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AN EPOXY-TRINOREUDESMANE SESQUITERPENE FROM THE LIVERWORT *LOPHOCOLEA BIDENTATA**

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Key Word Index—*Lophocolea bidentata*; *L. heterophylla*; liverworts; sesquiterpenes; epoxy-trinoreudesmane; 4,8a-dimethyl-1,2,3,4,6,7,8,8a-octalin.

Abstract—A new epoxy-trinoreudesmane sesquiterpene, (+)-(4*S*,4a*S*,5*R*,8a*S*)-*trans*-4,8a-dimethyl-4a,5-epoxy-decalin, was isolated from the liverwort *Lophocolea bidentata* collected in northern Germany. The structure of this compound was elucidated by means of spectroscopic methods and by independent synthesis. The configuration was proved by enantioselective gas chromatography. The previously described olefinic precursor was also identified as a constituent.

INTRODUCTION

The bryophyte plant group produces a remarkable variety of rare and previously unknown natural compounds. In particular, liverworts contain a large number of structurally diverse sesquiterpenes, which are the major constituents of their essential oils [1, 2]. We now report on the isolation and structural elucidation of a new nor-sesquiterpene from a certain chemotype of *Lophocolea bidentata* (L.) Dum.

RESULTS AND DISCUSSION

The hydrodistilled fresh plant material of *L. bidentata* was analysed by GC-mass spectrometry (MS). In addition to β-barbatene, α-selinene, diplophyllolide and some minor constituents the essential oil was found to contain compound **1** as a main component. The highly fragrant compound was isolated by preparative GC. The elemental composition ($C_{12}H_{20}O$) was determined by high resolution MS analysis.

The ^{1}H NMR spectrum of **1** indicated signals due to one tertiary (δ0.97, 3H, *s*, H-10) and one secondary methyl group (δ0.69, 3H, *d*, J = 6.1 Hz, H-9) and a methine proton (δ2.95, 1H, *s*, H-5). The presence of six CH_2 groups was shown by signals at δ1.95–1.15 and 0.85. The ^{13}C NMR of **1** contained two resonances of oxygenated carbons (δ66.8, C-4a, and δ55.8, C-5), which indicated the presence of an epoxy group. Signals of the tertiary and the secondary methyl groups were observed at δ20.7 (C-10) and δ14.3 (C-9). The ^{13}C NMR exhibited one quaternary carbon resonance (δ33.9, C-8a), six CH_2 resonances (δ37.7, 34.8, 33.1, 22.8, 21.8, 15.6) and one CH-resonance (δ29.8, C-4).

The synthesis of **1** (Scheme 1) was performed according to a slightly modified procedure of Marshall and Hochstetler [3, 4], starting with a Michael addition of methyl vinyl ketone (**5**) to 2,6-dimethylcyclohexanone (**6**) and leading almost exclusively to (*E*)-2,6-dimethyl-2-(3′-oxobutyl)cyclohexanone (**7**) [5]. Base catalysed cyclization afforded octalone (**8**), which was reduced by lithium aluminium hydride to the alcohol (**9**), which was directly converted into the acetate (**10**). Reduction of **10** using lithium in ethylamine formed the octalin (**11**) as a racemate, which was resolved by preparative GC using heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin as a chiral stationary phase [6]. The enantiomers were epoxidized individually by *m*-chloroperbenzoic acid to form the diastereoisomeric epoxides, **2** and **3**, and **1** and **4**, respectively, which again were separated by preparative GC. Co-injection onto capillary columns with a chiral stationary phase proved the epoxide **1** unambiguously to be identical with the natural product. Compound **1** was proved not to be an artefact by mild extraction methods such as cold solvent extraction and supercritical fluid extraction (SFE) with CO_2. With all methods, compound **1** was found to be one of the main components.

For assigning the configuration of **1** the epoxide was reduced with lithium aluminium hydride and converted into natural (−)-(4*S*,4a*S*,8a*R*)-geosmin (**12a**). This was established by co-injection of the reduction product **12a** with synthetic (+)-(4*R*,4a*R*,8a*S*)-geosmin (**12b**) (92% ee) [7] on a capillary column with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin as illustrated in Fig. 1 [8].

*Dedicated to Professor Hans Jürgen Bestmann on the occasion of his 70th birthday.

