PERGAMON

Phytochemistry 50 (1999) 1159–1165

PHYTOCHEMISTRY

Aromatic compounds from the liverwort *Plagiochila spinulosa*[1]

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Received 17 August 1998

Abstract

Two known and five novel 9,10-dihydrophenanthrene derivatives, two orsellinic acid derivatives and a lunularic acid derivative have been isolated from the liverwort *Plagiochila spinulosa* and identified following NMR studies. A further known 9,10-dihydrophenanthrene derivative has been identified using ^{1}H NMR fingerprinting of the extracts. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Plagiochila spinulosa*; Hepaticae; Liverwort; NMR fingerprinting; GC–MS; ^{1}H and ^{13}C NMR parameters; Aromatic compounds; 9,10-Dihydrophenanthrenes

1. Introduction

We have carried out phytochemical studies of a variety of liverwort species that are native to Scotland and have isolated a range of terpenoids, e.g. sesquiterpenoids from *Chiloscyphus pallescens* (Connolly, Harrison, & Rycroft, 1982) and *Saccogyna viticulosa* (Connolly, Harrison, & Rycroft, 1994) and diterpenoids from *Nardia scalaris* (Connolly, Harrison, Phillips, & Rycroft, 1984) and *Scapania undulata* (Huneck et al., 1986). Liverworts belonging to the genus *Plagiochila* are known to be rich sources of not only terpenoids but also aromatic compounds (Asakawa, 1995), and we have previously reported the presence of 1-(3,4-dihydroxy-5-methoxybenzyl)-3-methylbut-2-ene in *P. rutilans* from Cuba (Huneck, Connolly, Harrison, Joseph, & Pócs, 1984) and 3,4-dihydroxy-3′-methoxybibenzyl in *P. exigua* (Rycroft, Cole, & Aslam, 1998), as well as the sesquiterpenoids plagiochiline C and atlanticol in *P. atlantica* (Rycroft & Cole, 1998) and plagiochiline V in *P. porelloides* (Söderström et al., 1999). A recent report of a new and still unidentified *Plagiochila* species (Anton, Kraut, Mues, & Morales, 1997) that contains 9,10-dihydrophenanthrenes has prompted us to provide full details of our studies (Rycroft, 1990; Connolly, 1997) of the aromatic constituents of *P. spinulosa* (Dicks.) Dum., which is commonly encountered in the west of Britain (Watson, 1981). Several collections of *P. spinulosa* have been made and, using both GC–mass spectrometry and ^{1}H NMR fingerprinting of the crude extracts, it has been found that differences are minor and that all the compounds described herein are present in all the samples in similar proportions.

2. Results and discussion

This report deals with the nonterpenoid constituents of three representative samples (A–C) of *P. spinulosa* that were collected in the west of Scotland and in the English Lake District (the structural elucidation of the diterpenoid — bibenzyl conjugates spinuloplagins A, B (Rycroft, 1990) and C will be reported elsewhere). The ether extract of sample A was subjected to normal phase vacuum liquid chromatography to afford seven compounds described here. The first compound was 9,10-

[1] Part 7 in the series 'NMR Fingerprinting of Liverworts'. For part 6 see Söderström, Rycroft, Cole, and Wei (1999).

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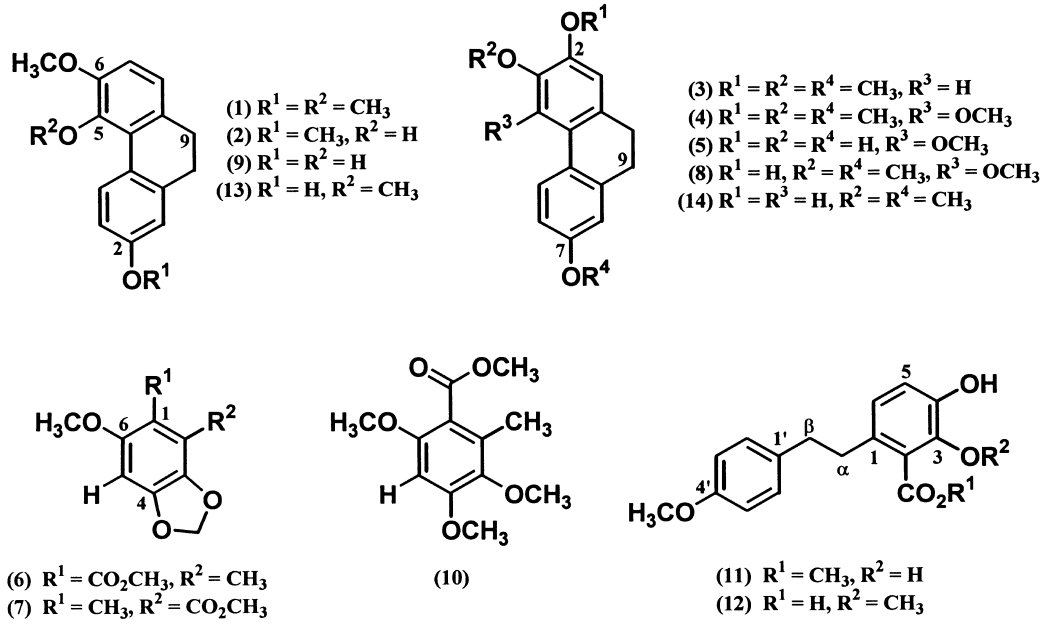
PII: S0031-9422(98)00665-7



