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Phytochemistry 65 (2004) 2045–2049
PHYTOCHEMISTRY
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Sesquiterpenes from the east African sandalwood *Osyris tenuifolia*

Andreas Th. Kreipl, Wilfried A. König *

Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

Received 8 March 2004; received in revised form 7 May 2004
Available online 20 July 2004

Abstract

The essential oil of the east African sandalwood *Osyris tenuifolia* was investigated by chromatographic and spectroscopic methods. Beside several already known sesquiterpenes four new compounds could be isolated by preparative gas chromatography and their structures investigated by mass spectroscopy and NMR techniques. Two of the new compounds – tenuifolene (**17**) and *ar*-tenuifolene (**15**) – show a new sesquiterpene backbone. 2,(7*Z*,10*Z*)-Bisabolatrien-13-ol (**23**) and the cyclic ether lanceoloxide (**21**) belong to the bisabolanes.
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Keywords: *Osyris tenuifolia*; Sandalwood; Sesquiterpene hydrocarbons; Tenuifolene; Oxygenated sesquiterpenes; 2,(7*Z*,10*Z*)-Bisabolatrien-13-ol; Lanceoloxide

1. Introduction

Osyris tenuifolia (East African sandalwood) belongs taxonomically to the Santalaceae. The small tree occurs in the equatorial region of Africa, where the extract of its small shoots is used as antipyretic agent by the Massai for cattle (Thanner, 1908). The essential oil of *O. tenuifolia* was investigated for the first time by Naves and Ardizio (1954), who could already identify the main constituent lanceol.

In the present work the essential oil of *O. tenuifolia* was analyzed by GC–MS. Four new sesquiterpenes – *ar*-tenuifolene (**15**), tenuifolene (**17**), 2,(7*Z*,10*Z*)-bisabolatrien-13-ol (**23**) and lanceoloxide (**21**) – could be isolated by preparative GC. The structures of the compounds were investigated by mass spectroscopy and NMR (^{1}H, ^{13}C, ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY). Compounds **17** and **21** show a sesquiterpene backbone which is reported for the first time.

2. Results and discussion

The commercially available essential oil of *O. tenuifolia* shows a complex fraction of sesquiterpenoids. Most of the known compounds (**1–14**, **16** and **20**) were identified by comparing their mass spectra and retention indices to a spectral library established under identical experimental conditions (Joulain and König, 1998). The already known sesquiterpenes *epi*-cyclosantalal (**18**) (Brunke and Vollhardt, 1995), (−)-*epi*-α-bisabolol (**19**) (Isaak et al., 1968; Kergomard and Veschambre, 1977), the main constituent (*S*)-(*Z*)-lanceol (**22**) (Naves and Ardizio, 1954) and four unknown sesquiterpenes (**15**, **17**, **21** and **23**) were isolated and identified by mass spectrometry and NMR spectroscopic investigation. The following compounds in order of their elution from a capillary column with polydimethylsiloxane could be identified (their relative concentrations of major compounds are given in parentheses, all other compounds were below 2% relative abundance; only identified compounds are given): 7-*epi*-α-cedrene (**1**), *cis*-α-bergamotene (**2**), α-cedrene (**3**), α-santalene (**4**), *trans*-α-bergamotene (**5**), *epi*-β-santalene (**6**), β-santalene (**7**), β-acoradiene (**8**), *ar*-curcumene (**9**), γ-curcumene (**10**), *trans*-β-bergamotene (**11**), (*Z*)-α-bisabolene (**12**), β-bisabolene (**13**, 3.7%),

* Corresponding author. Tel.: +49-4042-838-2824; fax: +49-4042-838-2893.
E-mail address: wkoenig@chemie.uni-hamburg.de (W.A. König).

