Terpenoids from the *in vitro* Cultured Liverwort *Riella helicophylla*[§]

Hans Becker* and Ulrike Martini

Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, D-66041 Saarbrücken, Germany. Fax: +49–681–302–2476. E-mail: pb13hb@rz.uni-sb.de

* Author for correspondence and reprint requests

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The liverwort *Riella helicophylla* was cultivated *in vitro* under aseptic conditions. The lipophilic extract of the plant material yielded seventeen monoterpenes and eleven diterpenes. Seven monoterpenes were hydroperoxides. From the diterpenes six belonged to the labdane type skeleton and one to the kaurane type, the other diterpenes were phytane derivatives.

Introduction

The liverwort *Riella helicophylla* (Borg et Montagne) Montagne is native to the western Mediterranean area (Spain, Algeria, Tunisia). It grows in saline lakes (pH 7.8–8) in a depth of about 70 cm (Müller, 1954). The propagation of the plant is either sexually by spores or vegetatively by gemmae. Because of its morphology and good regenerative power *R. helicophylla* has been the subject of many ontogenetic and physiological studies (e.g. Stange, 1977; Witt, 1992).

Little is known about the chemistry of this tiny plant. In earlier studies lunularic acid (Grotha and Schwabe, 1978) and some flavonoids (Markham *et al.*, 1976) have been detected and the GC analysis of the essential oil led to the identification of *p*-mentha-1,4(8)-diene (Buns, 1987). In continuation of our studies on *in vitro* cultured liverworts (Becker, 1994; Valcic *et al.,* 1997; Grammes *et al.,* 1997; Adam, 1999) we investigated a lipophilic extract of the plant for its chemical composition.

Results and Discussion

The liverwort was cultivated in 1 l glass cylinders covered with a glass lid. The medium was according to Viell (1980). Starting from about 250 gemmae of female plants, we obtained 260 g of dry plant material within one year. The lipophilic extract (combined ether and dichlormethane extract) was first subjected to column chromatography with Sephadex LH 20. Further fractionation was done by vacuum liquid chromatography (VLC) and HPLC.

Seven compounds from the isolated seventeen monoterpenes were already known. Their structures could be identified by comparison of their spectroscopic data with those from literature. Compound **1**, *p*-mentha-1,8-dien-4-ol, is well known for the essential oils of various plants, e.g. pepper -and spearmint (Chapman and Hall, 1996). It belongs to the *4R*- form, which could be proven by comparison of its optical rotation ($[\alpha]_D^{20}$ = + 20°) with those of the 4*R*- and 4*S*- enantiomers synthesised by Delay and Ohloff (1979). The hydroperoxides **2**, 4-hydroperoxy-*p*-mentha-1,8-dien, and **4**, 8-hydroperoxy-*p*-mentha-1,3,5-trien have been recently described by Buchanan *et al.* (1998) from the liverwort *Jungermannia obovata*. Compound **3**, *p*-mentha-1,3,5-triene-8-ol, first isolated from *Citrus reticulata* (Kugler and Kovats, 1963) is new for liverworts. The spectroscopic data from **6** were in agreement with *p*-mentha-1,3,5-triene-2,8-diol, first isolated from *Lavandula gibsonii* (Patwardhan and Gupta, 1983). **13**, *p*-menth-2-en-1α, 4β, 8- triol, was known from *Asiasari radix* (Yahara *et al.,* 1990) and the tris nor monoterpenoid **16**, 4-hydroxy-4-methyl-cyclohex-2-en-1-on, a degradation product of the hydroperoxide ascaridole had been isolated by Connolly (1990) for the first time.

Among the diterpenes 16-kaurene (**18**) was identified by GC-MS and compared with literature data (Stenhagen *et al.,* 1974). It has been previously described in various liverwort species (Asakawa, 1995). A second diterpene hydrocar-

