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Two New Monoterpenoid Hydroperoxides from the Liverwort *Jungermannia obovata*†

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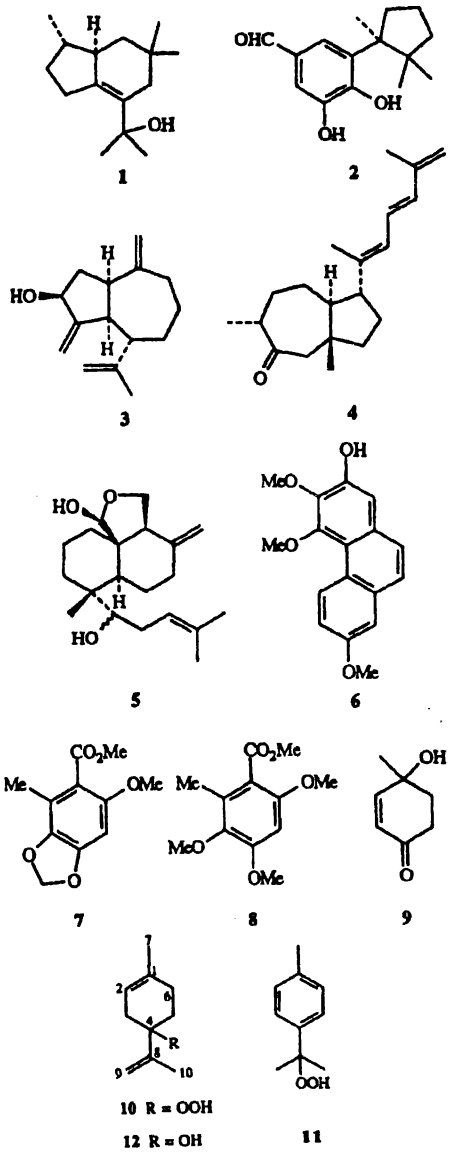
An ether extract of fresh *Jungermannia obovata* yielded two new monoterpenoid hydroperoxides, 4-hydroperoxy-*p*-mentha-1,8-diene (10) and 8-hydroperoxy-*p*-mentha-1,3,5-triene (11).

Liverworts, members of the Hepaticae class, have been the subject of considerable interest[1] over the years because of their impressive biosynthetic ability. They grow well in the cool, damp climate of Scotland. We have investigated many Scottish species and have found a wide range of sesquiterpenoids, diterpenoids and aromatic compounds. These may be exemplified by the brasilane sesquiterpenoid conocephalenol (**1**) from *Conocephalum conicum*,[2] 1,2-dihydroxyherberten-12-al (**2**) from *Herbertus aduncus*[3] and the zierane derivative saccogynol (**3**) from *Saccogyna viticulosa*.[4] Unusual diterpenoids include sphenolobanes, e.g. **4**, from *Anastrophyllum donnianum*[5] and sacculatanes, e.g. **5**, from *Porella platyphylla*[6]. Recently, we have isolated a series of dihydrophenanthrene derivatives, e.g. **6**, and two simple aromatic compounds **7** and **8** from *Plagiochila spinulosa*[7].

The liverwort *Jungermannia obovata* Nees belongs to the family Jungermanniaceae of the order Jungermanniales. It has a characteristic sweet, carrot-like odour due to the presence of monoterpenes. Previous work[8,9] has resulted in the identification of limonene, myrcene, α- and γ-terpinene, terpinolene, *p*-cymene and α- and β-pinene. The *trisnor*-monoterpenoid **9** has also been isolated[10]. Most of the Jungermanniaceae produce diterpenoids[1], which are normally regarded as chemical markers for this family. Sesquiterpenoids are less common in this family though *ar*-curcumene and α-cedrene have been detected[8] in *J. obovata*.



Results and Discussion

The liverwort *J. obovata*, while still moist, was extracted with diethyl ether. The extract was chromatographed over silica gel to give a monoterpene fraction together with two new oxygenated monoterpenoids **10** and **11**. Glc, gcms and ^{13}C nmr analyses of the hydrocarbon fraction led to the identification of the monoterpenes limonene, myrcene, *p*-cymene, α-phellandrene, α-terpinene and *p*-isopropyltoluene (see Experimental section).

The molecular formula, $C_{10}H_{16}O_2$ (M^+ at *m/z* 168.1135), of compound **10** was determined by hrms. Its ir spectrum showed hydroxyl absorption (ν_{max} 3600 cm^{-1}, strong and broad). The ^1H nmr spectrum revealed the presence of two vinyl methyl groups [δ_H 1.81 (t, *J* 1.1 Hz, 1.66 (br s)], a vinyl proton [δ_H 5.25 (m)], two exomethylene protons [δ_H

†Dedicated to Professor Sukh Dev on the occasion of his 75th. birth anniversary.