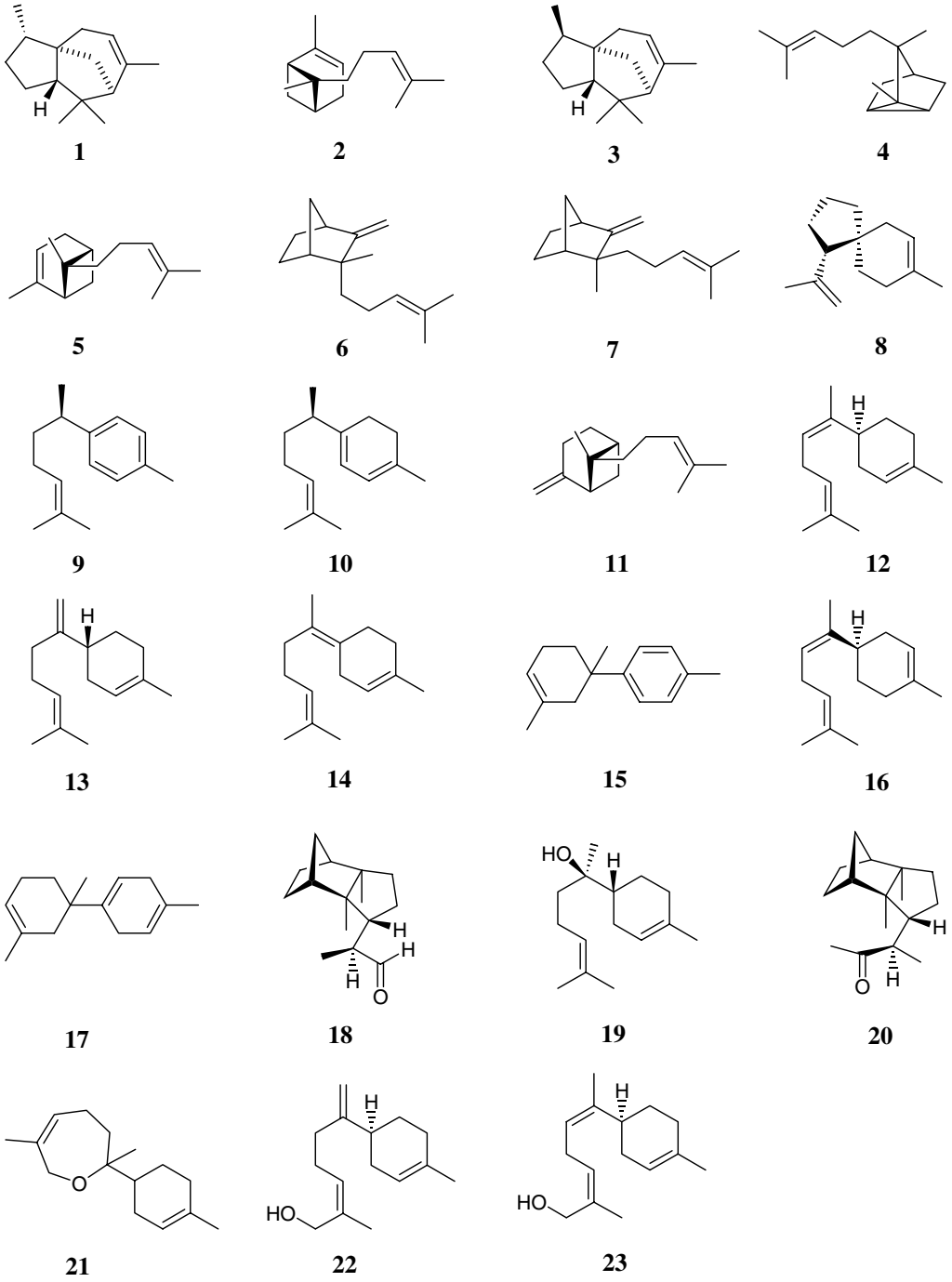
0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2004.05.007

(*Z*)-γ-bisabolene (**14**), *ar* -tenuifolene (**15**), (*E*)-α-bisabolene (**16**), tenuifolene (**17**), *epi*-cyclosantalal (**18**, 5.9%), (−)-*epi*-α-bisabolol (**19**, 5.1%), cyclosantalal (**20**, 2.6%), lanceoloxide (**21**, 3.9%), (*S*)-(*Z*)-lanceol (**22**, 18%) and 2,(7*Z*,10*Z*)-bisabolatrien-13-ol (**23**, 5.6%) (Scheme 1).

