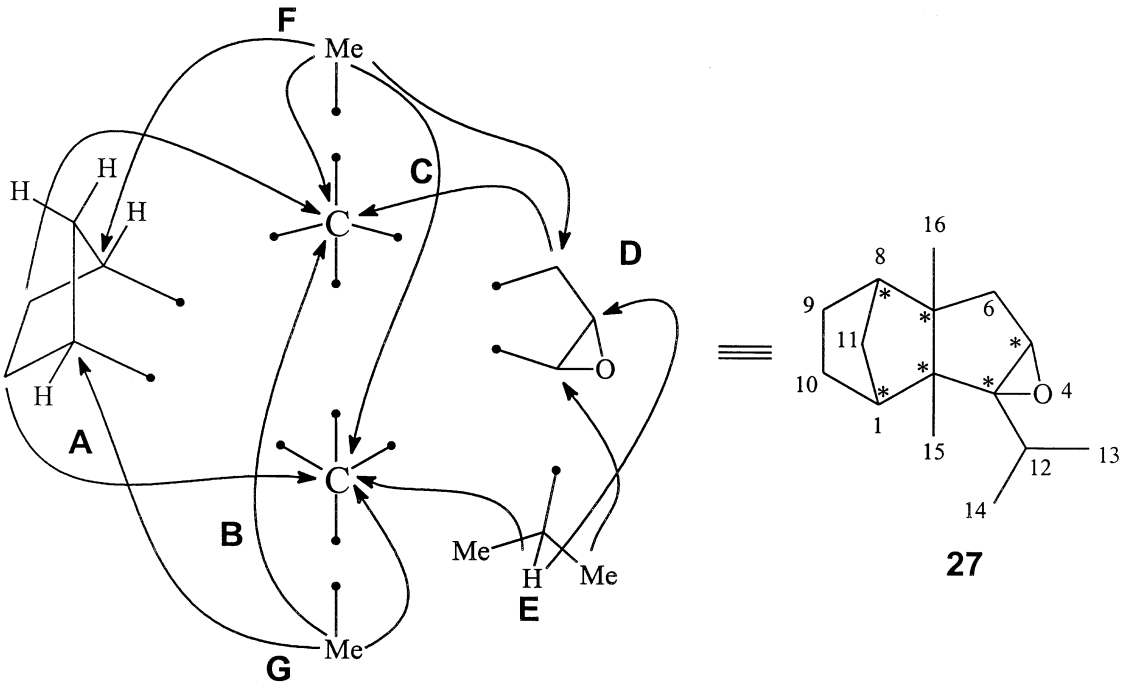

Fig. 4. Partial structures and HMBC correlations found for petasitene epoxide (**27**).

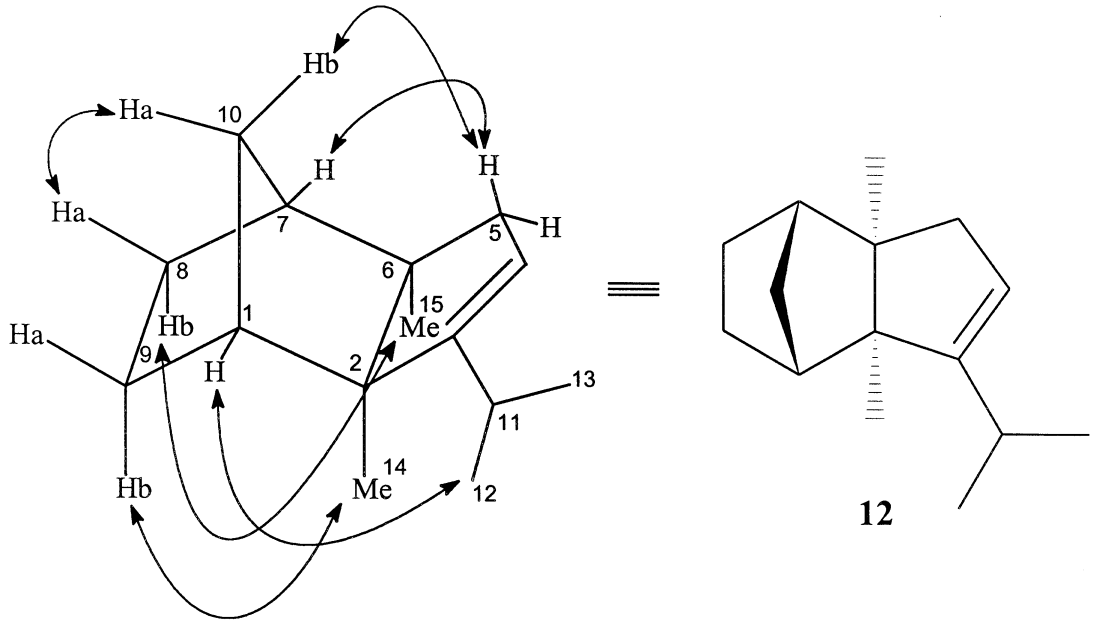

Fig. 5. NOESY correlations and relative configuration of petasitene (**12**).