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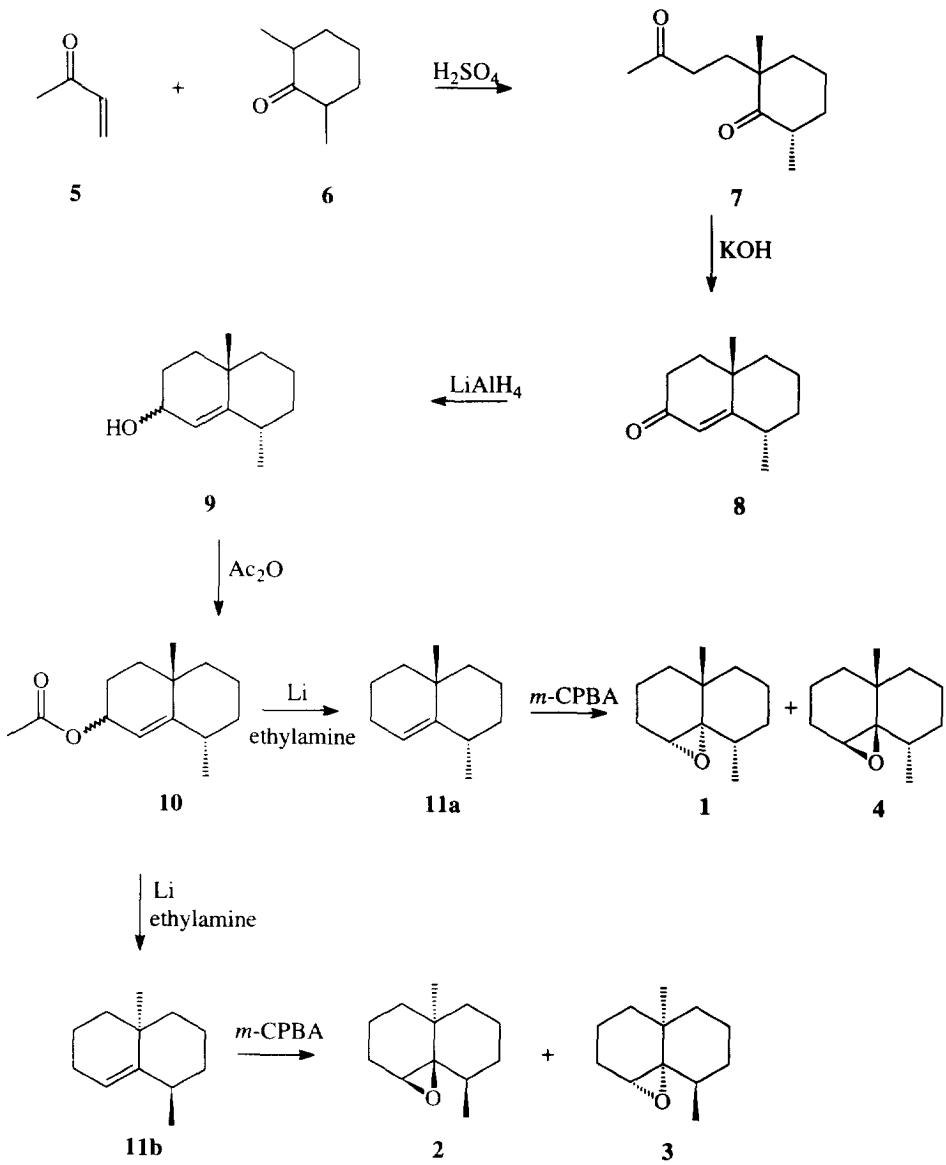

Scheme 1.

Traces of **1** were also detected in the essential oil of *L. heterophylla* (Schrad.) Dum. [9]. In addition, compound **11a**, most likely the biogenetic precursor of **1**, was identified in *L. bidentata* and *L. heterophylla*, both collected in northern Germany. The olefin **11a** has been described as a constituent of the liverwort *Bazzania fauriana* by Wu and Chang [10]. More recently it was found by Tabacchi *et al.* [11] as a constituent of *L. bidentata*. Enantiomeric separation of synthetic **11a** and **11b** by preparative GC allowed the correlation of configuration with optical rotation. GC investigations on a capillary column with the cyclodextrin derivative proved the natural olefin **11a** to be the (+)-enantiomer.