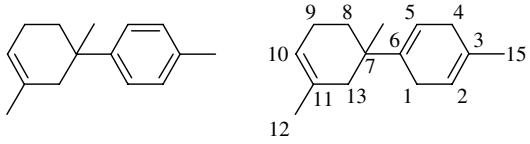
2.1. Tenuifolene (*17*) and ar-tenuifolene (*15*)

After the separation of the sesquiterpene hydrocarbon fraction by column chromatography (pentane), two new sesquiterpenes (**15** and **17**, Scheme 2) could be isolated by repetitive preparative GC using packed columns with different modified cyclodextrins as chiral stationary phases (Hardt and König, 1994).

The mass spectrum of **17** which is only present in 1% of the volatile constituents exhibits a molecular ion signal at *m*/*z* 202. According to the 22 proton and 15 carbon signals, the molecular composition is $C_{15}H_{22}$. The ^{1}H NMR shows three methine protons (δ 5.31, *m*; 5.43, *m*; 5.45, *m*). Together with the ^{13}C NMR showing



Scheme 1.



15 **17**

Scheme 2.

three olefinic double bonds and considering the elemental composition a, bicyclic system had to be assumed. In the ^{13}C NMR three primary (δ 22.8, 23.8 and 25.4), five secondary (δ 23.2, 25.6, 31.8, 32.0 and 41.3), three tertiary (δ 116.5, 119.3 and 119.9) and four quaternary carbons (δ 37.0, 130.9, 132.6 and 140.5) were found. The connectivity of the different groups was established by ^1H–^1H COSY, HMQC and HMBC spectra and leads to the constitution of **17**. A list of all important ^1H–^{13}C long-range HMBC couplings is given in Table 1.

Tenuifolene (**17**) is a new sesquiterpene hydrocarbon with a new sesquiterpene backbone that can formally be derived from bisabolene.

The second isolated hydrocarbon **15** shows a molecular ion signal at m/z 200. Together with the NMR data, a tenuifolene backbone could be established. Unlike tenuifolene, **15** shows two downfield shifted doublets at 7.11 and 7.20 ppm ($J = 13.1$ Hz) corresponding to four aromatic protons. This leads to the *para*-substituted aromatic structure of *ar*-tenuifolene (**15**).

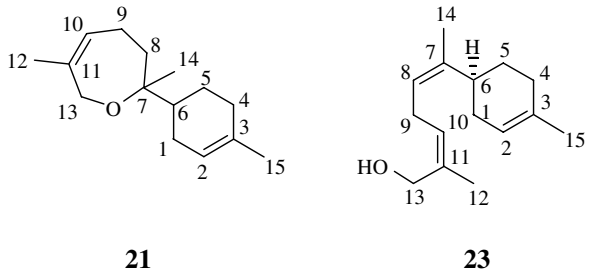
*2.2. 2,(7Z,10Z)-Bisabolatrien-13-ol (**23**) and lanceoloxide (**21**)*

From the oxygenated fraction of the essential oil five compounds (that could not be identified by GC–MS) were isolated by preparative GC as described above. Three of the compounds were the already known sesquiterpenes *epi*-cyclosantalal (**18**), (−)-*epi*-α-bisabolol (**19**) and the main constituent (*S*)-(*Z*)-lanceol (**22**). In addition, two unknown constituents **21** and **23** could be isolated (Scheme 3).