3. Experimental

3.1. Plant material

Ten kilograms of *Petasites hybridus* rhizomes were collected at the Alster in Hamburg (Germany) in May 1998 and were identified by Johannes Donath from the Botanical Garden of the University of Hamburg. The fresh material was washed, cut and submitted in 500 g portions to hydrodistillation for 2.5 h to yield the essential oil, which was collected in 1 ml of *n*-hexane and not further quantified.

3.2. NMR spectroscopy

NMR measurements were carried out with a Bruker WM 400 instrument for 1D spectra and with WM 500 instrument for 1D, ^{1}H–^{1}H-COSY, HMBC, HMQC

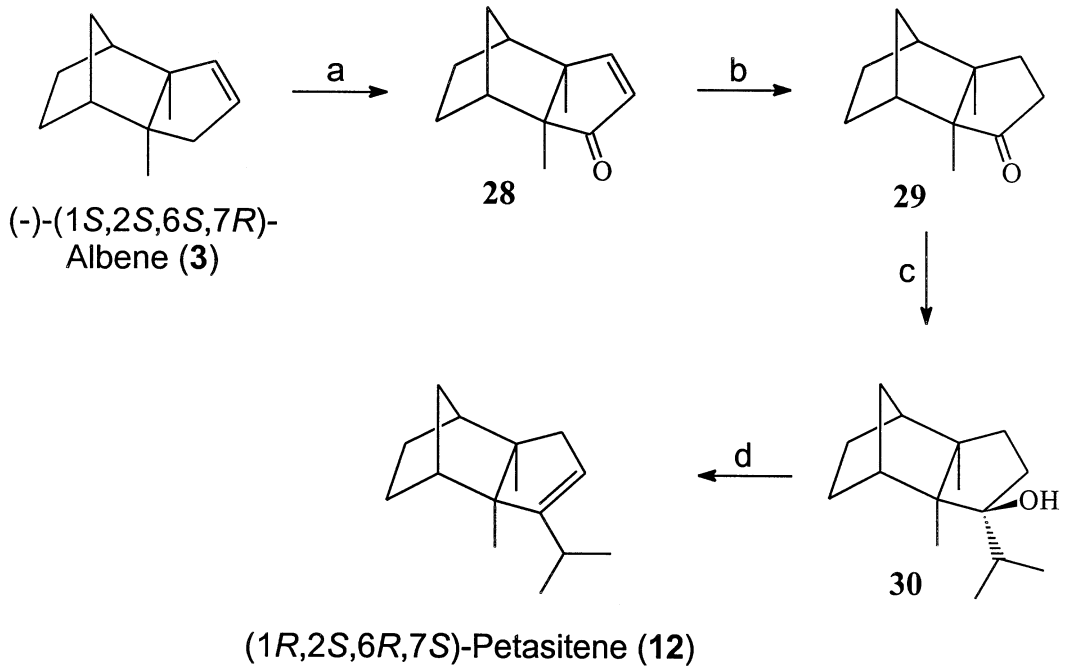

Fig. 6. Partial synthesis of petasitene (**12**) from (−)-albene (**3**). (a) Pyridinium dichromate, pyridine; (b) H_2/Pd, ether; (c) isopropyl-lithium, *n*-hexane; (d): H^+.

and NOESY spectra. TMS was used as an internal standard.

3.3. GC–MS

Electron impact (70 eV) GC–MS measurements were carried out on a Hewlett Packard HP 5890 gas chromatograph equipped with a 25 m CPSil 5 (Chrompack) fused-silica capillary column coupled to a VG Analytical 70-250S mass spectrometer. Ion source temp.: 200 °C.

3.4. Preparative GC

Preparative GC was carried out with a modified Varian 2800 gas chromatograph equipped with a stainless steel column (Silcosteel, Amchro) with 10% SE 30 on chromosorb W-HP, (1.85 m × 4.3 mm), 5% heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin – OV 1701 (1:1; w/w) on Chromosorb W-HP (1.85 m × 4.3 mm) [2,6-Me-3-Pe-β-CD], or 6.4% heptakis-(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin – SE 52 (1:1; w/w) on Chromosorb W-HP (1.95 m × 5.3 mm) [6-T-2,3-Me-β-CD]. Helium was used as carrier gas at a flow rate of 240 ml/min and a temperature program applied (initial: 80 °C, 2 °C/min, to 160 °C).

3.5. Polarimetry

A Perkin-Elmer 241 polarimeter was used. To avoid inaccuracies only the sense of the optical rotation was determined due to the small quantity of isolated material.