In a GC-MS investigation of the essential oil of the related liverwort *L. minor* Nees [2], collected in southern Germany, neither **1** nor **11a** were detected. In *L. bidentata*, collected from different sites in southern Germany, **1** and **11a** were not present either. This may indicate the occurrence of a specific chemotype of *L. bidentata* in northern Germany.

EXPERIMENTAL

Plant material. Northern German *L. bidentata* and *L. heterophylla* were collected in the Sachsenwald near Hamburg in March and September 1994. Southern German *L. bidentata* and *L. minor* were collected at Schwäbische Alb and Allgäu/Bavaria in September 1994. The collected liverworts are deposited in the Institut für Allgemeine Botanik, Universität Hamburg.

Hydrodistillation. The essential oils were prepared by steam distillation (2 hr) of aq. homogenates of fresh and

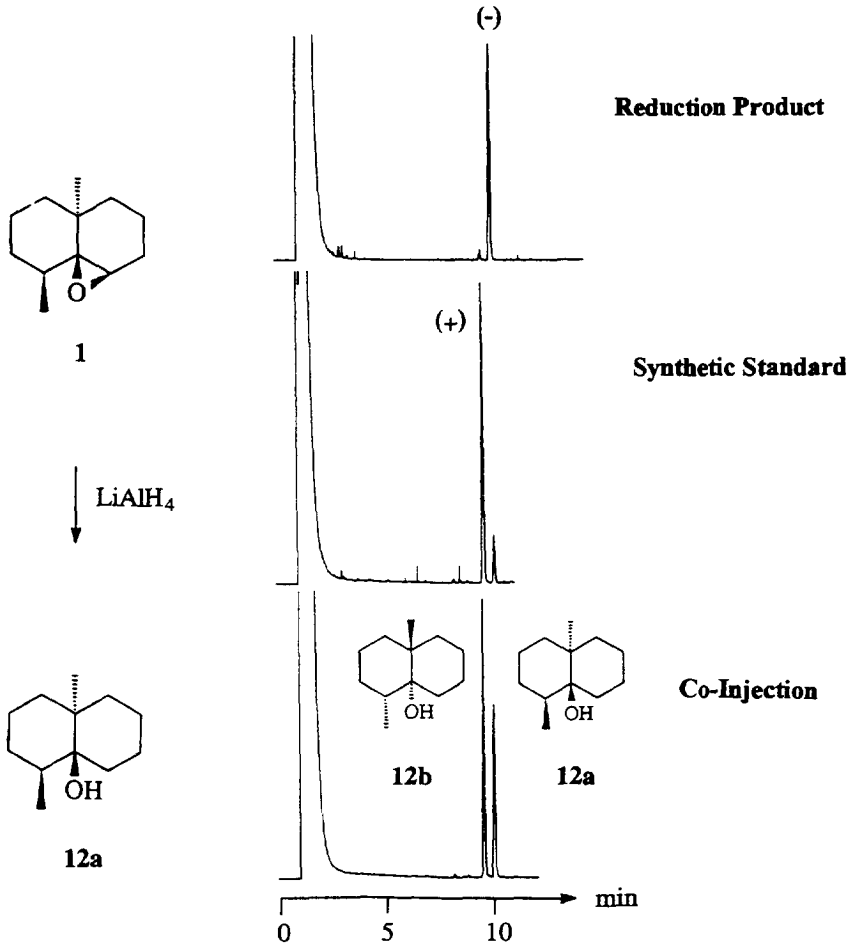

Fig. 1. Reduction of **1** to (−)-geosmin **12a** and assignment of configuration by comparison with a sample of synthetic (+)-geosmin **12b** by enantioselective GC on a 25 m capillary column with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (20% in polysiloxane OV-1701, w/w) at 120°.

green plants using *n*-hexane as collection solvent. The fresh material was not weighed.

Extraction. A cold extraction of fresh *L. bidentata* (Sachsenwald) was performed according to the method of Kubeczka [12]. SFE of fresh *L. bidentata* (Sachsenwald) was performed on a Suprex Prep Master with an Accu Trap using CO_2 at 400 atm/40° (10 min static, 50 min dynamic) and MeOH as collection solvent.