dihydro-2,5,6-trimethoxyphenanthrene (**1**). Its ^{13}C NMR spectrum (Table 1) contained 17 signals including three methoxyls, two methylenes, five aromatic methines and seven substituted aromatic carbons, three of which were oxygenated. These carbon signals and the high-resolution mass spectrum (*m/z* 270.1250) established the molecular formula as $C_{17}H_{18}O_3$, consistent with a dihydrophenanthrene. The ^1H NMR spectrum (Table 2) showed the expected four proton multiplet signal at δ_H 2.73 (H_2-9 and H_2-10) (Letcher & Nhamo, 1971), in addition to three methoxyl signals [δ_H 3.68 (5-OCH_3), 3.83 (2-OCH_3) and 3.88 (6-OCH_3)]. Two sets of coupled aromatic protons were present, an *ortho*-coupled pair [δ_H 6.74 (d, J_{AB}=8.2 Hz, H-8) and 6.92 (d, J_{AB}=8.2 Hz, H-7)] and the three spin system of a 1,2,4-trisubstituted aromatic ring [δ_H 6.83 (dd, J=2.8 and 8.6 Hz, H-3), 6.76 (d, J=2.8 Hz, H-1) and 8.36 (d, J=8.6 Hz, H-4)]. The most deshielded resonance [δ_H 8.36] was characteristic of H-4 (or H-5) of a 9,10-dihydrophenanthrene where C-5 (or C-4) bears an oxygen (Majumder & Joardar, 1985). Two of the methoxyl groups could therefore be placed at C-2 and C-5, whilst the third must be attached to C-6, because the ^{13}C NMR spectrum revealed that there were substituents in both of the positions *ortho* to one of the methoxyls ($\delta_C \approx 60$). Acquisition of a NOESY spectrum confirmed the substitution pattern. Thus, correlations were observed between the C-2 methoxyl group and both H-1 and H-3, as well as between the C-5 methoxyl and H-4 and the C-6 methoxyl and H-7. The ^{13}C NMR spectrum was assigned by comparison with similar compounds.

Table 1
^{13}C NMR data for compounds **1**, **2**, **8**, and **13**

	1	2	8	13
1	113.1	113.3	110.0	114.4
2	158.4	158.3	147.5	154.5
3	111.6	111.3	139.0	113.4
4	129.5	129.7	150.5	129.9
4a	125.3	125.6	120.3	125.6
4b	128.0	120.1	125.3	127.9
5	146.5	142.8	128.3	147.0
6	152.0	145.7	111.6	152.1
7	110.0	108.2	157.5	110.2
8	122.8	118.2	113.0	122.9
8a	131.4	131.6	139.4	131.4
9	29.5	29.7	30.2	29.5
10	30.5	30.5	30.1	30.4
10a	140.5	140.1	134.9	140.9
2-OCH_3	55.1	55.2	–	–
3-OCH_3	–	–	61.1	–
4-OCH_3	–	–	60.0	–
5-OCH_3	60.0	–	–	60.0
6-OCH_3	56.0	56.3	–	56.0
7-OCH_3	–	–	55.1	–

The second and third compounds were identified as 9,10-dihydro-2,6-dimethoxyphenanthren-5-ol (**2**) and 9,10-dihydro-2,3,7-trimethoxyphenanthrene (**3**), which have been reported recently from an unidentified Costa Rican *Plagiochila* species (Anton et al., 1997).