3.6. Isolation of (−)-petasitene (*12*)

(−)-(1*R*,2*S*,6*R*,7*S*)-2,6-Dimethyl-3-isopropyltricyclo-[5.2.1.0 ^{2,6}]-dec-3-ene. After prefractionation of the raw essential oil by dry column chromatography (elution with hexane) the compound was enriched from the hydrocarbon fraction by preparative GC on a 2,6-Me-3-Pe-β-CD column and finally isolated (approx. 5 mg total amount) through preparative GC with a 6-T-2,3-Me-β-CD column. ^{1}H NMR (400 MHz, C_6D_6): $\delta = 0.96$ (6 H, *s*, 14-Me, 15-Me), 1.00 (1 H, *d* (*br*), $^2J = 9.7$ Hz, 10-Ha), 1.11 (3 H, *d*, $J = 7.1$ Hz, 12-Me), 1.12 (3 H, *d*, $J = 6.6$ Hz, 13-Me), 1.28 (1 H, *m*, 8-Ha), 1.34 (1 H, *m*, 9-Ha), 1.56 (2 H, *m*, 8-Hb, 9-Hb), 1.74 (1 H, *d* (*br*), 10-Hb, $^2J = 9.7$ Hz), 1.79 (1 H, *d* (*br*), $J = 3.6$ Hz, 7-H), 1.95 (1 H, *d* (*br*), $J = 3.1$ Hz, 1-H), 2.06 (1 H, *qq*, $J_1 = J_2 = 6.6$ Hz, 11-H), 2.22 (2 H, *d*, $J = 2.6$ Hz, 5-H), 5.38 (1 H, *t*, $J = 2.6$ Hz, 4-H). ^{13}C NMR (125 MHz, C_6D_6) : δ 17.17 (*q*, 14-Me), 21.14 (*q*, 15-Me), 24.17, 24.40 (2 C, *t*, 8-C, 9-C), 25.41, 25.61 (2 C, *q*, 12-Me, 13-Me), 27.42 (*d*, 11-C), 34.89 (*t*, 10-C), 45.75 (*d*, 1-C), 47.84 (*s*, 6-C), 50.63 (*t*, 5-C), 51.39 (*d*, 7-C), 59.08 (*s*, 2-C), 121.47 (*d*, 4-C), 156.91 (*s*, 3-C). MS (EI, 70 eV), *m*/*z* (rel.int.) : 204 (4) [M^+], 189 (8), 161 (4), 147 (2), 137 (100), 121 (27), 105 (9), 95 (26), 79 (9), 77 (9), 75 (9), 67 (9), 57 (9), 41 (19).

3.7. Synthesis of (+)-(3*R*)-petasitenepoxide (*27*)

(+)-(1*R*,2*S*,3*R*,5*S*,6*R*,7*S*)-2,7-Dimethyl-3-isopropyl-4-oxa-tetracyclo-[6.2.1.0^{2,7}.0^{3,5}.]-undecane. To 3 mg of (−)-petasitene (**12**) in 2 ml $CHCl_3$ 5 mg of *m*-chloro perbenzoic acid were added. After 24 h the soln. was

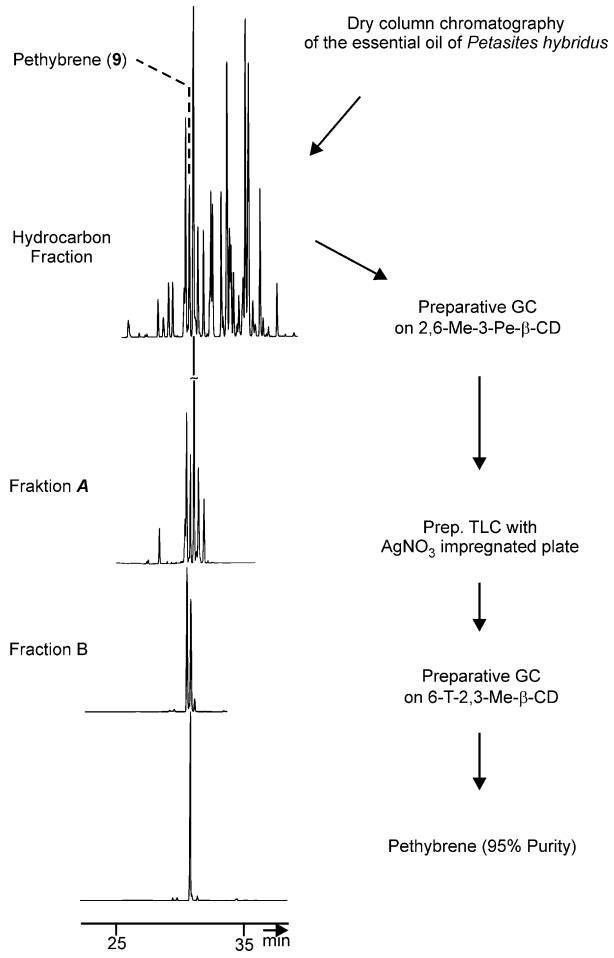

Fig. 7. Gas chromatographic control of the isolation procedure for pethybrene (**9**) from the hydrodistillation product of *P. hybridus*. 25 m Fused-silica capillary column with CPSil 5, 50 °C, 3 °C/min to 230 °C.