Preparative GC. Isolation of **1** and **11a** was performed by prep. GC on a Varian 1400 instrument, equipped with a stainless steel column (1.8 m × 4.3 mm) with 5% heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin–OV-1701 (1:1; w/w) (phase A) on Chromosorb W-HP. Synthetic products were isolated using a stainless steel column (Silcosteel, Amchro) (2.0 m × 5.3 mm) with 2.5% heptakis(6-*O*-dimethylthexylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin–SE-52 (20:80) (phase B) on Chromosorb G-HP; He was used as carrier gas at a flow rate of 240 ml min^{-1}.

NMR spectroscopy. NMR measurements were performed with a WM 400 (400 MHz) instrument (Bruker) using TMS as int. ståndard. In cases where diastereomeric mixtures were obtained the major diastereomer is described.

GC-MS. Electron impact (70 eV) GC-MS measurements were carried out on a Hewlett-Packard HP 5890 gas chromatograph coupled with a VG Analytical VG 70-250S mass spectrometer.

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

(+)-(4S,4*a*S,5R,8*a*S)-trans-4,8*a*-*Dimethyl*-4*a*,5-*epoxydecalin* (**1**) (*natural compound*). $[\alpha]_D^{22}$ + 55 (*c* 0.1 $CHCl_3$). ^{1}H NMR (C_6D_6): δ 2.95 (1H, *d*, *J* = 4.1 Hz), 1.95–1.15 (12H, *m*), 0.97 (3H, *s*, H-10), 0.85 (1H, *m*), 0.69 (3H, *d*, *J* = 6.8 Hz, H-9). ^{13}C NMR ($CDCl_3$): δ 66.8 (C-4a), 55.8 (C-5), 37.7 (CH_2), 34.8 (CH_2), 33.9 (C-8a), 33.1 (CH_2), 29.8 (C-4), 22.8 (CH_2), 21.8 (CH_2), 20.7 (C-10), 15.6 (CH_2), 14.3 (C-9). MS (EI, 70 eV), *m/z* (rel. int.): 180 $[M]^+$ (30), 109 (100), 81 (59), 67 (60), 55 (51), 41 (72).

(E)-2,6-*Dimethyl*-2-(3′-*oxobutyl*)*cyclohexanone* (**7**). A soln of 9.50 g (75 mmol) of **6** (mixture of stereoisomers) in 50 ml toluene was cooled under N_2 to 0°, and 5.25 g

(75 mmol) freshly distilled **5** and 1.5 ml conc. H_2SO_4 were portionally added over 1 day and left overnight. The reaction mixture was extracted with Et_2O, and the combined frs were washed once with 1M NaOH and 3 × with brine. The aq. solns were back-extracted with Et_2O, and the combined Et_2O phases were dried over $MgSO_4$. After removal of the solvent the reaction product was fractionated through a 28 cm Vigreux column (yield 2.9 g, 33% of **7**). ^{1}H NMR ($CDCl_3$): δ2.58 (1H, *m*), 2.42 (1H, *m*), 2.23 (1H, *m*), 2.12 (3H, *s*), 2.13–2.00 (2H, *m*), 1.95 (1H, *tq*, J_q = 13.2 Hz, J_t = 4.1 Hz), 1.86 (1H, *m*), 1.73–1.50 (3H, *m*), 1.32 (1H, *dq*, J_d = 4.1 Hz, J_q = 13.2 Hz), 0.99 (3H, *d*, J = 6.1 Hz), 0.98 (3H, *s*).

4,8*a*-*Dimethyl*-1,2,3,4,6,7,8,8*a*-*octal*-6-*one* (**8**). The diketone **7** (2.05 g, 10 mmol) was refluxed for 1.5 hr in a soln of 9 ml MeOH and 0.2 g KOH. To the reaction mixture 25 ml Et_2O was added and washed with water. The aq. phase was neutralized with 2% H_2SO_4 and extracted 5 × with Et_2O. The combined Et_2O phases were washed with brine and dried over $MgSO_4$. The product **8** was distilled, yielding 1.47 g (83%). ^{1}H NMR ($CDCl_3$): δ5.80 (1H, *d*, J = 1.5 Hz), 2.54–2.44 (1H, *ddd*, J_d = 6.1 Hz, J_d = 13.2 Hz, J_d = 16.8 Hz), 2.43–2.32 (1H, *m*), 2.34 (1H, *dt*, J_t = 4.6 Hz, J_d = 16.8 Hz), 1.94–1.60 (6H, *m*), 1.39 (1H, *dt*, J_t = 13.2 Hz, J_d = 4.6 Hz), 1.24 (3H, *s*), 1.16 (1H, *dq*, J_q = 12.7 Hz, J_d = 3.6 Hz), 1.07 (3H, *d*, J = 6.6 Hz).