The fourth compound was identified as 9,10-dihydro-2,3,4,7-tetramethoxyphenanthrene (**4**), $C_{18}H_{20}O_4$. The presence of a four proton multiplet at δ_H 2.74 (H_2-9 and H_2-10) in the ^1H NMR spectrum (Table 2) revealed that it was a dihydrophenanthrene. The ^1H NMR spectrum also had four methoxyl resonances at δ_H 3.76 (s, 4-OCH_3), 3.83 (s, 7-OCH_3), 3.87 (s, 2-OCH_3) and 3.90 (s, 3-OCH_3), as well as an aromatic singlet at δ_H 6.57 (s, H-1) and a three spin aromatic system [δ_H 6.81 (dd, J=2.8 and 8.7 Hz, H-6), 6.76 (d, J=2.8 Hz, H-8) and 8.21 (d, J=8.7

Table 2
^{1}H NMR parameters for 9,10-dihydrophenanthrenes **1**–**4**, **8**, **9** and **13**

	1	**2**	**3**	**4**	**8**	**9**	**13**
H-1	6.76 d 2.8	6.78 d 2.8	6.73 s	6.57	6.62	6.71 d 2.8	6.75 m
H-2	–	–	–	–	–	–	–
H-3	6.83 dd 2.8, 8.6	6.86 dd 2.8, 8.6	–	–	–	6.76 dd 2.8, 8.5	6.75 m
H-4	8.36 d 8.6	8.36 d 8.6	7.18 s	–	–	8.31 d 8.5	8.31 d 8
H-5	–	–	7.54 d 8.4	8.21 d 8.7	8.18 d 8.5	–	–
H-6	–	–	6.83 dd 2.8, 8.4	6.81 dd 2.8, 8.7	6.81 dd 2.8, 8.5	–	–
H-7	6.92 d 8.2	6.68 d 8.1	–	–	–	6.69 d 8.1	6.75 m
H-8	6.74 d 8.2	6.73 d 8.1	6.77 d 2.8	6.77 d 2.8	6.76 d 2.8	6.72 d 8.1	6.93 d 8.0
H-9,10	2.73 br s	2.75 br s	2.80 m	2.74 br s	2.71 br s	2.73 br s	2.73 br s
2-OCH_3	3.83 s	3.83 s	3.92 s	3.87 s	–	–	–
3-OCH_3	–	–	3.89 s	3.90 s	3.96 s	–	–
4-OCH_3	–	–	–	3.76 s	3.74 s	–	–
5-OCH_3	3.68 s	–	–	–	–	–	3.69 s
6-OCH_3	3.88 s	3.92 s	–	–	–	3.91 s	3.89 s
7-OCH_3	–	–	3.83 s	3.83 s	3.83 s	–	–
2-OH	–	–	–	–	5.76 br s	4.68 br s	4.80 br s
5-OH	–	6.20	–	–	–	6.19 br s	–

Hz, H-5)] similar to that of **1**. NOE difference experiments determined the positions of the methoxyl groups. On irradiation of the benzylic protons, NOEs were observed at H-1 (δ_H 6.57, 12%) and H-8 (δ_H 6.76, 8%) and thus established the presence of a proton (δ_H 6.57) at C-1. NOEs were also observed at H-6 (7%) and at H-8 (7%) upon irradiation of the methoxyl protons at δ_H 3.83 (7-OCH_3), confirming the position of this methoxyl and the substitution pattern of this compound. Whilst this is the first report of this compound in nature, it has previously been reported as a synthetic derivative of 9,10-dihydro-4-methoxyphenanthrene-2,3,7-triol (**5**) from the orchid *Bulbophyllum vaginatum*; its physical properties were in good agreement with those reported in the literature (Leong, Kang, Harrison, & Powell, 1997).

The next compound proved not to be a 9,10-dihydrophenanthrene but was identified as methyl 6-methoxy-2-methyl-3,4-methylenedioxybenzoate (**6**). Its ^{13}C NMR spectrum had 11 signals including an aromatic methyl [δ_C 12.4], a methyl ester [δ_C 168.0 (C═O) and 52.1 (OCH_3)], a methoxyl [δ_C 56.8 (6-OCH_3)], a methylenedioxy group [δ_C 101.2], an aromatic methine [δ_C 92.7 (C-5)], three oxygenated aromatic carbons [δ_C 140.0 (C-3), 148.4 (C-4) and 152.9 (C-6)] and two quaternary carbons [δ_C 117.7 (C-2) and 116.3 (C-1)]. These properties and the mass spectrum (*m*/*z* 224.0692) revealed the molecular formula to be $C_{11}H_{12}O_5$. The ^{1}H NMR spectrum contained a single aromatic proton resonance [δ_H 6.38 (s, H-5)], which indicated a pentasubstituted benzene ring structure. The strongly shielded nature of this proton suggested that it lay between two oxygen substituents. The ^{1}H spectrum also contained signals for an aromatic methyl [δ_H 2.14 (d, J=0.5 Hz)], two methoxyls [δ_H 3.74 (s, 6-OCH_3) and δ_H 3.86 (s, CO_2CH_3)] and a methylenedioxy group [δ_H 5.98 (s)]. These spectroscopic properties permitted only two possible structures, **6** and **7**. The crucial HMBC correlation (Fig. 1) from the aromatic methyl protons and the methylene protons to the same oxygenated aromatic carbon (C-3) was sufficient to preclude the latter structure. The other correlations were as expected and enabled the assignment of the ^{1}H and ^{13}C NMR spectra.