washed with 2 ml satd. Na_2SO_3 soln., dried over $MgSO_4$ and purified by preparative GC with a 2,6-Me-3-Pe-β-CD column to give 2 mg of (+)-(3*R*)-petasitenepoxide (**27**). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 0.83 (3 H, *s*, 16-H), 0.92 (6 H, *d*, J=7.1 Hz, 13-H, 14-H), 0.99 (3 H, *s*, 15-H), 1.09 (1 H, *d* (*br*), ^2J=9.7 Hz, 11-Ha), 1.24 (1 H, *m*, 9-Ha), 1.32 (1 H, *m*, 10-Ha), 1.43 (1 H, *m*, 9-Hb), 1.55 (1 H, *m*, 10-Hb), 1.62 (1 H, *d* (*br*), ^2J=9.7 Hz, 11-Hb), 1.71 (1 H, *dd*, J=14.6 Hz, J=2.0 Hz, 6-H), 1.77 (1 H, *d* (*br*), J=3.6 Hz, 8-H), 1.82 (1 H, *d*, J=14.6 Hz, 6-H), 2.18 (1 H, *d* (*br*), J=2.5 Hz, 1-H), 2.25 (1 H, *sept*, J=7.1 Hz, 12-H), 3.34 (1 H, *d*, J=2.0 Hz, 5-H). ^{13}C NMR (125 MHz, $CDCl_3$) : δ 13.89 (*q*, 15-C), 18.31, 19.69 (2C, *q*, 13-C, 14-C), 20.98 (*q*, 16-C), 21.45 (*t*, 9-C), 22.31 (*t*, 10-C), 24.76 (*d*, 12-C), 33.26 (*t*, 11-C), 42.62 (*d*, 1-C), 44.53 (*t*, 6-C), 48.91 (*s*, 7-C), 50.01 (*d*, 8-C), 52.93 (*s*, 2-C), 62.57 (*d*, 5-C), 77.61 (*s*, 3-C). MS (EI, 70 eV) *m*/*z* (rel.int.) : 220 (2) [M^+], 205 (4), 187 (2), 177 (4), 153 (100), 135 (10), 121 (5), 107 (16), 93 (11), 79 (10), 67 (10), 55 (10), 41 (17).

3.8. Isolation of (−)-albene (*3*)

(−)-(1*S*,2*S*,6*S*,7*R*)-2,6-Dimethyltricyclo-[5.2.1.0$^{2.6}$]-dec-3-ene. After prefractionation of the raw essential oil by dry column chromatography (Schwanbeck et al., 1982) with hexane as eluent the compound (approx. a total amount of 50 mg) was isolated from the hydrocarbon fraction by preparative GC using a 2,6-Me-3-Pe-β-CD column. ^{1}H NMR (400 MHz, $CDCl_3$) : δ 0.94 (6 H, *s*, 2-Me, 6-Me), 0.98 (1 H, *d* (*br*), ^2J=9.5 Hz, 10-Ha), 1.29 (2 H, *m*, 8-Ha, 9-Ha), 1.56 (2 H, *m*, 8-Hb, 9-Hb), 1.63 (1 H, *d* (*br*), ^2J=9.5 Hz, 10-Hb), 1.78 (1 H, *d* (*br*), J=5.1 Hz, 1-H), 1.80 (1 H, *d* (*br*), J=4.1 Hz, 7-H), 2.23 (2 H, *dd*, 5-H, J=5.6 Hz, J=2.4 Hz, 5-H), 5.26 (1 H, *dt*, J=6.1 Hz, J=5.6 Hz, 4-H), 5.56 (1 H, *dt*, J=6.1 Hz, J=2.4 Hz, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 18.09 (*q*, 2-Me), 20.65 (*q*, 6-Me), 23.77, 23.79 (2C, *t*, 8-C, 9-C), 34.16 (*t*, 10-C), 46.55 (*s*, 6-C), 47.02 (*d*, 7-C), 50.27 (*d*, 1-C), 51.75 (*t*, 5-C), 56.33 (*s*, 2-C), 128.30 (*d*, 4-C), 139.59 (*d*, 3-C). MS (EI, 70 eV), *m*/*z* (rel.int.) : 162 (2) [M^+], 147 (4), 133 (2), 119 (7), 105 (9), 95 (100), 94 (41), 79 (24), 67 (13), 53 (7), 41 (17).

3.9. Synthesis of (−)-albenone (*28*)

(−)-(1*R*,2*S*,6*S*,7*S*)-2,6-Dimethyltricyclo-[5.2.1.0$^{2.6}$]-dec-4-en-3-one. To 20 mg of (−)-albene (**3**) in 2 ml of pyridine 700 mg of pyridinium dichromate and 10 mg of molecular sieve 3 Å were added. After heating for 24 h under reflux 2 ml of H_2O were added. The reaction product was extracted by shaking with Et_2O, washed with 1 M HCl, $NaHCO_3$ and satd. NaCl soln., dried over $MgSO_4$, evaporated and purified by column chromatography (hexane–Et_2O, 10:1) to give 19 mg of (−)-albenone (**28**). ^{1}H NMR (500 MHz, C_6D_6): δ 0.62 (3H,*s*, 6-Me), 0.66 (1 H, *d* (*br*), ^2J=10.4 Hz, 10-Ha), 0.85 (3 H,