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bon, labda-8(17),13(16),14-triene (**19**) was identified through its ^{1}H-NMR spectrum in comparison with the respective 19-carboxy acid (Carman and Deeth, 1971. 8α,15-dihydroxy-13- labdene (**20**) and 3β, 8α,15-trihydroxy-13- labdene **21** were characterised through their ^{1}H NMR and ^{13}C NMR data (Forster *et al.,* 1985). The 3β-acetoxy derivative (**22**) and the 3β,15-diacetoxy derivative (**23**) of **21** were found to be new natural products. The ^{1}H NMR and ^{13}C NMR data of **24** were identical with 19-acetoxy-8α,15-dihydroxy-13-labdene previously isolated from *Juniperus sabina* (Feliciano *et al.,* 1991). The following phytane derivatives were detected and characterised as 2-phyten-1-ol (**25**), (2*E*, 2′*E*)-phyt-2′-enyl phyt-2-enoate **26** (Spörle *et al.,* 1991), (2*E*)-phyt-2-enyl phytanoate **27** (Buchanan *et al.,* 1995) and (2*E*)-phyt-2-enyl hexadecanoate **28** (Rasool *et al.,* 1991).

Compound **5** showed on TLC a positive reaction with each of three hydroperoxide specific spray reagents (Rieche and Schulz, 1958; Abraham *et al.,* 1957; Huber and Fröhlke, 1972). Its ^{1}H NMR revealed the signals of a 2,5 disubstituted cyclohexa-1,4-diene (δ_H 5.77, H-2, and δ_H 5.45, H-5, both *br s*; δ_H 2.68, *m*, 4H, 2H-3 and 2H-6), three singlet methyls of which one (δ_H = 1.66, 3H-7) is linked to a double bond and two (δ_H 1.33, 6H, 3H-9 and 3H-10) belong to a dimethyl carbinol and one proton at δ_H 7.48 (*s*), exchangeable with D_2O. On the basis of the above evidence compound **5** is 8-hydroperoxy-*p*-mentha-1,4-diene. **5** is not very stable. After a few days in solution, its ^{1}H NMR showed the presence of 8-hydroperoxy-*p*-mentha-1,3,5-trien (**4**), due to the aromatisation of the cyclohexa-1,4-diene ring. Its EIMS spectrum only showed the molecular ion peak of the dehydrated product at *m/z* =166 (4%) with a base peak at *m/z* = 133 which is indicative for a loss of an OOH group.

Compound **7**, 8-hydroperoxy-*p*-mentha-1,3,5-trien-2-ol, also gave a positive reaction with the hydroperoxide spray reagents. Its ^{1}H NMR spectrum was nearly identical with that of *p*-mentha-1,3,5-triene-2,8-diol (**6**), however an additional proton signal appeared at δ_H 7.27, indicating a hydroperoxide group. The position of the hydroperoxide at C-8 could easily be concluded from its ^{13}C NMR spectrum, in which C-8 appears at δ_C 83.8, in good accordance to the chemical shift of the hydroperoxyl substituted C-8 in **5** (see Table I).

Compound **8** was obtained as a colourless oil. The molecular formula, $C_{10}H_{16}O_3$, was determined by DCI mass spectrometry ($[M+H]^+$ *m/z* 185). Its ^{1}H and ^{13}C NMR spectra (Table I) revealed a derivative of the known monoterpene peroxide ascaridole (Nitz *et al.* 1989; Bohlmann and Zeisberg, 1974) with an additional hydroxyl group at C-8 (δ_C 72.4, *s*, C-8; δ_H 1.28, 6H, *s*, 3H-9 and 3H-10). Therefore **8** could be deduced as 8-hydroxyascaridole.

The structure of compound **9** followed immediately from the comparison of its ^{1}H and ^{13}C NMR spectra with those of **8**. The downfield shift of C-8 (δ_C 83.9, $\Delta\delta_{C\text{-}8}$ 11.8) in the ^{13}C NMR spectrum and the appearance of a singlet at δ_H 8.18, exchangeable with D_2O, in the ^{1}H NMR spectrum proved **9** to be 8-hydroperoxyascaridole. The DCI mass spectrum with ions at *m/z* 201 ($[M+H]^+$) and *m/z* 168 ($[M+H]^+$-OOH) supported the proposed structure.

Table I. ^{13}C NMR spectral data for compounds **5, 7–11, 14,** ($CDCl_3$).

