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3,4-DIHYDROXY-3′-METHOXYBIBENZYL FROM THE LIVERWORT *PLAGIOCHILA EXIGUA* FROM SCOTLAND†

DAVID S. RYCROFT,* W. JOHN COLE and NADIA ASLAM

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

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Key Word Index—*Plagiochila exigua*; Plagiochilaceae; Jungermanniales; Hepaticae; liverwort; NMR fingerprinting; ^{1}H NMR parameters; GC-MS; bibenzyl derivatives; 3,4-dihydroxy-3′-methoxybibenzyl.

Abstract—The ^{1}H NMR fingerprints of the $CDCl_3$ extracts of small quantities of three specimens of *Plagiochila exigua* from Scotland are very similar and are dominated by the spectrum of the new compound 3,4-dihydroxy-3′-methoxybibenzyl. This bibenzyl and its corresponding diacetate were isolated (approximately 15 μg and 70 μg respectively) from the crude and acetylated extracts using TLC. Characterization and structural elucidation by means of NMR and GC/GC-MS are reported. Comparison with literature data shows that a specimen of *P. exigua* from Peru differs considerably in its lipophilic constituents; the Scottish *P. exigua* is also distinct chemically, even more than the Peruvian, from *P. spinulosa*.

INTRODUCTION

The benefits of preparing a plant extract using the NMR solvent $CDCl_3$ and recording NMR spectra directly in conjunction with GC-MS and TLC investigations were demonstrated recently in a study of various specimens (both fresh and from herbaria) of the liverwort *Adelanthus decipiens* [1]. Application of NMR spectroscopy and the structural information derived from it greatly enhances the feasibility of determining the nature of secondary metabolites in small amounts of plant material [2] compared to the use of GC-MS alone. *Plagiochila exigua* (Tayl.) Tayl. is one of the nine species of *Plagiochila* found in Britain. It occurs occasionally in the western parts of Britain [3], but nowhere is it abundant; our new NMR technique is therefore ideal for investigating the chemistry of its oil bodies. This liverwort is not known to reproduce sexually: female plants are unknown in Britain although male plants are frequent [4]. It has been suggested that such populations of vegetatively reproducing liverworts are possibly derived from single clones [5, 6]. *P. exigua* also occurs in continental Europe and elsewhere [3, 4, 7]; in North America, *male* plants are unknown although a few female plants have been observed [6].

The only previous chemical study of *P. exigua* (as *P. corniculata*) is a GC-MS investigation of one specimen from southern Peru [8]; of the 11 compounds reported, only spathulenol and squalene were identified. A subsequent GC-MS study of *P. spinulosa* that reported 26 peaks and identified three (α-pinene, bicyclogermacrene and spathulenol) drew comparisons between *P. exigua* and *P. spinulosa* [9]. A major constituent with M_r 224 and a minor constituent with M_r 240 were reported to be common to both species. In addition there were statements to the effect that the gas chromatograms were "basically the same" and that chemically "both species are extremely similar"; however these statements are somewhat puzzling in relation to the published data, as the presence in *P. spinulosa* alone of a major diterpene with M_r 272 was mentioned as the "single important difference between [the] two species", but the presence of *P. exigua* alone of major peaks with M_r 300 and 330 was not discussed.

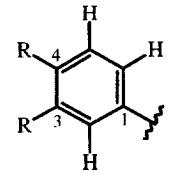
Here we report our investigations into the constituents of Scottish *P. exigua* and discuss chemical relationships both between Scottish and Peruvian material and with *P. spinulosa*.

RESULTS AND DISCUSSION

The ^{1}H NMR spectra of the $CDCl_3$ extracts of the three dried samples of *P. exigua* investigated were

† Part 3 in the series "NMR Fingerprinting of Liverworts". For Part 2 see Ref. [1].