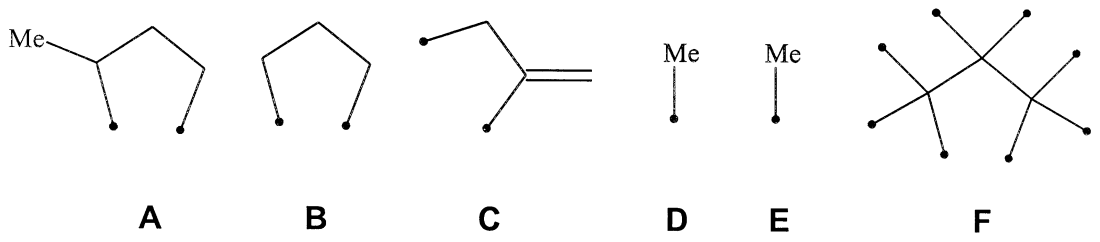

Fig. 8. Partial structures of pethybrene (**9**) derived by NMR investigations.

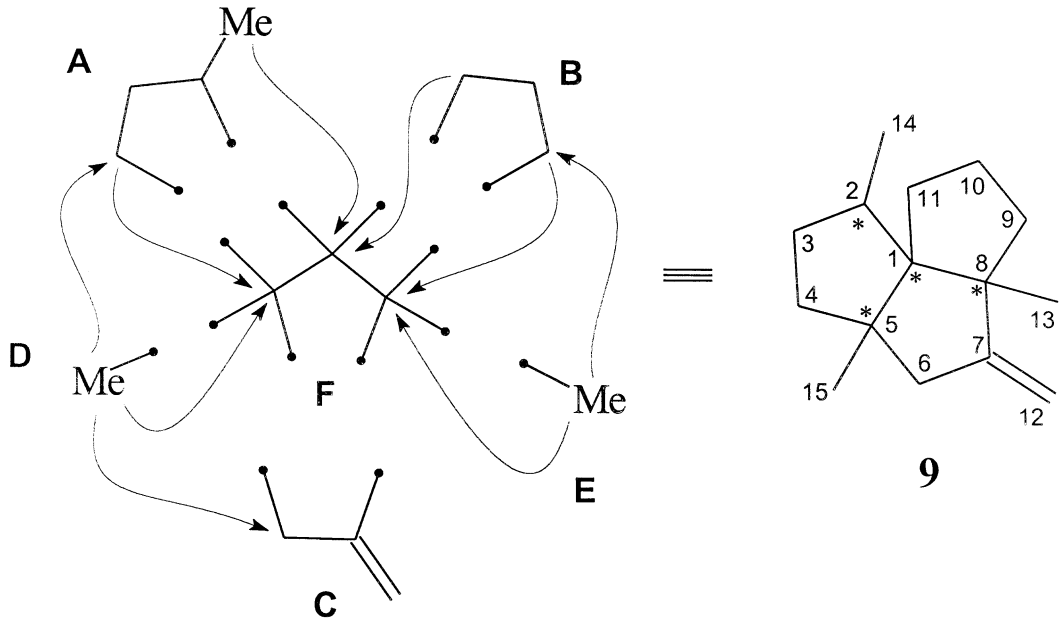

Fig. 9. Partial structures and HMBC correlations found for pethybrene (**9**).

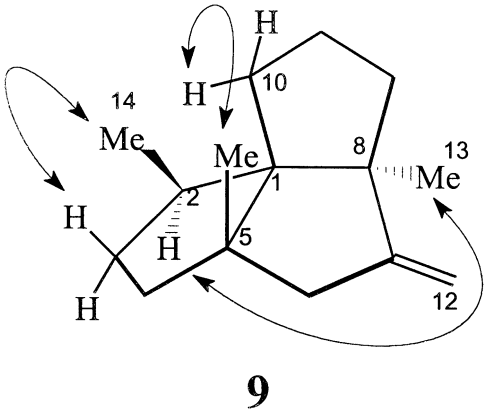

Fig. 10. NOESY correlations and relative configuration of pethybrene (**9**).

s, 2-Me), 1.07 (1 H, *m*, 8-Ha), 1.13 (1 H, *m*, 9-Ha), 1.16 (1 H, *d* (*br*), $^2J = 10.4$ Hz, 10-Hb), 1.32 (2 H, *m*, 8-Hb, 9-Hb), 1.50 (1 H, *d* (*br*), $J = 2.5$ Hz, 7-H), 2.14 (1 H, *d* (*br*), $J = 2.5$ Hz, 1-H), 6.04 (1 H, *d*, $J = 5.7$ Hz, 4-H), 6.58 (1 H, *d*, $J = 5.7$ Hz, 5-H). ^{13}C NMR (100 MHz, C_6D_6): δ 14.25 (*q*, 2-Me), 16.33 (*q*, 6-Me), 22.48 (*t*, 8-C), 23.30 (*t*, 9-C), 33.91 (*t*, 10-C), 44.07 (*d*, 7-C), 45.08 (*d*, 1-C), 52.94 (*s*, 2-C), 54.49 (*s*, 6-C), 134.08 (*d*, 4-C), 168.98 (*d*, 5-C), 213.53 (*s*, 3-C). MS (EI, 70 eV), *m*/*z* (rel.int.) : 176 (100) [M^+], 161 (63), 148 (31), 133 (41), 119 (28), 110 (68), 108 (68), 105 (41), 91 (41), 80 (41), 79 (35), 67 (31), 53 (16), 41 (31).