5.02 (2H, q, J 1.1 Hz)] and one proton at δ_H 7.27 (s), exchangeable with D_2O. The ^{13}C nmr spectrum showed resonances for ten carbons, including a trisubstituted double bond [δ_C 133.8 (s), 117.3 (d)], an exomethylene [δ_C 146.5 (s), 112.8 (t)], one oxygenated quaternary carbon [δ_C 84.0], three methylene carbons and two methyl groups. One of the oxygens cannot be bonded to carbon and this suggested the presence of a hydroperoxy group. Hrms gave ions at m/z 152.1185 (14%, $C_{10}H_{16}O$), 151.1119 (47%, $C_{10}H_{15}O$), 150.1044 (11%, $C_{10}H_{14}O$), 136.1235 (12%, $C_{10}H_{14}$), 135 (24%) and 134 (13%) which represent the fragment ions M^+-O, M^+-HO, M^+-H_2O, M^+-O_2, M^+-HO_2 and M^+-H_2O_2 respectively. These ms data support the presence of a hydroperoxy group[11,12]. The ^1H nmr signal at δ_H 7.27 (s) can be assigned to the hydroperoxy proton since these appear characteristically[11,13] at low field. Thus compound **10** is a hydroperoxy monocyclic monoterpenoid diene. NOEs between H-2 and 2H-3 (2.5%) and between 2H-9 and 2H-3 (1.5%) support a *p*-menthane framework with the hydroperoxide attached to C-4 as in **10**. The 2H-3 protons resonate at δ_H 2.20 (m) and 2.33 (m) as an AB type system with additional small couplings. The deshielded position of these protons is in agreement with their allylic nature. NOEs between 2H-9 (3.0%) and 3H-10 (1.0%) and between H-2 (6.0%) and 3H-7 (1.0%) readily distinguish the vinyl methyl resonances.

Initially we thought that the ms peak at m/z 152.1185 (14%, $C_{10}H_{16}O$) was the parent ion and that compound **10** was 4-hydroxy-*p*-mentha-1,8-diene (**12**). However this compound is known[14] and comparison of its ^1H and ^{13}C chemical shift data with those of compound **10** clearly showed that they are different. The most significant difference is the deshielding (11.9 ppm) of the oxygenated carbon C-4 in **10** relative to **12**. The chemical shift of C-4 (δ_C 84.0) is more characteristic[12,15,16] of an allylic carbon bearing a hydroperoxy group than of an allylic tertiary alcohol. Furthermore there is an upfield β shift of C-8 (3.9 ppm) and a downfield γ shift of C-9 (3.1 ppm) in **10** relative to **12**, again characteristic[16] of allylic hydroperoxides relative to allylic alcohols. On the basis of the above evidence compound **10** is 4-hydroperoxy-*p*-mentha-1,8-diene. The absolute configuration remains undetermined.

The second component, compound **11**, has the molecular formula $C_{10}H_{14}O_2$ (M^+ at m/z 166.0991). Its ^1H nmr spectrum shows resonances which suggest a *p*-disubstituted benzene ring [AA'BB' spin system : δ_H 7.35 (2H) and 7.18 (2H), both complex multiplets, $J_{AB} + J_{AB'}$ 8.3 Hz, $J_{AA'} + J_{BB'}$ 4.0 Hz]. The two proton multiplet at δ_H 7.18 has an additional small coupling (J 0.6 Hz) to an aromatic methyl group [δ_H 2.34 (br s)]. The spectrum also contains two other methyl groups with identical chemical shifts [δ_H 1.59 (s, 6H)] and a broad one proton singlet at δ_H 7.28. The ^{13}C nmr spectrum confirms the presence of a *p*-disubstituted benzene ring [δ_C 141.5 (s), 137.2 (s), 129.2 (d, 2C) and 125.4 (d, 2C)], an oxygenated quaternary carbon (δ_C 83.8) and three methyl groups [δ_C 26.1 (2C), 21.0]. It is evident from these spectral data that compound **11** is 8-hydroperoxy-*p*-mentha-1,3,5-triene. The fact that the ms indicates the presence of two oxygens while there is only one oxygenated carbon supports a hydroperoxy group. This is confirmed by the proton signal at δ_H 7.28 and the ms fragments at 150 [10%, M^+-O], 149 [2%, M^+-HO], 148 [1%, M^+-H_2O], 134 [13%, M^+-O_2] and 133 [100%, M^+-HO_2][11–13].

