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Killarniensolide, methyl orsellinates and 9,10-dihydrophenanthrenes from the liverwort *Plagiochila killarniensis* from Scotland and the Azores[1]

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Abstract

Methyl everninate has been identified as the major constituent of the deuterochloroform extract of ten specimens of the liverwort *Plagiochila killarniensis*. Using NMR fingerprinting and GC–MS, the new methyl 6-hydroxy-2-methyl-3,4-methylenedioxybenzoate and three other methyl orsellinate derivatives were identified as minor components. 9,10-Dihydro-3,5-dimethoxyphenanthren-2-ol and 9,10-dihydro-3-methoxyphenanthrene-4,5-diol, previously reported from a Neotropical *Plagiochila*, were also present. Killarniensolide, a new phthalide, was isolated; its structure was elucidated as 3-(2-hydroxy-4,5-dimethoxybenzyl)-7-methoxyphthalide by NMR spectroscopy, GC–MS and isolation of an acetate derivative. Chemical differences between specimens from Scotland and the Azores were relatively minor but characteristic; small differences between some of the Scottish materials were also observed.

Keywords: *Plagiochila killarniensis*; *Plagiochila bifaria*; Hepaticae; Liverworts; NMR fingerprinting; GC–MS; ^{1}H NMR parameters; Aromatic compounds; Methyl everninate

1. Introduction

We are engaged in a systematic chemical study of British liverworts, focussing on British representatives of the genus *Plagiochila* (Connolly et al., 1999; Rycroft, Cole & Aslam, 1998; Rycroft & Cole, 1998), and are applying our NMR fingerprinting method (Rycroft, 1996) in conjunction with GC/GC–mass spectrometry and TLC to identify the major lipophilic constituents in species that are generally available only in small quantities, either because of scarcity in the field or because the specimens under investigation are vouchers from herbaria (Rycroft, Cole, & Rong, 1998).

The liverwort known in recent years as *Plagiochila killarniensis* Pears. (Pearson, 1905) was described originally over 100 years ago (Carrington, 1874) but for most of this century was regarded, if it was distinguished at all, as a variety of *P. spinulosa*, until Paton (1977) established that it is indeed distinct specifically. A current proposal (Heinrichs, Grolle, & Drehwald, 1998) is that *P. killarniensis* is synonymous with the much older Neotropical *P. bifaria* (Sw.) Lindenb.; both names are retained for the purposes of this paper in view of the distinctive chemistry observed. *P. killarniensis* is known as one of the Atlantic species and is occasional, even locally frequent, in Britain, but restricted to areas close to the western seaboard (Averis, 1991). Grolle and Schumacker (1982) compared the ranges and distributions of *P. killarniensis* and *P. spinulosa* in Europe and Macaronesia and discovered that *P. killarniensis* has a more southern and *P. spinulosa* a more northern tendency. In Britain, *P. killarniensis*, at the northern limit of its range, is not known to produce sporophytes even though male and female plants have been found intermixed, but in the more favourable conditions of the Azores, not only is *P. killarniensis* abundant, it is also found commonly with sporophytes (Bates & Gabriel, 1997). *P. killarniensis* does not produce any specialized means of vegetative propagation and the regional difference in reproductive behaviour may result in regional genetic variation. Chemical investigation is of

[1] Part 8 in the series 'NMR Fingerprinting of Liverworts'. For Part 7 see Connolly et al. (1999).

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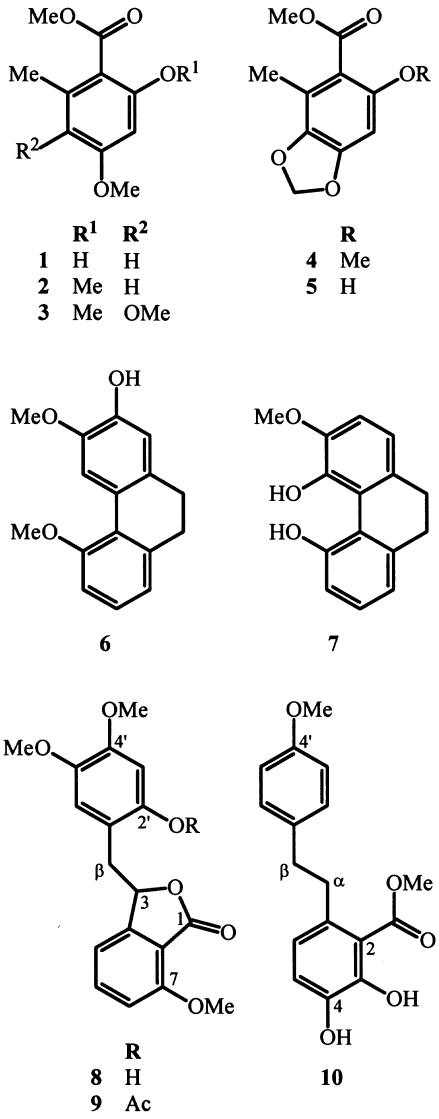
interest to see if there is any regional variation of the secondary metabolites.