3.10. Synthesis of (−)-albanone (*29*)

(−)-(1*R*,2*S*,6*R*,7*S*)-2,6-Dimethyltricyclo-[5.2.1.0$^{2.6}$]-decan-3-one. To 19 mg of (−)-albenone (**28**) in 2 ml hexane 20 mg of Pd-C (15%) were added and H_2 was passed through the soln. After 4 h the soln. was filtered to give 19 mg of (−)-albanone (**29**). ^{1}H NMR (500 MHz, C_6D_6) : δ 0.68 (3H, *s*, 6-Me), 0.75 (3 H, *s*, 2-Me), 0.84 (1 H, *d* (*br*), $^2J = 10.4$ Hz, 10-Ha), 1.10 (3 H, *m*, 8-Ha, 9-Ha, 10-Hb), 1.27 (4 H, *m*, 8-Hb, 9-Hb, 5-H), 1.65 (1 H, *d* (*br*), $J = 2.5$ Hz, 7-H), 1.89 (1 H, *dt*, $^2J = 17.9$ Hz, $J = 6.3$ Hz, 4-H), 2.01 (1 H, *dt*, $^2J = 17.7$ Hz, $J = 11.4$ Hz, 4-H), 2.35 (1 H, *d* (*br*), $J = 3.2$ Hz, 1-H). ^{13}C NMR (100 MHz, C_6D_6) : δ 16.13 (*q*, 2-Me), 20.93 (*q*, 6-Me), 22.79 (*t*, 8-C), 23.65 (*t*, 9-C), 35.29 (*t*, 5-C), 36.18, 36.33 (2C, *t*, 4-C, 10-C), 46.03 (*d*, 1-C), 46.10 (*s*, 6-C), 51.10 (*d*, 7-C), 56.41 (*s*, 2-C), 223.01 (*s*, 3-C). MS (EI, 70 eV), *m*/*z* (rel.int.) : 178 (20) [M^+], 163 (6), 156 (6), 135 (4), 122 (44), 111 (22), 94 (100), 79 (50), 77 (28), 67 (37), 53 (28), 41 (61).

3.11. Synthesis of (+)-(3*S*)-petasitan-3-ol (*30*)

(+)-(1*R*,2*S*,3*S*,6*R*,7*S*)-2,6-Dimethyl-3-isopropyltricyclo-[5.2.1.0 $^{2.6}$]-decan-3-ol. Under an argon atmosphere 19 mg of (−)-albanone (**29**) in hexane were added dropwise to 40 ml of boiling isopropyl-lithium (2.0 M) in hexane. After heating under reflux for 60 h the reaction mixture was slowly added to 100 g crushed ice. The reaction product was isolated with hexane, dried over $MgSO_4$, and purified by preparative GC with a SE 30 phase to give 2 mg of (+)-(3*S*)-petasitan-3-ol (**30**). ^{1}H NMR (500 MHz, C_6D_6) : δ 0.64 (3H, *s*, 14-Me), 0.78 (3 H, *d*, $J = 6.6$ Hz, 12-Me), 0.82 (3 H, *s*, 15-Me), 0.86 (3 H, *d*, $J = 6.9$ Hz, 13-Me), 1.05 (1 H, *d* (*br*), $^2J = 9.8$ Hz, 10-Ha), 1.13 (1 H, *m*, 4-Ha), 1.18 (1 H, *m*, 9-Ha), 1.22 (1 H, *m*, 5-Ha), 1.30 (1 H, *m*, 8-Ha), 1.38 (1 H, *m*, 4-Hb), 1.41 (2 H, *m*, 8-Hb, 9-Hb), 1.68 (1 H, *qq*, $J_1 = J_2 = 6.6$ Hz, 11-H), 1.80 (1 H, *d* (*br*), $J = 4.1$ Hz, 7-H), 1.84 (1 H, *m*, 5-Hb),