Compound **21** shows a molecular ion signal at m/z 220. Together with the NMR data this leads to the molecular composition $C_{15}H_{24}O$ and a bicyclic system can be assumed. The ^1H NMR shows three singlets of

Table 1
Important ^1H–^{13}C long-range HMBC couplings of tenuifolene (**17**)

Carbon	Hydrogen
C-3	H-15
C-6	H-1, H-4 and H-8
C-7	H-2, H-8
C-8	H-9, H-14
C-12	H-10, H-11 and H-13
C-15	H-2, H-4

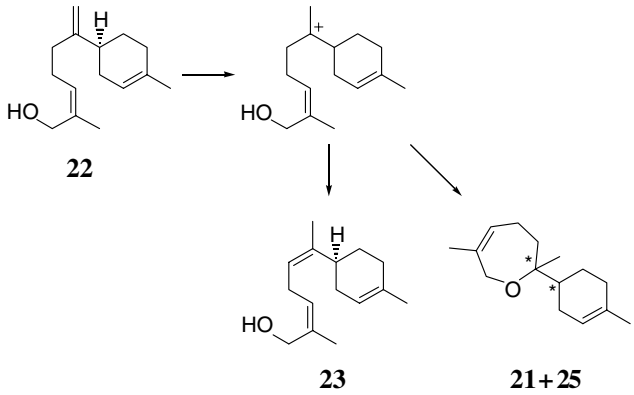


21 **23**

Scheme 3.

methyl groups connected to quaternary carbons (δ 1.05, 1.57 and 1.65). The strong downfield shift of two of them indicates that they are connected to a double bond. The two downfield shifted doublets δ 3.84 (H-13a) and 4.18 (H-13b, $J = 17.2$ Hz) were assigned to a diastereotopic allylic oxymethylene moiety. Information from $^1H^1H$ COSY, HMQC, HMBC in addition to the ^{13}C NMR and DEPT suggested structure **21** with a bisabolene backbone and a cyclic ether derived from (*S*)-(*Z*)-lanceol (**22**). The structure could be proved by the rearrangement reaction as described below. The absolute configuration has not been determined yet.

As the ^1H NMR data of **23** resemble those of (*S*)-(*Z*)-lanceol (**22**) with known absolute configuration (Ruegg et al., 1966) a related structure could be assumed. Compound **23** shows a molecular ion signal at m/z 220. A typical loss of water leads to a fragment ion at m/z 202. This together with the information from $^1H^1H$ COSY, HMQC, HMBC and in addition to the ^{13}C NMR and DEPT data suggested structure **23**. The configuration of the C-10,C-11 double bond was determined by a $^1H^1H$ NOESY experiment. A cross signal between H_2-9 (δ 2.78) and the CH_2OH group at C-13 (δ 4.16) established the (*Z*)-configuration. The structure could be proven by a chemical transformation (Scheme 4). Acid catalyzed rearrangement of (−)-(*S*)-lanceol resulted in the formation of **23**, hence establishing the (6*S*)-configuration.



22 **23** **21+25**

Scheme 4.

2.3. Rearrangement of **22**

A sample of **22** was treated with the acidic ion exchange resin Amberlyst 15 (Bülow and König, 2000) and the course of the reaction monitored by GC–MS. After 10 min **21** and **23** and several unknown sesquiterpenes could be detected. This shows that **21** and **23** could derive from the main constituent (−)-(*S*)-lanceol. After 1 h, **22** had almost disappeared and the reaction resulted in a complex mixture of rearrangement and dehydration products.

3. Experimental

3.1. General experimental procedures

3.1.1. 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, 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 approx. 1:30; FID; carrier gas 0.5 bar H_2; injector and detector temperatures were 200 and 250 °C, respectively.

3.1.2. Preparative GC

Modified Varian 1400 and 2800 instruments, equipped with stainless steel columns (1.85 m × 4.3 mm) with 10% polydimethylsiloxsane SE-30 on Chromosorb W-HP or with 2.5% octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin 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 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.

3.1.3. GC–MS

Electron impact (70 eV) and chemical ionization (NH_3) GC–MS was carried out using a Hewlett–Packard HP 5890 gas chromatograph coupled with a VG Analytical 70-250S magnetic field mass spectrometer.

3.1.4. NMR spectroscopy

NMR spectra were recorded with either a Bruker WM 400 or a Bruker WM 500 instrument in C_6D_6 and/or $CDCl_3$ using TMS as internal standard.