At first, as with compound **10**, we thought that compound **11** was the corresponding tertiary alcohol derivative but comparison of ^{13}C chemical shifts with abietane diterpenoids[17] containing the relevant part structure revealed that the oxygenated carbon (C-8) in compound **11** is substantially more deshielded (*ca.* 12 ppm) than expected for an alcohol. As expected the hydroperoxy group also shields the isopropyl methyl carbons (*ca.* 6 ppm)[17].

Both **10** and **12** are new natural products. This is the first report of monoterpenoid hydroperoxides in liverworts.

Experimental

Ir spectra were recorded in CCl_4 solution on a Perkin-Elmer 580 spectrophotometer. Low resolution mass spectra were determined using a VG updated MS 12 spectrometer while high resolution mass spectra were run on a modified Kratos MS 9 instrument. Glc separations were achieved with a Hewlett-Packard 5880A instrument equipped with a CP Sil 5 CB (chrompack) fused silica capillary column (25 m × 0.32 mm ID × 0.12 μm) and flame ionisation detector (FID). The Grob-type injector was operated in the split mode (50 : 1) and the helium carrier and make up gas flow rate was 2 ml/min. Linear temperature programmes were used in which the column temperature was raised from 50° (2 min) to 200° at 2°/min and then from 200° (5 min) to 250° (1 min) at 3°/min. The injection port and detector temperatures were both 57°. Gcms analyses were carried out with an AEI MS30 instrument interfaced to a PE Sigma 3 gas-liquid chromatograph. Separations were carried out with a 25 m BP 1 column using a temperature programme of 50° (3 min) to 260° at 5°/min. Retention times (T_R) were taken from the FID chromatograms. Nmr spectra were recorded at 298 K and at 4.7 T on Bruker WP 200SY and AM 200SY instruments (^1H, 200.132 MHz; ^{13}C, 50.32 MHz). Spectra were recorded for $CDCl_3$ solutions relative to $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_C 77.0 and chemical shifts are reported in ppm. ^{13}C multiplicities were obtained from DEPT spectra. NOE difference experiments were performed using a Bruker micro-

program and NOE enhancements are scaled up to represent 100% saturation of the irradiated proton. The silica gel used for cc and plc was Merck kieselgel GF_{254}. Analytical tlc was over Merck precoated silica gel 60 F_{254} plates (0.25 mm thick). Preparative and analytical plates were visualised under uv light (254 or 366 nm), by adsorption of iodine vapour or by spraying with 25% H_2SO_4 and then heating.

The liverwort was collected at Loch Doon in the West of Scotland by J.D.C. on the 7th June, 1991; the plant material was sterile and identification was reliant on vegetative characters and the characteristic smell. A voucher specimen is retained in the Chemistry Department, University of Glasgow.

The plant material was immersed in Et_2O while still moist, in order to capture the volatile constituents. The crude extract rapidly hardened to an insoluble mass due to the presence of polyunsaturated fats. Extraction of the solidified material with Et_2O afforded an oil (445 mg) which was subjected to column chromatography over silica gel. Plc of the less polar fractions gave a monoterpene hydrocarbon mixture (8 mg), 4-hydroperoxy-*p*-mentha-1,8-diene (**10**; 30 mg) and 8-hydroperoxy-*p*-mentha-1,3,5-triene (**12**; 33 mg).

Analysis of the monoterpene mixture : Glc, gcms and ^{13}C nmr analysis of the mixture enabled identification of the following compounds by comparison with published data[18,19] : myrcene (T_R = 6.04 min, *m/z* 136 [M^+]); *p*-cymene (T_R = 7.01 min, *m/z* 134 [M^+]); limonene (T_R = 7.71 min, *m/z* 136 [M^+]); α-phellandrene (T_R = 8.37 min, *m/z* 136 [M^+]); α-terpinene (T_R = 9.61 min, *m/z* 136 [M^+]); *p*-isopropyltoluene (T_R = 11.59 min, *m/z* 132 [M^+]). A major sesquiterpene hydrocarbon component remained unidentified.

4-Hydroperoxy-p-*mentha-1,8-diene* (**10**) was isolated as a gum [*m/z* 168.1135 (M^+); calculated for $C_{10}H_{16}O_2$: 168.1150]; ν_{max} (cm^{-1}) 3600, 2970, 2930, 1550; eims *m/z* (rel. int.) 168 [M^+] (7), 152 (14), 151 (47), 150 (11), 149 (51), 136 (12), 135 (24), 134 (13), 133 (27), 123 9 (32), 107 (85), 93 (100), 81 (74); nmr δ_H 7.27 (s, OOH), 5.25 (m, H-2), 5.02 (q, *J* 1.1 Hz, 2H-9), 2.20 (m, H-3), 2.33 (m, H-3), 1.6–2.2 (m, 2H-5 and 2H-6), 1.81 (t, *J* 1.1 Hz, 3H-10), 1.66 (br s, 3H-7); δ_C 146.5 (C-8), 133.8 (C-1), 117.3 (C-2), 112.8 (C-9), 84.0 (C-4), 33.2 (C-3), 27.6, 27.3 (C-5 and C-6), 23.2 (C-7), 18.5 (C-10).