C	5	7	8	9	10	11	14
1	130.6 s	123.0 s	74.7 s	74.7 s	140.5 s	72.1 s	73.5 s
2	120.7 d	154.0 s	136.4 d	136.4 d	122.3 d	75.0 d	130.3 d
3	25.8 t	112.3 d	132.8 d	132.4 d	65.2 d	125.5 d	136.5 d
4	137.2 s	143.8 s	82.0 s	82.8 s	67.6 s [a]	138.1 s	74.5 s
5	118.8 d	117.8 d	23.7 t	24.3 t	22.8 t	33.7 t	33.5 t
6	31.7 t	131.2 d	29.5 t	29.3 t	30.2 t	24.7 t	26.9 t
7	22.7 q	15.3 q	21.2 q	21.1 q [a]	23.2 q	20.6 q [a]	29.8 q
8	84.2 s	83.8 s	72.4 s	83.9 s	64.0 s [a]	142.5 s	66.7 s
9	23.8 q	26.1 q	24.8 q [a]	21.1 q [a]	21.1 q [b]	112.6 t	24.7 q [a]
10	23.8 q	26.1 q	25.2 q [a]	20.3 q [a]	20.9 q [b]	20.8 q [a]	24.4 q [a]

All assignments were confirmed by DEPT measurements.
[a,b] Values may be interchanged within the same column.

EIMS of **10** ($[M]^+$ at *m/z* 168) led to the molecular formula $C_{10}H_{16}O_2$. The ^{13}C NMR spectrum displayed resonances for ten carbons, including a trisubstituted double bond (δ_C 140.5, *s*, C-1; δ_C 122.3, *d*, C-2) two oxygenated quaternary (δ_C 67.6 and 64.0, C-4 and C-8) and one oxygenated secondary carbon (δ_C 65.2, C-3), two methylenes and three methyl groups. The ^1H NMR spectrum, recorded in $CDCl_3$, revealed the presence of a vinyl methyl group (δ_H 1.71, 3H-7) and two singlet methyls (δ_H 1.35, 6H, 3H-9 and 3H-10) of a geminal dimethyl carbinol. The vinyl proton (δ_H 5.52, H-2) shows a coupling (*J* 4.9 Hz) to the oxygen bearing methine at δ_H 3.90 (H-3). It is evident from these spectral data that **10** is a trioxygenated *p*-menth-1-ene with one hydroxyl and one ether or epoxide function located at C-3, C-4 or C-8. The position of the hydroxyl group could be taken