3.1.5. Polarimetry

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

3.1.6. Thin layer chromatography

Thin layer chromatography was effected using glass or aluminium supported plates of silica 60 F_{254} (Merck). An ethanolic solution of sulfuric acid (10%) and anisaldehyde was used as spray reagent.

3.1.7. Acidic rearrangement

It was carried out with ca. 1 mg of sample in 0.5 ml of dry hexane and 1 mg of Amberlist® 15. Samples were taken after 10 min and 1 h, respectively, and analyzed by GC–MS.

3.2. Plant material and essential oil

The essential oil of *O. tenuifolia* is commercially available and was obtained from Paul Kaders GmbH, Hamburg, where it was prepared by hydrodistillation of the dry wood.

3.3. Isolation of single constituents from the essential oil

All isolations were carried out using SE30- and /or SE-52-columns combined with at least one cyclodextrin phase column.

3.4. Tenuifolene (**17**)

4-(1,3-Dimethylcyclohexenyl)-1-methyl-1,4-cyclohexadiene; colorless oil; $RI_{CPSIL5} = 1570$; sense of optical rotation (chloroform): (−); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.95 (*s*, 3H, H-14), 1.42 (*m*, 1H, H-8a), 1.60 (*m*, 1H, H-8b), 1.65 (*s*, 3H, H-12), 1.66 (*s*, 3H, H-15), 1.74 (*d*, 1H, H-13a, $J = 12.9$ Hz), 1.95 (*m*, 2H, H-9), 2.08 (*d*, 1H, H-13b, $J = 12.9$ Hz), 2.67 (*m*, 2H, H-4), 2.61 (*m*, 2H, H-1), 5.31 (*m*, 1H, H-10), 5.43 (*m*, 1H, H-2), 5.45 (*m*, 1H, H-5); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 22.8 (*q*, C-15), 23.2 (*t*, C-9), 23.8 (*q*, C-12), 25.4 (*q*, C-14), 25.6 (*t*, C-4), 31.8 (*t*, C-8), 32.0 (*t*, C-1), 37.0 (*s*, C-7), 41.3 (*t*, C-13), 116.5 (*d*, C-5), 119.3 (*d*, C-2), 119.9 (*d*, C-10), 130.9 (*s*, C-3), 132.6 (*s*, C-11), 140.5 (*s*, C-6); MS (EI, 70 eV): *m*/*z* (rel. int.) 202 [M^+](85), 187 (27), 173 (25), 159 (45), 145 (35), 132 (62), 119 (100), 105 (60), 91 (65), 77 (40), 67 (35), 63 (5), 53 (25), 41 (50).

3.5. ar-Tenuifolene (**15**)

4-(1,3-Dimethylcyclohexenyl)-1-methylbenzene; colorless oil; $RI_{CPSIL5} = 1528$; sense of optical rotation (chloroform): (−); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.23 (*s*, 3H, H-14), 1.65 (*m*, 2H, H-9), 1.66 (*m*, 1H, H-8a), 1.72 (*d*, 3H, H-12), 1.84 (*m*, 1H, H-8b), 2.00 (*m*, 1H, H-13a), 2.25–2.31 (*m*, 1H, H-13b), 2.32 (*s*, 3H, H-15), 5.34 (*m*, 1H, H-10), 7.11 (*d*, 2H, H-2, H-4, $J = 13.1$ Hz), 7.20

(*d*, 2H, H-1, H-5, $J = 13.1$ Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 20.9 (*q*, C-15), 23.8 (*q*, C-12), 28.9 (*q*, C-14), 34.3 (*t*, C-9), 34.7 (*t*, C-8), 36.6 (*s*, C-7), 42.6 (*t*, C-13), 120.5 (*d*, C-10), 132.8 (*s*, C-11), 134.9 (*s*, C-3), 146.5 (*s*, C-6), 125.6 (*d*, C-1), 125.6 (*d*, C-5), 128.7 (*d*, C-2), 128.7 (*d*, C-4); MS (EI, 70 eV): *m*/*z* (rel. int.) 200 [M^+] (18), 185 (2), 178 (1), 171 (1), 165 (1), 157 (2), 151 (1), 141 (1), 132 (100), 117 (20), 105 (8), 91 (10), 77 (5), 65 (4), 53 (4), 41 (5).