The ^{1}H NMR spectrum (Table 2) of the next compound, 9,10-dihydro-3,4,7-trimethoxyphenanthren-2-ol (**8**), $C_{17}H_{18}O_4$, differed from that of **4** only in the presence of three methoxyls [δ_H 3.74 (s, 4-OCH_3), 3.83 (s, 7-OCH_3) and 3.96 (s, 3-OCH_3)], and one hydroxyl [δ_H 5.76 (s, 2-OH) instead of four methoxyls. The ^{13}C NMR spectrum revealed that two of the methoxyls were *ortho*-disubstituted [δ_C 55.1 (7-OCH_3), 60.0 and 61.0 (4-OCH_3 and 3-OCH_3)]. This indicated that the two compounds possessed the same oxygenation pattern. The position of the free hydroxyl group was shown to be C-2, since saturation of one of the methoxyl groups [δ_H 3.83 (7-

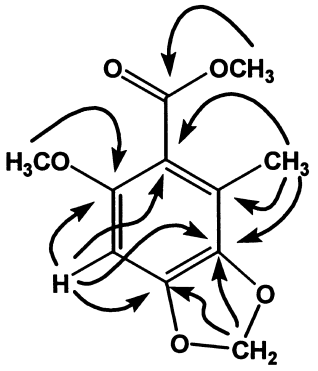


Fig. 1. HMBC correlations for **6**.

OCH_3)] gave NOEs at H-6 (4%) and H-8 (4%) and the other two methoxyls were *ortho*-disubstituted. Further NOEs from H-1 to H_2-10 (1%) and the phenolic hydroxyl proton (1%), from the phenolic hydroxyl proton to H-1 (2%) and to 3-OCH_3 (1%), from 3-OCH_3 to the phenolic hydroxyl (3%) and 4-OCH_3 (0.3%), from 4-OCH_3 to 3-OCH_3 (0.6%) and H-5 (5%), and from H-5 to H-6 (10%) and 4-OCH_3 (1%) were in accordance with the proposed structure. The carbon assignments are based on comparison with data reported for similar compounds, as well as HMQC and HMBC spectra.

9,10-Dihydro-6-methoxyphenanthrene-2,5-diol (**9**), $C_{15}H_{14}O_3$, was isolated from sample A only, but has been observed in several NMR and GC–mass spectrometry fingerprints. It again showed the characteristic four proton multiplet at δ_H 2.73 (H_2-9 and H_2-10) due to a 9,10-dihydrophenanthrene system. The ^{1}H NMR spectrum (Table 2) was almost identical to those of **1** and **2**, apart from the presence of only one methoxyl [δ_H 3.91 (s, 6-OCH_3)], as well as two phenolic hydroxyls [δ_H 4.68 (s, 2-OH) and δ_H 6.19 (s, 5-OH); both exchangeable with D_2O]. It seemed therefore that **9** possessed the same oxygenation pattern as **1** and **2**. NOE difference experiments readily confirmed this and enabled the position of the methoxyl to be established. Irradiation of the four proton multiplet at δ_H 2.73 (H_2-9 and H_2-10) showed NOEs at H-1 (15%) and at H-8 (δ_H 6.71, 1%). Irradiation of H-8 enhanced the H-7 signal and gave a NOE at δ_H 2.73 (H-9 and 10, 1%). Irradiation of the phenolic proton at δ_H 4.68 afforded NOEs at H-3 (2%) and H-1 (3%) indicating that the hydroxyl group at δ_H 4.68 was attached to C-2. Irradiation of the remaining phenolic hydroxyl at δ_H 6.19 gave NOEs at H-4 (2%) and at the methoxyl group (0.2%), revealing that the substitution pattern of ring A was shown as in structure (**9**).