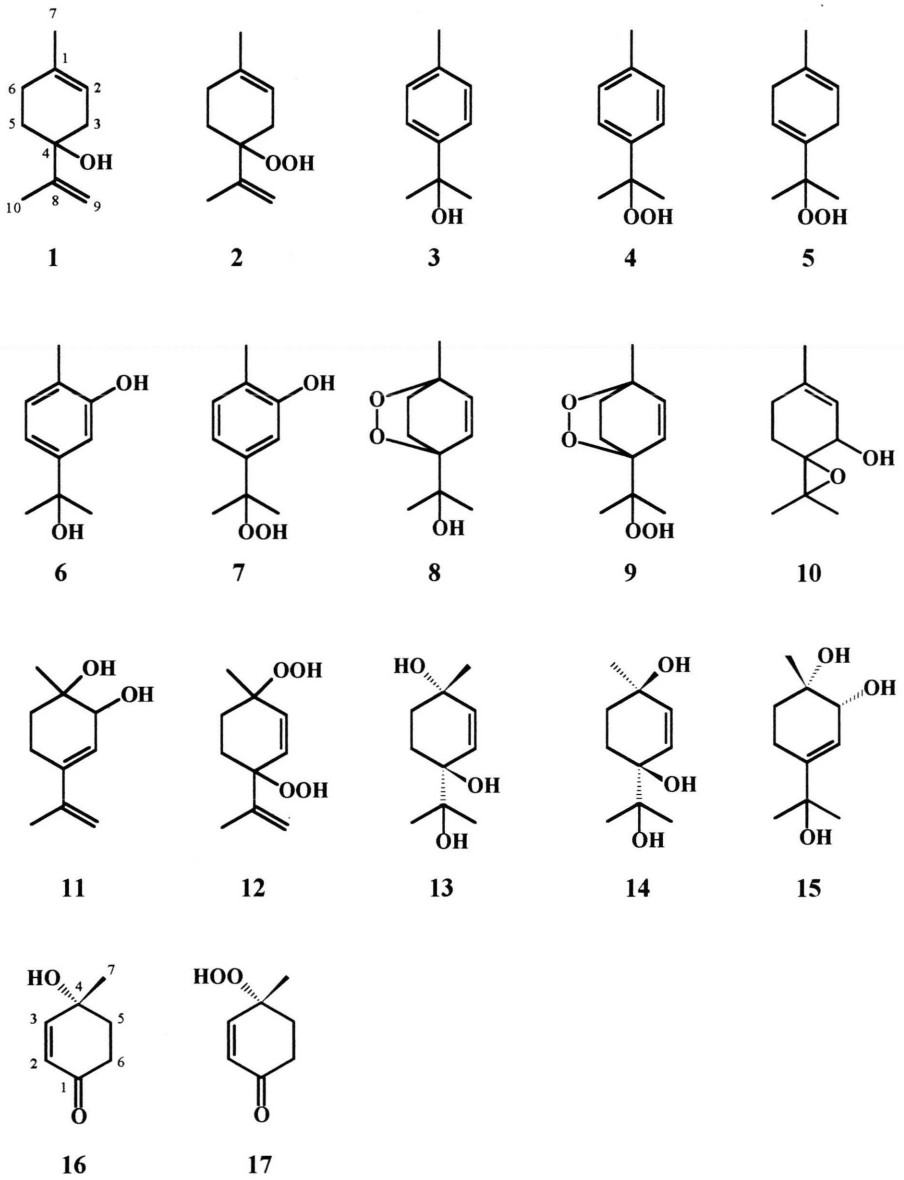

Fig. 1. Monoterpenoids from *R. helicophylla*.

from the ^1H spectrum recorded in acetone-d_6, where the OH proton was visible (δ_H 3.40, *br s*) and showed a weak coupling to H-3 (δ_H 3.82, *br d*) in the H-H-COSY. Consequently C-4 and C-8 belong to an epoxide and **10** therefore represents 4,8-epoxy-3-hydroxy-*p*-menth-1-ene. Because of its instability the relative configuration of **10** remains undetermined.

The molecular formula of **11** ($[M]^+$ at m/z = 168) was determined by EIMS. Its ^1H and ^{13}C NMR spectra displayed one exomethylene, one trisubstituted double bond, a secondary and a tertiary alcohol, two methylenes together with a vinyl methyl and a methyl carbinol. This assumption led to a derivative of *p*-mentha-3,8-dien-1-ol with an additional hydroxyl group. Its position at C-2 followed immediately from its multiplicity and that of the vinyl proton H-3 which both appeared as broad singlets in the ^1H NMR spectrum indicating that they are vicinal neighbours with a dihedral angle of ca 90°. Due to the instability of **11** the relative configuration of *p*-mentha-3,8-dien-1,2-diol could not be determined.

Compound **12** gave positive reactions with the peroxide reagents. The compound was unstable, however the structure could be determined from its ^1H NMR spectrum, which gave rise to a monoterpene with a *p*-menthane skeleton. At δ_H = 7.38 and δ_H 7.34 two peroxide protons appear as singlets. The chemical shift of the methyl group H-7 (δ_H = 1.33, 3H, *s*) places one peroxide at position C-1. The signals at δ_H = 5.04 and δ_H = 5.07 (H-9a and H-9b, both *s*) belong to an exomethylene which is part of an isopropylidene group. The corresponding methyl group appears at 1.83 ppm (H-10, 3H, *s*). The remaining signals reveal two methylene groups (δ_H = 2.01 and 1.80, both 2H, both *m*, 2H-5 and 2H-6) and a *cis* configured double bond (δ_H = 6.03 and 5.89, both *d*, H-2 and H-3, $J_{2,3}$ = 10.3 Hz). These data can only be correlated with 1,4-dihydroperoxy-*p*-mentha-2,8-diene.

MS and ^1H NMR spectra of **14** are nearly identical to those of *p*-menth-2-en-1α, 4β, 8- triol (**13**), however the optical rotation of both compounds are quite different (**14**: $[\alpha]_D^{20}$ = + 50°; **13**: $[\alpha]_D^{20}$ = + 2.3°) and the ^{13}C NMR shifts of C-2, C-3, C-6, C-7 and C-8 differ about 2–3 ppm. These facts indicate that **14** is a diastereomer of 13. The hydroxyl groups at C-1 and C-4 in **14** should be *cis* orientated in contrast to **13** were they are *trans* .