*Author to whom correspondence should be addressed. E-mail: D.Rycroft@chem.gla.ac.uk



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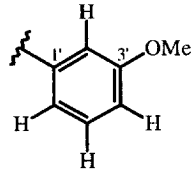

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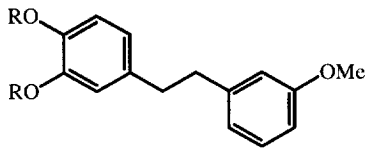
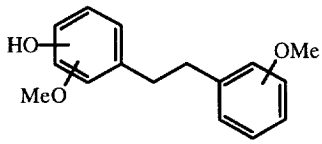
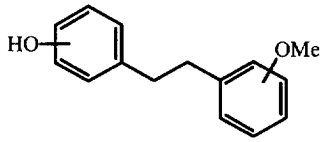


R	
3	H
4	Ac



5a,b



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extremely similar to each other. They indicated the existence of one dominant compound in concentrations of the order of 1 mM (equivalent to 0.2–0.7% dry weight of plant material) with, *inter alia*, one methoxyl group and seven aromatic protons. A spectral simulation based on the spin systems shown in **1** and **2** gave a good match to the observed spectrum. The methoxyl position was deduced from a NOE difference experiment. The suspicion at this stage was that R in **1** was OH and that fragments **1** and **2** were joined by CH_2CH_2 (consistent with signals at δ 2.8) to form the catechol-containing bibenzyl **3**. Some of the GC and GC-MS traces were adequate to observe a major compound, derive a retention index and measure a mass spectrum with a peak with highest $m/z = 244$. Acetylation and trimethylsilylation resulted in traces showing one dominant compound with highest $m/z = 328$ and 388 respectively, and confirmed the presence of two hydroxyl groups. Integration of the GC-MS TIC chromatograms showed that, apart from variable amounts of several fatty acids, the second most abundant compound was present to the extent of only 4% of the main compound (**3**). GC/GC-MS characterization of a methaneboronate derivative (M^+ 268, with the expected boron isotopic pattern) confirmed that **3** is a catechol derivative.

Attempts to isolate the new compound **3** using TLC were hampered by the tenacious hold of the catechol on the silica and recovered yields were low. Characterization using GC was possible by first acetylating the isolated material. A ^1H NMR spectrum of approximately 15 μg of isolated **3** was also measured (the weight of **3** was derived from comparison with the integral of the residual $CHCl_3$ signal). HR EI-MS of the recovered NMR sample confirmed the formula to be $C_{15}H_{16}O_3$.

Isolation of **4**, the diacetate of **3**, using TLC (of acetylated crude extract) was more straightforward. The ^1H NMR spectrum of approximately 70 μg of isolated diacetate contained readily interpreted aromatic proton signals as the three protons in the ring containing the two acetates are deshielded compared to their position in **3** and overlap is no longer a problem. The dominant component observed in the NMR fingerprint of *P. exigua* is therefore concluded to be 3,4-dihydroxy-3′-methoxybibenzyl (**3**). This compound is new, although close analogues are well known in liverworts [10]; the corresponding *E*-stilbene was isolated recently from a Colombian sample of the liverwort *Marchesina bongardiana* [11].

MS fragmentation patterns are particularly informative in studies of bibenzyls such as **3**: an important cleavage point is the centre of the 1,2-disubstituted ethane to form two substituted tropylium moieties. Thus the distribution of substituents between the two halves of a bibenzyl is readily determined. In the case of **3** the base peak has m/z 123 and arises from $[M-121]^+$, involving loss of the methoxybenzyl fragment from **3**. Although the minor constituents of the plant extracts have not been studied in detail, observation of this characteristic fragmentation has enabled three of the minor components present in all three extracts to be identified as bibenzyls. The ^1H NMR spectra show a methoxyl signal at δ 3.839 with intensity 5% of that of the methoxyl signal of **3**. The only peak in the GC-MS TIC trace of the acetylated extracts that can be related to this NMR signal has an intensity 4% of that of **4**; this peak gave a parent ion at m/z 300 and showed sequential loss of 42 and 121 to give the base peak at m/z 137 and indicating loss of a methoxybenzyl fragment as was observed in **3**. The corresponding peak in the unacetylated extracts gave a parent ion at m/z 258 and a base peak at m/z 137. Therefore this compound has one hydroxyl group and consists of methoxybenzyl and hydroxymethoxybenzyl fragments making up the bibenzyl **5a**, where the substitution patterns of the two rings are unknown. A second minor component, 0.3–0.6% of the abundance of **4**, showed similar acetylation and MS fragmentation behaviour and is assigned an isomeric bibenzyl structure, designated **5b**. Interestingly, the elution order of the trimethylsilyl ethers of **5a** and **5b** was reversed compared to that of the acetates. The third minor bibenzyl, 1–2% of the abundance of **4**, showed a parent ion at m/z 270 in the acetylated extract, compared to 228 before acetylation, with a base peak at m/z 107 again indicating loss of a methoxybenzyl fragment; this compound was therefore assigned the general structure **6**. Specific