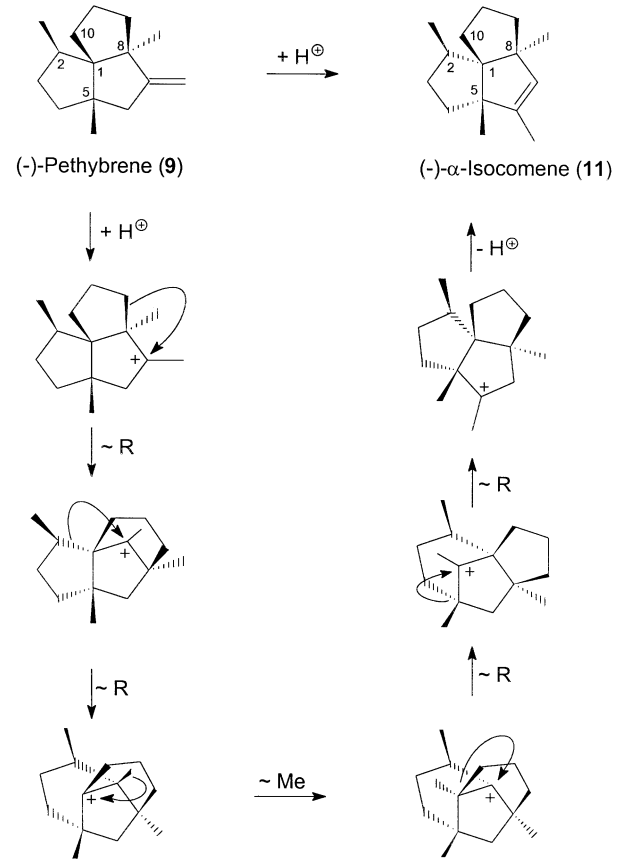

Fig. 11. Tentative rearrangement mechanism of pethybrene (**9**) to (−)-α-isocomene (**11**).

1.87 (1 H, *d* (*br*), $J=4.4$ Hz, 1-H), 2.51 (1 H, *d* (*br*), $^2J=9.8$ Hz, 10-Hb). ^{13}C NMR (100 MHz, C_6D_6) : δ 17.61 (*q*, 12-Me), 19.00, 19.06 (2 C, *q*, 14-Me, 13-Me), 20.71 (*q*, 15-Me), 23.34 (*t*, 8-C), 25.27 (*t*, 9-C), 33.25 (*d*, 11-C), 36.58 (*t*, 4-C), 37.85 (*t*, 10-C), 38.58 (*t*, 5-C), 44.62 (*d*, 1-C), 46.41 (*d*, 7-C), 53.01 (*s*, 6-C), 53.59 (*s*, 2-C), 88.55 (*s*, 3-C). MS (EI, 70 eV), *m*/*z* (rel.int.) : 222 (1) [M^+], 189 (1), 179 (16), 161 (7), 151 (1), 137 (11), 123 (100), 111 (16), 95 (24), 94 (33), 81 (34), 67 (34), 55 (24), 41 (85).

3.12. Preparation of isopropyl-lithium

To 2 g of Li in 30 ml boiling hexane 9 ml of 2-chloropropane in 20 ml hexane were added. After heating under reflux for 3 h the reaction mixture was filtered to give a 2.0 M isopropyl-lithium soln. (hexane was dried over Na and freshly distilled under Ar; 2-chloropropane was dried over P_2O_5 and freshly distilled under Ar).

3.13. Synthesis of (−)-petasitene (*12*)

(−)-(1*R*,2*S*,6*R*,7*S*)-2,6-dimethyl-3-isopropyltricyclo-[5.2.1.0 ^{2,6}]-dec-3-ene. To 2 mg of (+)-(3*S*)-petasitan-3-ol (**30**) in 1 ml hexane 3 mg of Amberlyst™ 15 were added. After standing at room temperature for 12 h the mixture was filtered and the dehydration product isolated through preparative GC with a SE 30 phase to give 1 mg (−)-petasitene identical to the natural product isolated from *P. hybridus*.

3.14. Isolation of (−)-pethybrene (*9*)

(−)-(1*S*,2*R*,5*S*,8*S*)-7-methylen-2,5,8-trimethyltricyclo-[6.3.0.0^{1,5}]-undecane. After prefractionation of the raw essential oil by dry column chromatography (Schwanbeck et al., 1982) with hexane as eluent the hydrocarbon fraction was fractionated by preparative GC using a 2,6-Me-3-Pe-β-CD column. The compound was enriched from the first sesquiterpenoid fraction by preparative TLC with $AgNO_3$-silica gel (impregnated with ca. 0.5 g of $AgNO_3$ in 100 ml EtOH–H_2O (1 : 4) for 1 h, dried at 110 °C) with *n*-hexane as eluent and was finally purified by preparative GC with a 6-T-2,3-Me-β-CD column. ^{1}H NMR (500 MHz, C_6D_6): δ 1.09 (3 H, *d*, $J=7.1$ Hz, 14-Me), 1.26 (3 H, *s*, 13-Me), 1.31 (3 H, *s*, 15-Me), 1.33 (1 H, *m*, 3-H), 1.41 (1 H, *m*, 11-H), 1.50 (1 H, *m*, 4-H), 1.60 (1 H, *m*, 9-H), 1.63 (2 H, 2*s*, 6-H), 1.68 (1 H, *m*, 4-H), 1.74 (1 H, *m*, 9-H), 1.78 (2 H, *m*, 10-H), 1.89 (1 H, *q*, $J=7.1$ Hz, 2-H), 2.07 (1 H, *m*, 3-H), 2.14 (1 H, *m*, 11-H), 4.80 (1 H, *s*, 12-CH), 5.04 (1 H, *s*, 12-CH). ^{13}C NMR (100 MHz, C_6D_6): δ 17.90 (*q*, 14-Me), 19.53 (*q*, 13-Me), 21.26 (*t*, 10-C), 24.89 (*q*, 15-Me), 27.59 (*t*, 3-C), 30.31 (*t,* 11-C), 37.03 (*d*, 2-C), 40.14 (*t*, 4-C), 44.00 (*t*, 9-C), 45.60 (*s*, 5-C), 48.66 (*t*, 6-C), 48.95 (*s*, 8-C), 62.32 (*s*, 1-C), 99.17 (*t*, 12-CH_2), 162.37 (*s*, 7-C). MS (EI, 70 eV), *m*/*z* (rel.int.) : 204 (8) [M^+], 189 (22), 175 (7), 161 (15), 149 (100), 133 (15), 119 (15), 107 (19), 105 (19), 93 (19), 91 (19), 79 (15), 67 (9), 55 (16), 41 (28).