The ^1H NMR data of **15** led to *p*-meth-2-en-1α, 2α, 8-triol. Its 2α-acetyl derivative had already been described from *Asiasari radix* (Yahara *et al.* 1990). The *cis* orientation of the hydroxyls at C-1 and C-2, based on the correlation between methyl H-7 and H-2 in the NOESY spectrum, is in agreement with the published structure.

Apart from the known 4-hydroxy-4-methyl-cyclohex-2-en-1-on **16** (Connolly, 1990) we found a similar compound **17** as a minor component. In contrast to **16,** compound **17** gave a positive reaction in our peroxide tests and its ^1H NMR showed an additional hydroperoxy proton at δ_H = 7.68 (*s*). Therefore **17** is 4-hydroperoxy-4-methyl-cyclohex-2-en-1-on.

It is known that terpene hydroperoxides are formed from unsaturated terpenes in the presence of light, oxygen and chlorophyll. Therefore the question arose if the isolated products were genuine or artefacts. To test this fresh plant material was extracted with dichlormethane in the dark and chlorophyll omitted by gelfiltration with Sephadex LH 20. Fractions containing monoterpenes were chromatographed on TLC silica plates with n-hexane/ethylacetate (80:20 v/v) together with the isolated compounds. The plates were sprayed with the peroxide reagents mentioned above. The test proved that the peroxides were present in the extract. A further evidence that the peroxides are genuine. is the amount isolated in relation to the respective alcohol. E.g. 4-hydroperoxy-*p*-mentha-1,8-dien (**2**) was isolated in an amount of 80 mg compared to 3 mg of *p*-mentha-1,8-dien-4-ol (**1**).

Experimental

Plant material

Gemmae of female plants from *Riella helicophylla* (Borg et Montagne) Montagne were kindly provided by Prof. Stange, Kassel, Germany and cultivated aseptically in 1 l glass cylinders covered with a glass lid. The medium was according to Viell (1983). The plants were kept for 4 to 5 weeks in 2000 lux 12 h/12 h dark at 20 °C. The plants were harvested separately from the gemmae and dried at room temperature with a fan. The gemmae were used as seed material for new cultures. The plant material was stored at −15 °C before extraction. A voucher specimen is retained in the department of Pharmacognosy and Analytical Phytochemistry, University of Saarland, Saarbrücken, Germany.