8-Hydroperoxy-p-*mentha-1,3,5-triene* (**12**) was obtained as a gum [*m/z* 166.0991 (M^+); calculated for $C_{10}H_{14}O_2$: 166.0994]; eims *m/z* (rel. int.) 166 [M^+] (1), 150 (10), 149 (2), 148 (1), 134 (13), 133 (100), 119 (15), 105 (37), 91 (40), 85 (20), 65 (19); nmr δ_H 7.35 (2H, m), 7.28 (s, OOH), 7.18 (2H, m), 2.34 (br s, 3H-7), 1.59 (s, 3H-9 and 3H-10); δ_C 141.5 (C-4), 137.2 (C-1), 129.2 (C-2 and C-6), 125.4 (C-3 and C-5), 83.8 (C-8), 26.1 (C-9 and C-10), 21.0 (C-7).

References

1. Y. ASAKAWA in "Progress in the Chemistry of Organic Natural Products", eds. W. HERZ, G. W. KIRBY, R. E. MOORE, W. STEGLICH and CH. TAMM, Springer, Vienna, 1995, Vol. 65.
2. M. TORI, K. NAKASHIMA, Y. ASAKAWA, J. D. CONNOLLY, L. J. HARRISON, D. S. RYCROFT, J. SINGH and N. WOODS, *J. Chem. Soc., Perkin Trans. 1*, 1995, 593.
3. M. S. BUCHANAN, J. D. CONNOLLY and D. S. RYCROFT, *Phytochemistry*, 1996, **43**, 1245.
4. J. D. CONNOLLY, L. J. HARRISON and D. S. RYCROFT, *J. Chem. Res.*, 1994, 284.
5. M. S. BUCHANAN, J. D. CONNOLLY and D. S. RYCROFT, *Phytochemistry*, 1996, **43**, 1297.
6. M. S. BUCHANAN, J. D. CONNOLLY and D. S. RYCROFT, *Phytochemistry*, 1996, **43**, 1249.
7. J. D. CONNOLLY, D. S. RYCROFT, D. L. SRIVASTAVA, W. J. COLE, P. IFEADIKE, S. F. KIMBU, J. SINGH, M. HUGHES, C. THOM, U. GERHARD, A. J. ORGAN, R. J. SMITH and L. J. HARRISON, *Phytochemistry*, in press.
8. Y. ASAKAWA, C. SUIRE, M. TOYOTA, N. TOKUNAGA, R. MATSUDA, T. TAKEMOTO, S. HATTORI and M. MIZUTANI, *J. Hattori Bot. Lab.*, 1980, **48**, 285.
9. L. SVENSSON, *Phytochemistry*, 1974, **13**, 651.
10. J. D. CONNOLLY in "Bryophytes : Their Chemistry and Chemical Taxonomy", eds. H. D. ZINSMEISTER and R. MUES, Oxford University Press, 1990, p. 41.
11. G.-C. ZHENG, A. ICHIKAWA, M. O. ISHITSUKA, T. KUSUMI, H. YAMAMOTO and K. KAKISAWA, *J. Org. Chem.*, 1990, **55**, 3677.
12. K. ITO, Y. SAKAKIBARA and M. HARUNA, *Chem. Lett.*, 1979, 1503.
13. B. M. HOWARD, W. FENICAL, J. FINER, K. HIROTSU and J. CLARDY, *J. Am. Chem. Soc.*, 1977, **99**, 6440.
14. F. DELAY and G. OHLOFF, *Helv. Chim. Acta*, 1979, **62**, 2168.
15. F. S. EL-FERALY, Y.-M. CHAN, E. H. FAIRCHILD and R. W. DOSKOTCH, *Tetrahedron Lett.*, 1977, 1973.
16. F. S. EL-FERALY, Y.-M. CHAN, G. A. CAPITON, R. W. DOSKOTCH and E. H. FAIRCHILD, *J. Org. Chem.*, 1979, **44**, 3952.
17. T. OHMOTO, K. KANATANI and K. YAMAGUCHI, *Chem. Pharm. Bull.*, 1987, **35**, 229.
18. R. RYHAGE and E. VON SYDOW, *Acta Chem. Scand.*, 1963, **17**, 2025.
19. F. BOHLMANN, R. ZEISBERG and E. KLEIN, *Org. Magn. Reson.*, 1975, **7**, 426.