3.15. Rearrangement of (−)-pethybrene (*9*)

To 0.2 mg of (−)-pethybrene in 500 µl of *n*-hexane 0.5 mg of Amberlyst™ 15 were added. After stirring at room temperature for 2 h the solution was filtered to give (−)-α-isocomene (**11**) identical to the natural product from *Silphium perfoliatum*.

Acknowledgements

We thank Dr. V. Sinnwell, University of Hamburg, for his support in the NMR measurements and Mrs. A. Meiners and Mr. M. Preusse for technical assistance. The financial support of the Fonds der Chemischen Industrie is gratefully acknowledged.

References

Aebi, A., Büchi, J., Waaler, T., Eichenberger, E., Schmutz, J., 1955. Inhaltsstoffe von *Petasites hybridus* (L.) Fl. Wett. Pharmaceutica Acta Helvetiae 29, 277–279.

Baldwin, J.E., Barden, T.C., Ciauciosi, S.J., 1986. Reinterpretation of two degradations of (−)-albene. Journal of Organic Chemistry 51, 1133–1135.

Bohlmann, F., Jakupovic, J., 1980. Neue Sesquiterpen-Kohlenwasserstoffe mit anomalem Kohlenstoffgerüst aus *Silphium*-Arten. Phytochemistry 19, 259–265.

Bohlmann, F., Le Van, N., Pickardt, J., 1977. Über ein anomales Sesquiterpen aus *Berkheya radula*. Chemische Berichte 110, 3777–3781.

Brunke, E.-J., Hammerschmidt, F.-J., Schmaus, G., 1992. Headspace analysis of selected European medicinal plants. Dragoco Report, pp. 105–124.

Hardt, I.H., König, W.A., 1994. Preparative enantiomer separation with modified cyclodextrins as chiral stationary phases. Journal of Chromatography A 666, 611–615.

Hochmannová, J., Novotny, L., Herout, V., 1962. Hydrocarbons from *Petasites albus* (L.) Gaerth. rhizomes. Collection of Czechoslovak Chemical Communications 27, 2711–2713.

Joulain, D., König, W.A., 1998. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. E.B.-Verlag, Hamburg.

Kreiser, W., Janitschke, L., Voss, W., Ernst, L., Sheldrick, W.S., 1979. Synthese des (±)-Isoalbens. Chemische Berichte 112, 397–407.

Kreiser, W., Janitschke, L., 1979. Erste Totalsynthese von (−)-"Alben". Chemische Berichte 112, 408–422.

Neuenschwander, M., Neuenschwander, A., Steinegger, E., Engel, P., 1979. Struktur der Sesquiterpene von *Petasites hybridus* (L.) G. M. et SCH.: Petasol- und Isopetasol-Abkömmlinge. Helvetica Chimica Acta 62, 609–626.

Schwanbeck, J.E., Koch, V., Kubeczka, K.-H., 1982. In: Kubeczka, K.-H. (Ed.), Ätherische Öle, Analytik, Physiologie, Zusammensetzung. Thieme-Verlag, Stuttgart, p. 70.

Stoll, A., Morf, R., Rheiner, A., Renz, J., 1956. Über Inhaltsstoffe aus Petasites officinalis Moench. I. Petasin und die Petasolester B und C. Experientia 12, 360–362.

Willuhn, G., 1997. Petasitidis folium. In: Wichtl, M. (Ed.), Teedrogen und Phytopharmaka, 3rd. Edition. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Zalkov, L.H., Harris III., R.N., van Derveer, D, Bertrand, J.A., 1977. Isocomene: a novel sesquiterpene from *Isocoma wrightii*. X-ray structure of the corresponding diol. Journal of the Chemical Society, Chemical Communications, 456–457.