A detail of chemical significance that has not been published widely (Bates, 1994) is that *P. killarniensis* has a reputation amongst bryologists for a distinctive earthy smell that becomes apparent while working with relatively new material from herbarium packets, and that is not encountered when dealing with *P. spinulosa*. However, it should be noted that the field experience of the nose of D.S.R. (and others) is that both *P. killarniensis* and *P. spinulosa* (and also, incidentally, the related *P. punctata*) produce a strong 'aromatic' smell when fresh material is crushed; this may be attributable to β-phellandrene, that we have found to be present in all three species.

We have investigated material of *P. killarniensis* from various substrates in several localities in Scotland and the Azores, and now report the identification and isolation of methyl everninate as the dominant lipophilic constituent. In addition, four minor methyl orsellinate derivatives (including one new one), two known 9,10-dihydrophenanthrenes and some common terpenes have been identified. We also report the isolation and structural elucidation of killarniensolide, a new phthalide derivative.



2. Results and discussion

Six specimens of *P. killarniensis* from Scotland and four from the Azores were investigated. The GC–mass spectrometry data of the 20 most abundant compounds in the $CDCl_3$ extracts are summarized in Table 1. Data were obtained for both the untreated and the acetylated extracts (except No. 96088 and 96194); because of strong adsorption on the column, peaks from some of the polar components were weak or absent unless the extract was acetylated. Extract 98013 was treated with methoxylamine hydrochloride in pyridine but no oxime derivatives were observed by GC–mass spectrometry.

The methoxyl region of the ^{1}H NMR spectrum of the $CDCl_3$ extract of each sample was dominated by two signals of equal intensity. Signals of appropriate intensity were also observed for a chelated hydroxyl proton (δ_H 11.77) and a pair of shielded *meta*-coupled aromatic protons (δ_H 6.29 and 6.33), showing long-range coupling to an aromatic methyl substituent (δ_H 2.50). Peak 5 in the GC–mass spectrum confirmed the presence of a dominant constituent in the extracts, with $[M^+]$ m/z 196; acetylation increased the retention time and gave $[M^+]$ m/z 238. These data indicated that the compound was methyl everninate (**1**); confirmation was obtained by isolating it using TLC ($[M^+]$ m/z 196.0746) and comparing ^{1}H NMR data with the literature (Sala & Sargent, 1981).

The GC–mass spectrometry data in Table 1 indicated the presence of variable amounts of several minor aromatic and terpenoid components in the extracts. β-Phellandrene, germacrene D and bicyclogermacrene were assigned by comparison of retention indices and mass spectral fragmentation patterns with the literature (Adams, 1995). Peaks 2 and 7 are substantial and presumed to be of sesquiterpenoid origin but, along with several other peaks, are unidentified. Peak 6 is very small and does not move on acetylation; structure **2**, the methyl ether of methyl everninate (apparently unreported previously in nature), was assigned on the basis of the GC–MS data and confirmed by co-injection with an authentic sample. Peaks 12 and 9 arise from compounds **3** and **4** with the structures that we have reported (Rycroft et al., 1998a; Connolly et al., 1999) to be assignable to two components observed previously in *P. spinulosa* and a Peruvian specimen of *P. exigua* (Inoue & Asakawa, 1988). Peak 8 arises from a new compound (**5**) closely related to **4**, but movement on acetylation shows the presence of a hydroxyl group. Both **4** ($[M^+]$ m/z

Table 1
Occurrence (based on GC–MS) of twenty components[a] in ten $CDCl_3$ extracts of *Plagiochila killarniensis*