The constituents of sample B were isolated by preparative reverse-phase HPLC of the MeOH extract. Three compounds were isolated in addition to **1**–**4**, **6** and **8**. The first of these was a tetrasubstituted methyl benzoate, $C_{12}H_{16}O_5$. Its ^{1}H and ^{13}C NMR spectra (see Section 3) were similar to those of **6**, except that the methylenedioxy group signals had been replaced by those of two methoxyl groups. This suggested that the compound was methyl 3,4,6-trimethoxy-2-methylbenzoate (**10**). Confirmation of the proposed structure was achieved using HMBC (Fig. 2). The methoxyl group at C-3 (identified because of the correlations involving the aromatic methyl protons) resonated at δ_C 60.5 and therefore had to be *ortho* to another methoxyl at C-4. The signals for C-4 and C-6 (along with the attached methoxyls) could not be assigned unambiguously, but were made by comparison with those of **6**.

The second compound, methyl 4-hydroxy-4′-*O*-methyllunularate (**11**), was also a methyl ester [δ_H 4.00 (s, CO_2CH_3); δ_C 172.4 (CO_2CH_3) and 52.9 (CO_2CH_3)] but the occurrence of 12 aromatic carbon signals in the ^{13}C

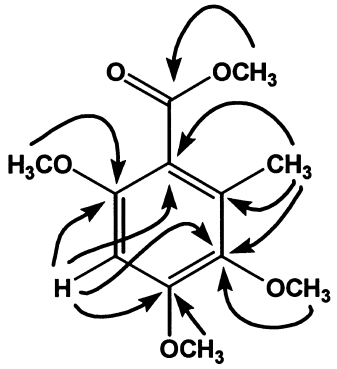


Fig. 2. HMBC correlations for **10**.

NMR spectrum (see Experimental), as well as ^{1}H NMR signals for a *para*-disubstituted aromatic ring [δ_H 7.07 and 6.83 (AA′BB′, J_{AB} = 8.0 Hz) H-2′, H-3′, H-4′, H-5′] excluded both 9,10-dihydrophenanthrene and simple benzoic acid structures. The molecular formula, $C_{17}H_{18}O_5$, which was derived from EI-mass spectrometry and ^{13}C NMR, and the presence of two deshielded methylene groups [δ_H 3.10 (m, H_2-α) and 2.75 (m, H_2-*β*); δ_C 38.7 (C-α) and 38.2 (C-*β*)] suggested a bibenzyl structure. The second aromatic ring was tetrasubstituted, since a pair of *ortho* aromatic protons was also present [δ_H 6.98 (d, J = 8.1 Hz, H-5) and 6.61 (d, J = 8.1 Hz, H-6)]. One methoxyl [δ_H 3.80 (s, 4′-OCH_3); δ_C 55.7 (4′-OCH_3)] and two hydroxyl groups [δ_H 11.50 (s, 2-OH) and 5.65 (br s, 3-OH)] were present in addition to the methoxycarbonyl. One of the hydroxyl groups was placed *ortho* to the ester; hydrogen bonding then accounted for the deshielded nature of the hydroxyl proton. The relative disposition of the substituents was readily determined using HMBC (Fig. 3). Thus, the second methoxyl group was attached to the disubstituted aromatic ring. The more shielded benzylic methylene and the nonchelated hydroxyl proton correlated to different aromatic methine carbons and this established that the groups were mutually *para*. The remaining correlations of the hydroxyl proton to two oxygenated aromatic carbons revealed that the hydroxyl groups were *ortho* and that the compound was methyl 4-hydroxy-4′-*O*-methyllunularate (**11**). The other cor-

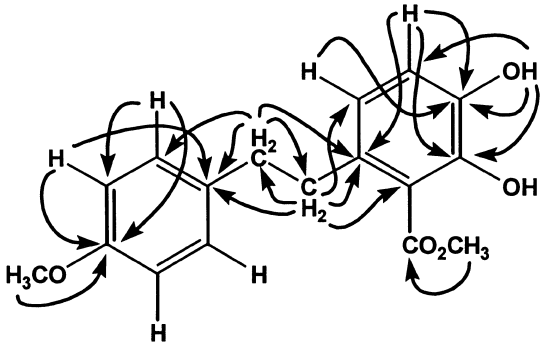


Fig. 3. HMBC correlations for **11**.

relations observed were consistent with the proposed structure. Recently, Mues and coworkers reported (Anton et al., 1997) a compound with identical spectroscopic properties to **11** from an unidentified Costa Rican *Plagiochila* species but proposed a different structure (**12**). In consultation with the Saarbrücken group, we have concluded that structure **12** is untenable, because it would give a ^{13}C NMR resonance at ca. δ 60 for the *ortho*-disubstituted methoxyl, rather than at δ_C 52.4 as observed; identity of the compounds was confirmed by comparison with GC–mass spectrometry data of the acetylation product of a sample of the bibenzyl provided by Professor Mues.