6-*Hydroxy*-4,8*a*-*dimethyl*-1,2,3,4,6,7,8,8*a*-*octalin* (**9**). To a suspension of **8** (0.95 g, 5.3 mmol) in 5 ml dry Et_2O, 0.28 g (7.3 mmol) $LiAlH_4$ in 25 ml dry Et_2O were added and the mixture stirred for 3 hr. The reaction mixture was treated with 0.45 ml 10% NaOH and 0.55 ml H_2O and left overnight. After filtering and washing the residue with Et_2O the soln was dried over $MgSO_4$. The product **9** was purified by column chromatography on silica gel (*n*-pentane–EtOAc, 2:1) yielding 0.85 mg (81%) of the product. ^{1}H NMR ($CDCl_3$): δ5.28 (1H, *d*, J = 1.5 Hz), 4.23 (1H, *m*), 2.19 (1H, *mq*, J = 6.6 Hz), 1.91 (1H, *m*), 1.83–1.74 (1H, *m*), 1.68 (1H, *tq*, J_t = 3.6 Hz, J_q = 13.2 Hz), 1.67–1.25 (5H, *m*), 1.47 (1H, *bs*), 1.19 (1H, *dt*, J_d = 4.1 Hz, J_t = 13.2 Hz), 1.11 (3H, *s*), 0.99 (3H, *d*, J = 6.6 Hz), 0.96 (1H, *dq*, J_d = 4.1 Hz, J_q = 13.2 Hz).

6-*Acetoxy*-4,8*a*-*dimethyl*-1,2,3,4,6,7,8,8*a*-*octalin* (**10**). The alcohol **9** (0.76 g, 4.2 mmol) was dissolved in 6 ml dry pyridine under N_2, 1.73 g (17 mmol) Ac_2O was added and the mixture stirred for 24 hr. The reaction mixture was poured into H_2O and extracted with Et_2O. The combined Et_2O extracts were washed with H_2O, 2% H_2SO_4, again with H_2O and dried over $MgSO_4$. The product was purified by column chromatography on silica gel (petrol–EtOAc, 25:1) yielding 0.83 g (89%) of **10**. ^{1}H NMR ($CDCl_3$): δ5.32 (1H, *m*), 5.21 (1H, *bs*), 2.20 (1H, *mq*, J = 6.6 Hz), 2.06 (3H, *s*), 1.90 (1H, *m*), 1.83–1.41 (6H, *m*), 1.43 (1H, *dt*, J_d = 2.5 Hz, J_t = 13.2 Hz), 1.22 (1H, *dt*, J_d = 4.1 Hz, J_t = 13.2 Hz), 1.13 (3H, *s*), 0.98 (3H, *d*, J = 6.6 Hz), 0.97 (1H, *dq*, J = 4.1 Hz, J = 12.7 Hz).

4,8*a*-*Dimethyl*-1,2,3,4,6,7,8,8*a*-*octalin* (**11a**, **11b**). Compound **10** (0.52 g, 2.34 mmol) was cooled under N_2 to − 30° and then 20 ml ethylamine was condensed into the flask. Lithium powder (0.2 g, 30 mmol) was added in portions to the reaction mixture. The soln was allowed to warm up to − 5° within 2 hr. After adding 20 mg NH_4Cl the soln was warmed up to room temp. Water (5 ml) and Et_2O (5 ml) were poured into the flask and left overnight. The aq. phase was acidified with 2% H_2SO_4 to pH 4 and extracted with Et_2O. The combined Et_2O phases were washed with H_2O, 2% H_2SO_4, H_2O and brine and dried over $MgSO_4$. The product was purified by column chromatography on silica gel (hexane), yielding 0.27 g (70%) **11**. The two enantiomers were separated by prep. GC on phase A (column temp. 75° isothermal). Enantiomeric purity was proved by capillary GC, using chiral and achiral stationary phases and by optical rotation measurements. ^{1}H NMR ($CDCl_3$): δ5.29 (1H, *dt*, J_d = 2.0 Hz, J_t = 3.6 Hz), 2.18 (1H, *m*), 1.99 (2H, *m*), 1.78–1.7 (1H, *m*), 1.67 (1H, *tq*, J_q = 13.2 Hz, J_t = 3.6 Hz), 1.60–1.45 (5H, *m*), 1.35 (1H, *ddd*, J_d = 12.2 Hz, J_d = 14.8 Hz, J_d = 7.1 Hz), 1.22 (1H, *dt*, J_t = 13.2 Hz, J_d = 4.1 Hz), 1.07 (3H, *s*), 0.96 (3H, *d*, J = 6.1 Hz), 0.93 (1H, *dq*, J_q = 13.2 Hz, J_d = 4.1 Hz).