Peak No.[d]	Assignment and structure No.	R_i	M_r	Base peak	Collection No. (Relative abundance from GC–MS TIC integration[e])									
					96088[b,c]	96146	96194[c]	96288	97032	98013	RG 3126	RG 3127	RG 3129	RG 3130
1	*β*-phellandrene	1015	136	93	+	9	7	9	10	3	5	4	5	5
2	–	1386	222	137	8	30	–	16	19	22	20	15	20	16
3	germacrene D	1457	204	161	–	2	2	+	+	+	1	1	2	1
4	bicyclogermacrene	1475	204	121	–	4	5	4	5	–	3	3	5	4
5	methyl everninate (**1**)	1546	196	164	100	100	100	100	100	100	100	100	100	100
5-Ac		1686	238	164										
6	Me 2,4-$(OMe)_2$-6-Me-benzoate (**2**)	1575	210	179	+	2	1	2	2	2	+	–	–	+
7	–	1581	220	107	1	32	35	16	19	19	17	20	16	15
8	Me 6-OH-2-Me-3,4-(OCH_2O)-benzoate (**5**)	1618	210	178	22	13	14	10	12	10	2	2	3	2
8-Ac		1761	252	178										
9	Me 6-OMe-2-Me-3,4-(OCH_2O)-benzoate (**4**)	1643	224	193	26	16	15	29	23	23	1	+	1	1
10	–	1681	236	109	+	14	7	10	10	1	2	2	2	2
11	–	1695	236	109	+	12	14	6	+	9	8	9	10	8
12	Me 3,4,6-$(OMe)_3$-2-Me-benzoate (**3**)	1706	240	240	+	1	1	2	1	1	+	–	+	+
13	–	1812	272	135	–	1	+	3	2	–	2	1	2	2
14	–	1828	270	68	+	1	1	4	2	+	+	+	1	+
15	fusicoccadiene	1963	272	135	–	1	3	2	1	+	2	+	1	2
16	–	2203	304	147	–	2	1	1	+	2	3	1	3	2
17	4,5-$(OH)_2$-3-OMe-9,10-DHP (**7**)		242		–	–	–	–	–	–	–	–	–	–
17-Ac_2		2227	326	242		7		1	9	13	8	9	14	8
18	2-OH-3,5-$(OMe)_2$-9,10-DHP (**6**)	2216	256	256	–	7	9	1	2	4	6	6	3	2
18-Ac		2375	298	256										
19	2-CO_2Me-3,4-$(OH)_2$-4′-OMe-bibenzyl (**10**)		302		–	–	–	–	–	–	–	–	–	–
19-Ac_2		2622	386	121		+		+	3	–	–	–	–	–
20	killarniensolide (**8**)	2775	330	167	–	+	4	14	+	5	4	4	9	4
20-Ac	(**9**)	2806	372	167		8		20	12					

[a] Peaks are included if the integrated intensity in any extract was $\geq 2\%$ of that of methyl everninate (peak 5).
[b] The composition of extract 96088 changed considerably between the GC and GC–MS runs: e.g. a large peak (31% of peak 5) attributed to methyl chloroeverninate appeared, and peak 1 almost disappeared.
[c] Extracts 96088 and 96194 were not acetylated.
[d] Data for peaks with the suffix-Ac refer to the acetylated extracts.
[e] + Indicates that this peak was detectable at a low level.

224.0679) and **5** ([M$^+$] m/z 210.0536) were present as minor components in the sample of methyl everninate (**1**) isolated using TLC. Structure **5** was assigned by considering the GC–mass spectrometry data and comparing the ^{1}H NMR data of **4** and **5**, which show that the hydroxyl proton is chelated as in methyl everninate (**1**) and that the oxygen substitution pattern in **5** is the same as in **4**.

GC peaks 17 and 18 correlate with respect to intensity variation between samples with some ^{1}H NMR signals that are observed between δ 6.8 and 8.0, that correspond with data reported for compounds **6** and **7**, two of the 9,10-dihydrophenanthrenes isolated from an unidentified *Plagiochila* species from Costa Rica (Anton, Kraut, Mues, & Morales, 1997). The structural assignments were confirmed by GC–mass spectrometric comparison with authentic materials.

An aromatic proton signal was distinctive in the ^{1}H NMR spectra of all the extracts as a doublet of doublets (J=8.2, 7.6 Hz) at δ 7.58. The set of ^{1}H NMR signals relating to this signal and corresponding to the GC peak 20 from compound **8** was also deduced by comparison of peak intensities in the spectra of the different extracts, the low abundance of **6** and **7** in 96088 and 96288 being particularly fortuitous. The mass spectrum ([M$^+$] m/z 330) indicated that the compound was a bibenzyl derivative, with central cleavage giving m/z 167 and 163 fragments. Acetylation demonstrated the presence of one hydroxyl group and produced a more satisfactory peak shape, thus providing a more reliable means of detecting the compound. **8** was isolated (ca. 150 μg) using TLC and characterized using GC–mass spectrometry and ^{1}H NMR; the acetylation product of this material was also isolated (ca. 15 μg) using TLC and characterized using GC–mass spectrometry, ^{1}H NMR and HREI mass spectrometry, which confirmed the molecular formulae of the acetate to be $C_{20}H_{20}O_7$, of **8** to be $C_{18}H_{18}O_6$, and of the benzyl fragments to be $C_9H_{11}O_3$ and $C_9H_7O_3$. An ABX-system was observed for the protons of the trisubstituted ethane moiety of the bibenzyl, with a proton at δ 5.69 geminal to oxygen. The five aromatic protons are present as two mutually *para* protons in one ring and three adjacent protons in the other. Incorporation of two of the three methoxyl groups into a hydroxydimethoxybenzyl group generates a m/z 167 fragment. The m/z 163 fragment has two degrees of unsaturation (additional to those of a benzyl group) that can be accommodated in a methoxyphthalide fragment. The position of substituents was based partly on the results of NOE difference experiments, that were undertaken using one of the $CDCl_3$ plant extracts, rather than the isolated compound for reasons of sensitivity. NOEs showed that in the substituted benzyl group each aromatic proton is *ortho* to a different methoxyl group, so that the pattern is either 2-hydroxy-4,5-dimethoxy or 4-hydroxy-2,5-dimethoxy. The placing of the hydroxyl group was decided by comparing the chemical shifts of the benzylic and phthalide protons in **8** and its acetate **9**; if the hydroxyl group were *para* to the benzylic methylene, very little change would be expected on acetylation, whereas acetylation of a hydroxyl group *ortho* to the benzylic methylene could perturb conformational equilibria of the benzyl group. The benzylic and phthalide protons of **8** do shift slightly on acetylation and the hydroxyl was therefore placed in the *ortho*-position. In the phthalide, a proton at position 7 would be deshielded by the carbonyl group; as both doublets of the aromatic three spin system have similar chemical shifts, the methoxyl was placed on C-7 and H-6 assigned by correlation through a NOE. Compound **8** is therefore 3-(2-hydroxy-4,5-dimethoxybenzyl)-7-methoxyphthalide, to which we have given the name killarniensolide. The absolute configuration is unknown.