The final compound isolated proved to be another 9,10-dihydrophenanthrene because it gave NMR signals (Tables 1–2) for a pair of benzylic methylenes [δ_H 2.73 (br s, H_2-9 and H_2-10); δ_C 30.4 and 29.5 (C-9 and C-10)], as well as for the proton at H-4 of a 5-oxygenated 9,10-dihydrophenanthrene [δ_H 8.31 (d, $J=8$ Hz, H-4)]. The NMR shifts were very similar to those of **1**, **2** and **9**, again indicating a 2,5,6-trioxygenation pattern. The substituents were identified as two methoxyls [δ_H 3.89 (s, 6-OCH_3) and 3.69 (s, 5-OCH_3); δ_C 60.0 (5-OCH_3) and 56.0 (6-OCH_3)], as well as a hydroxyl group [δ_H 4.80 (br s)]. Although the overlapped nature of the aromatic proton resonances reduced the usefulness of the HMBC spectrum, it was still possible to determine the compound's structure. Since H-4 showed a correlation to an oxygenated carbon which did not, in turn, correlate with any of the methoxyl protons, C-2 must be hydroxylated. The two methoxyl groups were therefore attached to C-5 and C-6 and the compound was 9,10-dihydro-5,6-dimethoxyphenanthren-2-ol (**13**). The ^{13}C NMR spectrum Table 1 was assigned using HMBC and by comparison with known compounds.

During the ^1H NMR and GC–mass spectrometric analysis of sample C, a further compound (which was not isolated) was identified as 9,10-dihydro-3,7-dimethoxyphenanthren-2-ol (**14**) by comparison of its ^1H NMR shifts with those of the same compound isolated by Mues and coworkers (Anton et al., 1997).

This is the first report of the isolation of the metabolites of *P. spinulosa*. Compound **8** is the major aromatic compound in all specimens of *P. spinulosa* that we have studied. An earlier paper (Inoue & Asakawa, 1988) detailed a GC–mass spectrometric study of Belgian *P. spinulosa*, in which a number of unidentified compounds were assigned as sesqui- and diterpenoids simply on the basis of their M_rs. The compounds in the Belgian sample that have $[M]^+$ m/z 224 (peak 106), m/z 240 (peak 107) and m/z 286 (peak 3) are likely to be **6**, **10** and **8**, respectively, from the present study. Compounds **6** and **10** have also been found in *P. killarniensis* (Rycroft, Cole, Aslam, Lamont, & Gabriel, 1999).

Although the presence of aromatic compounds in the Hepaticae is well established (Asakawa, 1995), only a few phenanthrene derivatives have been recorded. 3,7-Dimethoxyphenanthren-2-ol has been found in Indian *Marchantia polymorpha* (Asakawa, Tori, Takikawa, Krishnamurty, & Kar, 1987), 3-methoxyphenanthrene-2,7-diol, 2,3-dimethoxyphenanthren-7-ol and three biphenanthrenes in cultured *M. polymorpha* (Adam & Becker, 1994), jakinenol (sic; 9,10-dihydro-3,4-dimethoxyphenanthren-5-ol) in *Riccardia jackii* (Matsuo, Nozaki, Suzuki, & Nakayama, 1985), eight 9,10-dihydrophenanthrenes (including **2**, **3** and **14**) and one phenanthrene in an unidentified *Plagiochila* species from Costa Rica (Anton et al., 1997); in addition, two of the 9,10-dihydrophenanthrenes from the Costa Rican *Plagiochila* (but not including **2**, **3** or **14** from *P. spinulosa*) have been found in *P. killarniensis* (Rycroft et al., 1999).

3. Experimental

3.1. General

Mps: uncorr. IR: $CHCl_3$ soln. EIMS: 70 eV. ^1H (^{13}C) NMR: 200 (50) and 360 MHz in $CDCl_3$ relative to $CHCl_3$ and $CDCl_3$ at δ_H 7.25 and δ_C 77.0, respectively or 400 (100) MHz in $CDCl_3$ relative to TMS.

3.2. Plant material

Voucher samples are deposited in the University of Glasgow herbarium (GL). Sample A: collected by JDC and DSR, Loch Ard Forest, Aberfoyle, Stirlingshire, 7 January 1995, det. DSR (confirmed by D. G. Long, Royal Botanic Garden Edinburgh); sample B: collected by JDC and DSR, Loch Katrine, Stirlingshire, 15 August 1992, det. DSR; sample C: collected by DSR, Rydal Beck, English Lake District, 11 September 1997, det. DSR, voucher No. DSR97137.