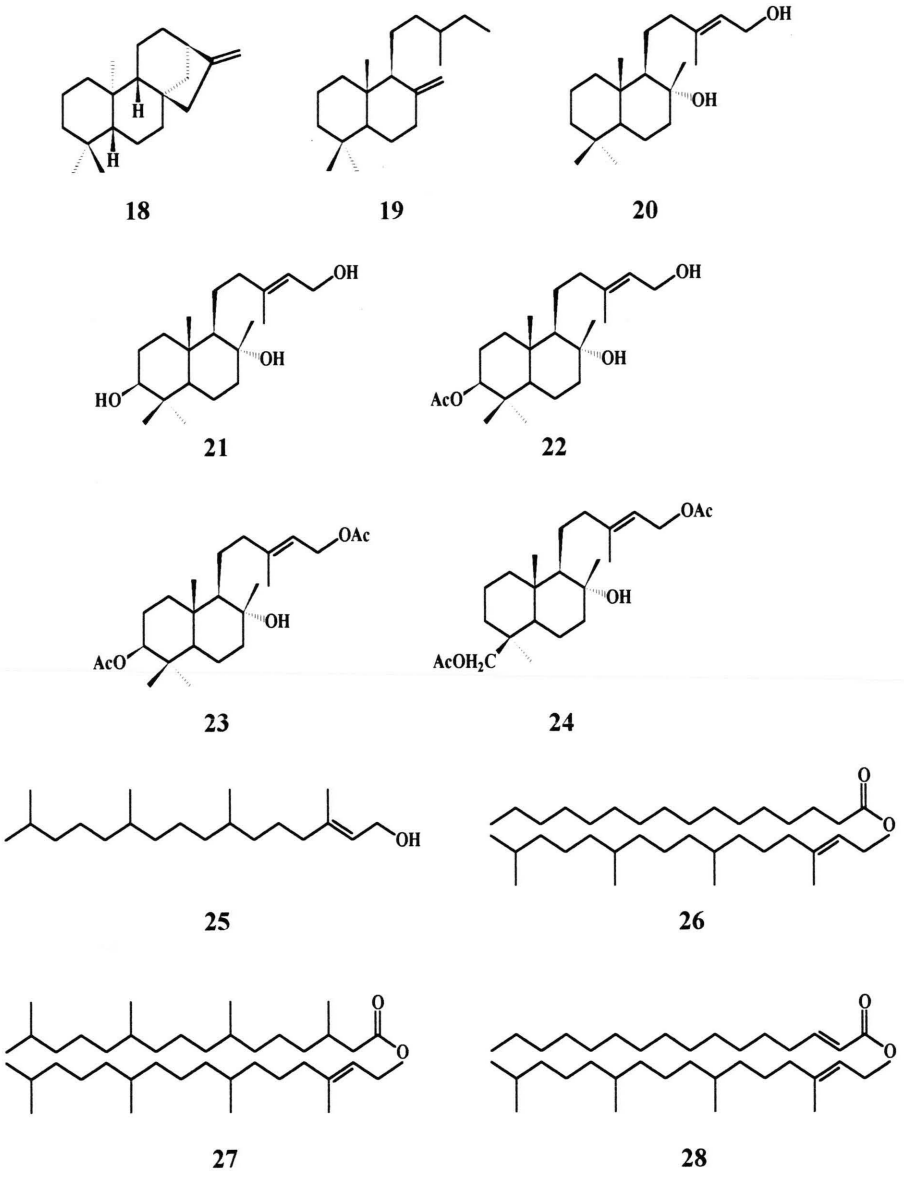

Fig. 2. Diterpenoids from *R. helicophylla*.

Extraction and isolation

General: All HPLC separations were performed isocratic. The composition of the mobile phase is given as v/v (in parenthesis).

260 g of *R. helicophylla* were pulverised and successively extracted with diethyl ether and CH_2Cl_2. Since the diethyl ether and the CH_2Cl_2 extracts exhibited identical TLC and HPLC chromatograms, they were combined to yield 15.8 g crude lipophilic extract. This extract was subjected to SEC on Sephadex LH 20 using CH_2Cl_2/MeOH (1:1) as mobile phase to give five main fractions (I-V). Fraction I mainly consisted of chlorophyll, carotenoids and fat. Fraction II was rechromatographed under the same conditions to yield a chlorophyll free fraction. This fraction was subjected to VLC on silica gel in a n-hexane/ethyl acetate gradient (0–100%) to yield 10 subfractions II.2.1–10. Fraction II.2.1, containing the hydrocarbons,

was separated by HPLC on silica gel (100% n-hexane) to yield **19** (2 mg). GC-MS of the same fraction led to **18**. HPLC of fraction II.2.2 (silica gel, n-hexane/EtOAc 99.5/0.5) resulted in **26** (10 mg), **27** (3.3 mg) and **28** (2.4 mg). Fraction II.2.4 was found to be pure **25** (252 mg). HPLC of fraction II.2.6 (silica gel, n-hexane/EtOAc 98/2) gave **10** (18.5 mg) and **23** (3.5 mg). Fraction II.2.7 was separated on DIOL modified silica gel via HPLC (n-hexane/EtOAc 75/25) to give **20** (123.5 mg). HPLC on CN modified silica gel led for fraction II.2.8 (n-hexane/EtOAc 75/25) to **22** (126.5 mg) and **24** (62 mg) and for fraction II.2.9 (n-hexane/EtOAc 70/30) to **21** (46.5 mg). Fraction III was further separated by VLC (silica gel, n-hexane/ethyl acetate gradient, 0–100% EtOAc) to give **3** (44.5 mg) and 7 subfractions III.1–7. Fraction III.1 upon HPLC (silica gel, n-hexane/EtOAc 95/5) gave **1** (3 mg), **2** (80 mg) **4** (20.5 mg) and **5** (4 mg). Fraction III.3 was almost pure and gave **9** (23 mg) after HPLC on silica gel (n-hexane/EtOAc 80/20). Fraction III.4 was purified by HPLC (Si, n-hexane/EtOAc 75/25) and gave rise to **7** (2 mg), **8** (6 mg), **12** (3 mg) and **17** (1 mg). Fraction III.5 on HPLC (Si, n-hexane/EtOAc 70/30) gave **6** (2 mg). Fraction III.6 was separated with HPLC on CN modified silica gel (n-hexane/EtOAc 85/15) and gave rise to **11** (3 mg) and **16** (7.5 mg). HPLC on DIOL modified silica gel led for fraction III.7 (n-hexane/EtOAc 50/50) to **14** (3 mg) and for fraction III.8 (n-hexane/EtOAc 50/50) to **13** (4 mg) and **15** (2.5 mg).