compounds agreeing with these structures have been reported previously from liverworts, namely 3-hydroxy-4,3′-dimethoxybibenzyl [12] and 4-hydroxy-3′-methoxybibenzyl [13–15].

The lipophilic constituents of the Scottish samples of *P. exigua* investigated here show little resemblance to those of *P. spinulosa* or to those of the Peruvian sample of *P. exigua* studied previously. For the latter sample there is a minor component shown [8] (base peak in parentheses) as *m/z* 258 (137) that may correspond to **5a** or **5b**. Some bibenzyl derivatives have been found in *P. spinulosa* [16], but the Scottish *P. exigua* does not contain any of the dihydrophenanthrenes that are perhaps more characteristic of *P. spinulosa* [17, 18], and neither the compound shown as *m/z* 224 (193) observed previously in both *P. exigua* [8] and *P. spinulosa* [9] as a major component, that has been demonstrated to be methyl 2-methyl-3,4-methylenedioxy-6-methoxybenzoate [17, 18], nor the minor component shown as *m/z* 240 (240) that we now recognize as methyl 2-methyl-3,4,6-trimethoxybenzoate [Hughes, M., Connolly, J. D. and Rycroft, D. S., unpublished results]. The other two major compounds found previously [8] in *P. exigua* were described as *m/z* 300 (179) and 330 (179); these are strong candidates for assignment as bibenzyls but, whatever their structures are, they clearly provide additional means to distinguish the Peruvian from the Scottish material (and, incidentally, from *P. spinulosa*). In view of this indication of population diversity in *P. exigua* it would be of interest to use the present results to make a chemical comparison of these Scottish *P. exigua* specimens collected from a limited area with additional material collected further afield.

EXPERIMENTAL

General

TLC, GC, GC-MS, HR EI-MS, and ^{1}H NMR (360 MHz, $CDCl_3$) were performed as described previously [1]. The Bruker program PANIC was used for spectral simulations.

Plant material

Three samples of *Plagiochila exigua* were collected by D.S.R. from different locations in Glen Creran, Argyll, Scotland; voucher specimens are deposited in the University of Glasgow Herbarium (GL). The specimens studied were: (i) no. 96144, collected 13th August 1996, 78 mg extracted 6th September 1996; (ii) no. 96267, collected 28th September 1996, 184 mg extracted 6th February 1997; (iii) no. 96140, collected 13th August 1996, 24 mg extracted 6th February 1997. Extracts were prepared by triturating dried plant material with sufficient $CDCl_3$ to produce 0.6–0.7 ml of a filtered soln. The concentration of **3** in extracts (i), (ii) and (iii) was 1, 3 and 1 mM respectively. Minor compounds in the extracts and derivatives were characterized *in situ* using GC/GC-MS.

*3,4-Dihydroxy-3′-methoxybibenzyl (**3**).* Isolated from a crude liverwort extract using TLC (ca 15 μg). GC: R_i 2250. HR EI-MS *m/z* (rel. int.): found 244.1084 $[M]^+$ (32) ($C_{15}H_{16}O_3$ requires 244.1099), 123.0447 (100), 121.0649 (19), 91.0539 (15). ^{1}H NMR: δ 2.827 (4H, *s*, CH_2CH_2), 3.784 (3H, *s*, MeO); chemical shifts from spectral simulation: δ 6.616 (H-6), 6.699 (H-2), 6.710 (H-2′), 6.739 (H-4′), 6.766 (H-6′), 6.769 (H-5), 7.191 (H-5′); coupling constants from spectral simulation: *J* H-2/H-6 = 1.9, H-2/H-5 = 0.5, H-5/H-6 = 8.1, H-2′/H-4′ = 2.65, H-2′/H-5′ = 0.5, H-2′/H-6′ = 1.6, H-4′/H-5′ = 8.3, H-4′/H-6′ = 1.0, H-5′/H-6′ = 7.6 Hz. Bis(trimethylsilyl ether): GC: R_i 2260. GC-MS *m/z* (rel. int.): 388 $[M]^+$ (31), 373 (4), 267 $[M-121]^+$ (100), 179 (27), 149 (4), 121 (3). Methaneboronate: GC: R_i 2007. GC-MS *m/z* (rel. int.): 268 $[M]^+$ (23), 267 (5), 147 $[M-121]^+$ (100), 146 (27), 121 (5), 77 (10), 51 (5).