3.3. Isolation

VLC was performed using a column of silica gel G_{254} with a step gradient of Et_2O-petrol as eluent and 50 ml frs were collected. Prep. TLC was carried out on silica gel GF_{254} with an appropriate concn of Et_2O in petrol as eluent. Prep. HPLC was performed using a Hypersil 5 μm ODS column (20×250 mm, 20 ml min^{-1}) and a solvent gradient of 30–60% MeCN–H_2O (both components contained 0.1% TFA) over 65 min. The detection wavelength was 215 nm.

3.4. GC–MS

Sample C (29 mg) was extracted with $CDCl_3$ and the NMR fingerprint (Rycroft, 1996) was recorded. The same solution was used for the GC study, which gave the RIs reported for the individual compounds. GC–MS con-

ditions were generally as reported previously (Rycroft, Cole, & Rong, 1998).

3.5. Compound characterization

3.5.1. Sample A

Dried and powdered plant material (221 g) gave a crude extract (4.4 g) on extraction with Et_2O. VLC of the crude extract followed by prep. TLC of the crude compounds gave, in order of increasing polarity, **1** (14 mg), **2** (12 mg), **3** (2 mg), **4** (2 mg), **6** (70 mg), **8** (250 mg) and **9** (4 mg).

*3.5.1.1. 9,10-Dihydro-2,5,6-trimethoxyphenanthrene (**1**).* Mp 74–76°C (*ex* petrol-$CHCl_3$). R_i 2204. EI–MS (rel. int.): 270 $[M]^+$ (100), 255 (60), 227 (33), 212 (21), 197 (23), 196 (17), 195 (20), 181 (13), 169 (20), 165 (19), 153 (17), 152 (24), 141 (22), 139 (21), 115 (24); HREI–MS: m/z 270.1250 ($C_{17}H_{18}O_3$ requires m/z 270.1256). ^1H (200 MHz) and ^{13}C (50 MHz) NMR: Tables 1 and 2.

*3.5.1.2. 9,10-Dihydro-2,6-dimethoxyphenanthren-5-ol (**2**).* Mp 118–120°C (*ex* petrol). EI–MS (rel. int.): 256 $[M]^+$ (100), 241 (28), 223 (16), 213 (17), 197 (13), 195 (20), 181 (34), 169 (17), 165 (12), 153 (33), 152 (36), 151 (12), 141 (22), 139 (18), 115 (29); HREI–MS: m/z 256.1113 ($C_{16}H_{16}O_3$ requires m/z 256.1099). ^1H (200 MHz) and ^{13}C (50 MHz) NMR: Tabs. 1–2. *Acetate* (prepared in situ): R_i 2355.

*3.5.1.3. 9,10-Dihydro-2,3,7-trimethoxyphenanthrene (**3**).* Gum. R_i 2359. EI–MS: m/z 270 $[M]^+$ (100), 255 (55), 227 (29), 212 (16), 195 (20), 165 (22), 152 (27), 115 (19). ^1H (360 MHz) NMR: Table 2.

*3.5.1.4. 9,10-Dihydro-2,3,4,7-tetramethoxyphenanthrene (**4**).* Mp 134–138°C. R_i 2363. EI–MS (rel. int.): 300 $[M]^+$ (100), 285 (29), 271 (15), 257 (23), 242 (39), 232 (18), 227 (33), 226 (19), 225 (20), 199 (23), 198 (26), 197 (10), 174 (11), 171 (56), 165 (12), 156 (14), 153 (10), 152 (15), 139 (26), 128 (47), 115 (12); HREI–MS: m/z 300.1352 ($C_{18}H_{20}O_4$ requires m/z 300.1362). ^1H (360 MHz) NMR: Table 2.

*3.5.1.5. Methyl 6-methoxy-2-methyl-3,4-methylenedioxybenzoate (**6**).* Amorphous, mp 79–80°C. IR ν_{max} cm^{-1}: 1733. R_i 1642. EI–MS (rel. int.): 224 $[M]^+$ (62), 193 (100), 178 (23), 135 (14), 123 (10), 119 (12), 107 (10), 79 (25), 77 (33); HREI–MS: m/z 224.0692 ($C_{11}H_{12}O_5$ requires m/z 224.0685). ^1H (200 MHz) and ^{13}C (50 MHz) NMR: see text.

*3.5.1.6. 9,10-Dihydro-3,4,7-trimethoxyphenanthren-2-ol (**8**).* Mp 139–142°C (ex Et_2O–petrol). R_i 2313 (2455 after acetylation). EI–MS (rel. int.): 286 $[M]^+$ (88), 271 (32), 256 (25), 253 (16), 243 (14), 239 (50), 228 (54), 227 (12), 225 (29), 213 (34), 211 (67), 199 (38), 197 (21), 185 (37), 183 (30), 181 (21), 171 (39), 168 (22), 165 (15), 157 (34), 155 (17), 153 (24), 152 (36), 141 (29), 140 (30), 139 (58), 128 (100), 127 (58), 115 (47); HREI–MS: m/z 286.1181 ($C_{17}H_{18}O_4$ requires m/z 286.1205). ^1H (200 MHz) and ^{13}C (50 MHz) NMR: Tabs. 1–2.