Spectroscopic methods

NMR-spectroscopy: BRUKER AM 400, $CDCl_3$, ambient temperature, 400 MHz (^1H), 100 MHz (^{13}C); chemical shifts are given in δ values (ppm) relative to $CHCl_3$ at δ_H 7.24 or $CDCl_3$ at δ_C 77.0 and acetone-d6 at δ_H 2.04. mass spectrometry: VARIAN MAT 311 (DCI); GC-MS (EIMS) was performed on a HP-1 capillary column with a G 1800A GCD (HP).

Spectroscopic data

8-Hydroperoxy-p-mentha-1,4-diene (**5**): colourless oil, ^1H NMR ($CDCl_3$): δ_H 7.19 (s, OOH), 5.77 and 5.45 (both br s, H-2 and H-5), 2.68 (m, 2H-3 and 2H-6), 1.66 (s, 3H-7), 1.33 (s, 3H-9 and 3H-10); ^{13}C NMR: see Table I; EIMS *m/z* (rel. int.) = 166 (4) $[M-2H]^+$, 135 (76), 133 (100), 119 (86), 115 (35), 105 (89), 93 (56), 91 (85), 77 (30), 65 (54).

8-Hydroperoxy-p-mentha-1,3,5-trien-2-ol (**7**): colourless oil, ^1H NMR ($CDCl_3$): δ_H 7.27 (s, OOH), 7.10 (d, *J* 7.8 Hz, H-6), 6.91 (dd, *J* 7.8 and 1.7 Hz, H-5), 6.88 (d, *J* 1.7 Hz, H-3), 2.22 (s, 3H-7), 1.56 (s, 3H-9 and 3H-10); ^{13}C NMR: see Table I; EIMS, *m/z* (rel. int.) = 182 (8) $[M]^+$, 165 (42), 149 (100), 148 (24), 135 (7), 121 (12), 109 (5), 91 (3), 77 (4).

8-Hydroxyascaridole (**8**): colourless oil, $[\alpha]_D^{20}$ = +3.2° (*c* = 0.5);^1H NMR ($CDCl_3$): δ_H 6.66 (d, *J* 8.6 Hz, H-3), 6.42 (d, *J* 8.6 Hz, H-2), 2.3–1.4 (m, 2H-5 and 2H-6), 1.38 (s, 3H-7), 1.28 (s, 3H-9 and 3H-10); ^{13}C NMR: see Table I; DCIMS, *m/z* (rel. int.) = 185 (11) $[M+H]^+$, 167 (43), 152 (100), 151 (87), 137 (85), 133 (54), 111 (43), 110 (84), 109 (100), 59 (65).

8-Hydroperoxyascaridole (**9**): colourless oil, ^1H NMR ($CDCl_3$): δ_H 8.18 (s, OOH), 6.65 (d, *J* 8.6 Hz, H-3), 6.42 (d, *J* 8.6 Hz, H-2), 2.3–1.4 (m, 2H-5 and 2H-6), 1.36, 1.35 and 1.31 (all s, 3H-7, 3H-9 and 3H-10); ^{13}C NMR: see Table I; DCIMS, *m/z* (rel. int.) = 201 (9) $[M+H]^+$, 183 (63), 168 (86), 167 (70), 152 (84), 151 (98), 149 (77), 136 (82), 135 (100), 133 (88), 109 (74).