4,8*a*-*Dimethyl*-4*a*,5-*epoxydecalin* (**1**, **4**). To a soln of 5 mg (0.03 mmol) of the (+)-enantiomer **11a** in 1 ml CH_2Cl_2, *m*-chloroperbenzoic acid (85%) was added at 0° and the mixture stirred for 2 hr. The reaction mixture was washed with 2% $Na_2S_2O_3$ soln, 6 × with 10% Na_2CO_3 soln and with brine, and dried over $MgSO_4$. The yield according to GC was 98%. The diastereomers were separated by prep. GC (phase B, 110° isothermal), yielding 2 mg (73%) of each of the diastereoisomers **1** and **4**. Product **1** was identical in all spectroscopic data with those of the natural product. The optical rotation of compound **4** was $[\alpha]_D^{22}$ − 44 (*c* 0.13 $CHCl_3$). The optical rotations of the isomers **2** and **3** were $[\alpha]_D^{22}$ − 54 (*c* 0.06 $CHCl_3$) and $[\alpha]_D^{22}$ + 43 (*c* 0.07 $CHCl_3$).

(−)-(4*S*,4a*S*,8a*R*)-*Geosmin* (**12a**). To a soln of 5 mg (0.03 mmol) of the natural compound **1** in 1 ml dry THF, 1 mg (0.03 mmol) $LiAlH_4$ in 1 ml dry THF was added. After 1 hr at 60°, 1 ml H_2O was added to the cooled mixture. After concn of the soln, 1 ml $CHCl_3$ was added and the organic phase was dried over $MgSO_4$. The product was isolated by prep. GC (phase A, 120° isothermal), yielding 3.5 mg (64%) **12a** $[\alpha]_D^{22}$ −16 (*c* 0.07 $CHCl_3$), which was found to be identical to natural (−)-(4*S*,4a*S*,8a*R*)-geosmin in all spectroscopic data. The configuration was proved by co-injection of **12a** with synthetic (+)-(4*R*,4a*R*,8a*S*)-geosmin **12b** (92% ee) on a capillary column with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (20% in OV-1701, 25 m, column temp. 120° isothermal). Optical rotation measurements of **12b** were impossible because of the small amount of material available.

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REFERENCES

1. Huneck, S. and Klein, E. (1967) *Phytochemistry* **6**, 383.
2. Asakawa, Y. (1982) *Progress in the Chemistry of Organic Natural Products*, Vol. 42, p. 1. Springer, Vienna.
3. Marshall, J. A. and Hochstetler, A. R. (1965) *J. Org. Chem.* **31**, 1020.
4. Marshall, J. A. and Hochstetler, A. R. (1968) *J. Org. Chem.* **33**, 2593.
5. Still, W. C. and van Middlesworth, F. L. (1977) *J. Org. Chem.* **42**, 1258.
6. Hardt, I. and König, W. A. (1994) *J. Chromatogr. A* **666**, 611.
7. Boland, W., Huber, U., König, W. A. and Gehrcke, B. (1993) *Helv. Chim. Acta* **76**, 1949.
8. König, W. A., Gehrcke, B., Icheln, D., Dönnecke, J. and Wang, W. (1992) *J. High Resol. Chromatogr.* **15**, 367.
9. Toyota, M., Asakawa, Y. and Frahm, J.-P. (1990) *Phytochemistry* **29**, 2334.
10. Wu, C. L. and Chang, S. J. (1988) *J. Hatt. Bot. Lab.* **64**, 151.
11. Tabacchi, R., Joulain, D., Huneck, S. and Herout, V. (1992) *Proc. 12th Int. Congress of Flavours, Fragrances and Essential Oils*, Vienna, p. 125.
12. Kubeczka, K.-H. (1982) in *Ätherische Öle* (Kubezcka, K.-H., ed.), p. 116. Thieme, Stuttgart.