*3.5.1.7. 9,10-Dihydro-6-methoxyphenanthrene-2,5-diol (**9**).* Gum. EI–MS (rel. int.): 242 $[M]^+$ (100), 227 (20), 209 (25), 199 (19), 181 (55), 169 (14), 153 (22), 152 (25), 151 (11), 141 (15), 139 (14), 115 (25); HREI–MS: m/z 242.0950 ($C_{15}H_{14}O_3$ requires m/z 242.0943). ^1H (360 MHz) NMR: Table 2. *Diacetate* (prepared in situ): R_i 2476.

3.5.2. Sample B

Extraction of dried and powdered plant material (161 g) with MeOH gave a crude extract (13.3 g). Prep. HPLC gave, in order of elution, **10**, **6**, **2**, **1**, **8**, **11**, **4**, **3** and **13**.

*3.5.2.1. Methyl 3,4,6-trimethoxy-2-methylbenzoate (**10**).* R_i 1706. EI–MS (rel. int.): 240 $[M]^+$ (100), 225 (50), 209 (45), 193 (52), 165 (46). ^1H NMR (400 MHz): δ 6.37 (s, H-5), 3.89 (s, CO_2CH_3), 3.88 (s, 4-OCH_3), 3.81 (s, 6-OCH_3), 3.72 (s, 3-OCH_3), 2.21 (s, H_3-8). ^{13}C NMR (100 MHz): δ 168.4 (C-7), 154.2 (C-4), 153.3 (C-6), 141.2 (C-3), 130.6 (C-2), 116.5 (C-1), 95.1 (C-5), 60.5 (3-OCH_3), 56.5 (6-OCH_3), 55.9 (4-OCH_3), 52.2 (CO_2CH_3), 12.9 (C-8).

*3.5.2.2. Methyl 4-hydroxy-4′-O-methyllunularate (**11**).* ^1H NMR (400 MHz): see text. ^{13}C NMR (100 MHz): δ 172.4 (C=O), 158.3 (C-4′), 150.2 (C-3), 144.0 (C-4), 135.6 (C-1), 134.5 (C-1′), 129.6 (C-2′ and C-6′), 122.6 (C-6), 119.0 (C-5), 114.2 (C-3′ and C-5′), 111.8 (C-2), 55.7 (4′-OCH_3), 52.9 (CO_2CH_3), 38.7 (C-α), 38.2 (C-β). *Diacetate* (prepared in situ): GC–MS R_i 2619: 386 $[M]^+$ (15), 302 (5), 284 (2), 270 (4), 269 (2), 181 (2), 149 (3), 121 (100), 91 (2), 78 (3), 77 (3), 43 (17).

*3.5.2.3. 9,10-Dihydro-5,6-dimethoxyphenanthren-2-ol (**13**).* ^1H (400 MHz) and ^{13}C NMR (100 MHz): Tables 1–2. *Acetate* (prepared in situ): GC–MS R_i 2344: 298 $[M]^+$ (39), 256 (100), 241 (40), 225 (2), 198 (4), 197 (5), 181 (8), 169 (3), 165 (5), 152 (4), 141 (4), 139 (2), 137 (6), 115 (6), 43 (21).

3.5.3. Sample C

In addition to the compounds described above, 9,10-dihydro-3,7-dimethoxyphenanthren-2-ol (**14**) was observed in the ^1H NMR spectrum of the $CDCl_3$ extract (concn approximately the same as that of **3**) and in the GC–MS of the acetylated extract. The compound was identified by comparison with ^1H NMR data in the literature (Anton et al., 1997). *Acetate* of **14** (prepared in situ):

GC–MS R_i 2479: 298 $[M]^+$ (22), 256 (100), 241 (26), 181 (1), 165 (1), 152 (4), 139 (2), 115 (1), 43 (14).

Acknowledgements

We thank Guillaume Hoizey for extracting sample B, David Long for examining several liverwort specimens, Professor Rüdiger Mues and Mr. Hermann Anton (Saarbrücken) for discussions and for providing a reference sample of the bibenzyl **11**, the Nepal–UK Forestry Research Project and the British Council for financial support (to DLS), and the Royal Society and the Association of Commonwealth Universities for the award of fellowships (to SFK and JS, respectively).

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