3.6. Lanceoloxide (*21*)

1,5-Dimethyl-1-(4-methylhexenyl)-4-cycloheptenyl-ether; Colorless oil; $RI_{CPSIL5} = 1695$; sense of optical rotation (chloroform): (−); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (*s*, 3H, H-14), 1.25 (*m*, 1H, H-1a), 1.53 (*m*, 1H, H-1b), 1.57 (*s*, 3H, H-12), 1.65 (*s*, 3H, H-15), 1.80–1.90 (*m*, 1H, H-4a), 1.85 (*m*, 2H, H-8), 1.93 (*m*, 1H, H-6), 1.90–2.10 (*m*, 2H, H-5), 2.05 (*m*, 1H, H-9a), 2.10 (*m*, 1H, H-4b), 2.35 (*m*, 1H, H-9b), 3.84 (*d*, 1H, H-13a, $J = 17.2$ Hz), 4.18 (*d*, 1H, H-13b, $J = 17.2$ Hz), 5.41 (*m*, 1H, H-10), 5.42 (*m*, 1H, H-2); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 19.2 (*q*, C-14), 21.1 (*q*, C-12), 23.2 (*t*, C-9), 23.4 (*q*, C-15), 25.5 (*t*, C-1), 25.8 (*t*, C-4), 31.4 (*t*, C-5), 38.5 (*t*, C-8); 40.8 (*d*, C-6), 64.4 (*t*, C-13), 79.1 (*s*, C-7), 121.3 (*d*, C-2), 125.2 (*d*, C-10), 132.5 (*s*, C-3), 136.4 (*s*, C-11); MS (EI, 70 eV): *m*/*z* (rel. int.) 220 [M^+](2), 202 (3), 187 (2), 173 (1), 159 (2), 145 (10), 132 (20), 125 (65), 119 (15), 107 (25), 93 (20), 79 (20), 67 (25), 55 (20), 43 (100).

3.7. 2,(7Z,10Z)-Bisabolatrien-13-ol (*23*)

Colorless oil; $RI_{CPSIL5} = 1806$; sense of optical rotation (chloroform): (−); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.49 (*m*, 1H, H-5a), 1.61 (*s*, 3H, H-14), 1.64 (*s*, 3H, H-15), 1.73 (*m*, 1H, H-5b), 1.80 (*s*, 3H, H-12), 1.85–2.01 (*m*, 4H, H-1, H-4), 2.05 (*m*, 1H, H-6), 2.78 (*t*, 2H, H-9), 4.16 (*s*, 2H, H-13), 5.11 (*t*, 1H, H-8), 5.31 (*t*, 1H, H-10), 5.39 (*m*, 1H, H-2); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 14.2 (*q*, C-14), 21.3 (*q*, C-12), 23.5 (*q*, C-15), 26.5 (*t*, C-9), 27.9 (*t*, C-5), 30.7 (*t*, C-4), 30.6 (*t*, C-1); 42.8 (*d*, C-6), 61.7 (*t*, C-13), 120.8 (*d*, C-2), 121.2 (*d*, C-8), 127.4 (*d*, C-10), 133.7 (*s*, C-3), 134.2 (*s*, C-11), 140.0 (*s*, C-7); MS (EI, 70 eV): *m*/*z* (rel. int.) 220 [M^+](2), 202 (30), 187 (10), 173 (6), 159 (10), 145 (15), 134 (40), 119 (100), 107 (60), 93 (80), 84 (75), 79 (60), 67 (40), 55 (55), 41 (60).

Acknowledgements

We gratefully acknowledge financial support of "Fonds der Chemischen Industrie". We although thank Dr. V. Sinnwell, University of Hamburg, for his support in recording the NMR spectra and Mrs. A. Meiners and Mr. M. Preusse for GC–MS measurements.

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