*3,4-Diacetoxy-3′-methoxybibenzyl (**4**).* A crude liverwort extract was acetylated and **4** isolated using TLC (approximately 70 μg). GC: R_i 2345. HR EI-MS *m/z* (rel. int.): found 328.1306 $[M]^+$ (47) ($C_{19}H_{20}O_5$ requires 328.1311), 244.1080 (100), 123.0442 (83), 121.0645 (25). ^{1}H NMR: δ 2.281, 2.284 (3H × 2, *s* × 2, AcO × 2), 2.899 (4H, *s*), 3.779 (3H, *s*, MeO), 6.699 (1H, *dd*, *J* = 2.5, 1.8 Hz, H-2′), 6.750 (1H, *ddd*, *J* = 8.0, 2.5, 0.8 Hz, H-4′), 6.775 (1H, *ddd*, *J* = 7.6, 1.8, 0.8 Hz, H-6′), 7.017 (1H, *d*, *J* = 1.9 Hz, H-2), 7.047 (1H, *dd*, *J* = 8.3, 1.9 Hz, H-6), 7.087 (1H, *d*, *J* = 8.3 Hz, H-5), 7.203 (1H, *dd*, *J* = 8.0, 7.6 Hz, H-5′).

*Methoxy[2-(methoxyphenyl)ethyl]phenol (**5a**).* GC: R_i 2099. GC-MS *m/z* (rel. int.): 258 $[M]^+$ (24), 137 $[M-121]^+$ (100), 122 (8), 121 (6). Monoacetate: GC: R_i 2224. GC-MS *m/z* (rel. int.): 300 $[M]^+$ (10), 258 $[M-42]^+$ (26), 137 $[M-42-121]^+$ (100), 122 (5), 121 (11), 91 (5), 43 (14). Trimethylsilyl ether: GC: R_i 2195. GC-MS *m/z* (rel. int.): 330 $[M]^+$ (29), 209 $[M-121]^+$ (100), 179 (16), 149 (5), 121 (6).

*Methoxy[2-(methoxyphenyl)ethyl]phenol (**5b**).* GC: R_i 2120. GC-MS *m/z* (rel. int.): 258 $[M]^+$ (22), 137 $[M-121]^+$ (100), 122 (4). Monoacetate: GC: R_i 2240. GC-MS *m/z* (rel. int.): 300 $[M]^+$ (6), 258 $(M-42]^+$ (21), 137 $[M-42-121]^+$ (100), 43 (15). Trimethylsilyl ether: GC: R_i 2186. GC-MS *m/z* (rel. int.): 330 $[M]^+$ (26), 209 $[M-121]^+$ (100), 179 (33).

*[2-(Methoxyphenyl)ethyl]phenol (**6**).* GC: R_i 2027. GC-MS *m/z* (rel. int.): 228 $[M]^+$ (21), 122 (4), 121 (7), 107 $[M-121]^+$ (100), 91 (5), 77 (11). Monoacetate: GC: R_i 2090. GC-MS *m/z* (rel. int.): 270 $[M]^+$ (13), 228 $[M-42]^+$ (9), 121 (7), 107 $[M-42-121]^+$ (100), 43 (10). Trimethylsilyl ether: GC: R_i 2075. GC-MS *m/z* (rel. int.): 300 $[M]^+$ (12), 179 $[M-121]^+$ (100), 121 (2).

Acknowledgements—D.S.R. thanks Gordon Rothero for demonstrating *Plagiochila exigua* during the first week of the British Bryological Society 1996 Summer Field Meeting.

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