4,8-Epoxy-3-hydroxy-p-menth-1-ene (**10**): colourless oil, $[\alpha]_D^{20}$ = −0.5° (*c* = 1.5);^1H NMR ($CDCl_3$): δ_H 5.52 (d, *J* 4.9 Hz, H-2), 3.90 (d, *J* 4.9 Hz, H-3), 2.27 (m, H-6_a), 2.08 (m, H-6_b), 1.71 (s, 3H-7), 1.48 (m, 2H-5), 1.35 (s, 3H-9 and 3H-10); ^1H NMR (acetone-d6): δ_H 5.50 (d, *J* 4.7 Hz, H-2), 3.82 (d, *J* 4.7 Hz, H-3), 3.40 (br s, OH), 2.26 (m, H-6_a), 2.10 (m, H-6_b), 1.69 (s, 3H-7), 1.40 (m, 2H-5), 1.28 and 1.27 (both s, 3H-9 and 3H-10); ^{13}C NMR: see Table I; EIMS *m/z* (rel. int.) = 168 (57) $[M]^+$, 107 (45), 97 (60), 84 (69), 83 (57), 80 (100), 70 (52), 69 (38), 67 (67), 55 (40).

p-Mentha-3,8-dien-1,2-diol (**11**): colourless oil, ^1H NMR ($CDCl_3$): δ_H 5.72 (br s, H-3), 5.05 (s, H-9_a), 4.96 (s, H-9_b), 4.19 (br s, H-2), 2.40 (ddd, *J* 17.3, 2.3, 2.3 Hz, H-5_a), 2.29 (ddd, *J* 17.3, 8.9, 2.3 Hz, H-5_b), 1.91 (s, 3H-7), 1.20 (s, 3H-10); ^{13}C NMR: see Table I; EIMS *m/z* (rel. int.) = 168 (7) $[M]^+$, 126 (1), 125 (100), 111 (12), 110 (42), 97 (25), 83 (15), 67 (62), 68 (9), 53 (8).

1,4-Dihydroperoxy-p-mentha-2,8-diene (**12**): colourless oil, ^1H NMR ($CDCl_3$): δ_H 7.38 and 7.34 (both s, both OOH), 6.03 and 5.89 (both d, *J* 10.3 Hz, H-2 and H-3), 5.07 and 5.04 (both s, H-9_a and H-9_b), 2.01 and 1.80 (both m, 2H-5 and 2H-6), 1.83 (s, 3H-10), 1.33 (s, 3H-7).

p-Menth-2-en-1β, 4β, 8- triol (**14**): colourless oil, $[\alpha]_D^{20}$ = +50° (*c* = 0.2); ^1H NMR ($CDCl_3$): δ_H 5.95 (d, *J* 11.7 Hz, H-2), 5.81 (d, *J* 11.7 Hz, H-3), 2.15–1.65 (m, 2H-5 and 2H-6), 1.36, 1.25 and 1.17 (all s, 3H-7, 3H-9 and 3H-10); ^{13}C NMR: see Table I; DCIMS, *m/z* (rel. int.) = 185 (5) $[M+H]^+$, 169 (19), 152 (48), 151 (100), 133 (77), 123 (19), 111 (16), 110 (18), 109 (17).

p-Meth-2-en-1α, 2α, 8-triol (**15**): colourless oil, $[\alpha]_D^{20} = +8.3°$ (c = 0.25); ^{1}H NMR ($CDCl_3$): δ_H 5.71 (d, J 1.7 Hz, H-3), 3.86 (d, J 1.7 Hz, H-2), 2.28, 2.05, 1.82, 1.58 (m, H-5_a, H-5_b, H-6_a, H-6_b) 1.32 (s, 3H-9 and 3H-10), 1.19 (s, 3H-7); DCIMS, *m/z* (rel. int.) = 185 (5) $[M+H]^+$, 169 (30), 151 (100), 133 (29), 126 (35), 123 (30), 110 (47), 109 (37), 107 (51), 95 (44).

4-Hydroperoxy-4-methyl-cyclohex-2-en-1-on (**17**): colourless oil, $[\alpha]_D^{20} = -100°$ (c = 0.05); ^{1}H NMR ($CDCl_3$): δ_H 7.68 (s, OOH), 6.83 (d, J 10.3 Hz, H-3), 6.00 (d, J 10.3 Hz, H-2), 2.67 (m, H-6_a), 2.37 (m, H-6_b), 2.00 (m, 2H-5), 1.44 (s, 3H-7); DCIMS, *m/z* (rel. int.) = 143 (66) $[M+H]^+$, 127 (100), 109 (92), 98 (53), 81 (80).

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