The GC peak 19-Ac_2, observed with only three of the acetylated extracts, arose from the diacetate of the lunularic acid derivative **10** that is abundant in *P. spinulosa* (Rycroft, 1990; Connolly et al., 1999). Observation of this compound raises the question of the significance of components present at very low levels and the question of the contribution made by contaminants in the plant material. The practicalities of handling leafy liverworts dictate that while it is possible to sort and check the identity of individual shoots, it is not feasible to remove contaminants from the plant surfaces. Extract 97032 was the only one with a nontrace amount of **10** and it may not be coincidental that this plant was actually growing through a mat of *P. spinulosa*; the presence of *P. spinulosa* debris adhering to the shoots of *P. killarniensis* cannot be excluded.

Killarniensolide (**8**) is a rare addition to the small 3-benzylphthalide class of natural products, that (apart from four alkaloidal members) is found only in liverworts (Dictionary of Natural Products on CD-ROM, 1997). NMR fingerprinting showed that it was present in rather similar proportions (ca. 10% of the methyl everninate) in all the plant specimens. This is the first time that methyl everninate (**1**) has been found as a major chemical constituent of a liverwort; previously it has been reported as a minor component of a *Blasia pusilla* extract, where it was thought it might have been derived from symbiotic *Nostoc* algal colonies that grow in the thalli (Yoshida et al., 1996). Methyl everninate is well-known (Ohloff, 1994) as one of the odour-impact compounds of oakmoss (the lichen, *Evernia prunastri*) and the large amount present in *P. killarniensis* is evidently responsible for the musty smell familiar to bryologists working with herbarium packets of the liverwort, whereas the more volatile monoterpene β-phellandrene (present in large amounts in essential oils of, for example, various species of *Eucalyptus*, *Pinus* and *Abies*) is probably more responsible for the smell produced by crushing fresh plant material in the field.

Comparison between data for material from Scotland and the Azores revealed relatively minor differences, and other differences were observed between some Scottish specimens. Compounds **4** and **5** were in low amount in Azores material and abundant (but with variable ratios) in Scottish material. Dihydrophenanthrenes **6** and **7** were abundant in all but two of the Scottish specimens (96088 and 96288), where, however, they were still detectable by NMR. Geographical separation and sexual reproduction in one area only has not generated large differences in the patterns of the secondary metabolites of *P. killarniensis* from Scotland and the Azores.

The methyl orsellinates and dihydrophenanthrenes identified provide chemical links from *P. killarniensis* to *P. spinulosa* (Connolly et al., 1999) and the Costa Rican *Plagiochila* (Anton et al., 1997), respectively. None of the dihydrophenanthrenes of *P. spinulosa* are significant in *P. killarniensis*, but some dihydrophenanthrenes and the bibenzyl **10** are common to both *P. spinulosa* and the Costa Rican *Plagiochila* (the revision of the structure reported in Anton et al. (1997) for bibenzyl **10** is presented in Connolly et al. (1999)). The chemical relationships between European and Neotropical *Plagiochilae* are of phytogeographical interest, particularly in the context of the chemical relationships that we have demonstrated recently between British and Neotropical *Adelanthus decipiens* (Rycroft et al., 1998b). The significance of the chemical similarities observed in the present study, should, however, be judged in the context of known differences. For example, cyclic *bis*bibenzyls have been found (Anton et al., 1997; Valcic, Zapp, & Becker, 1997) in Neotropical *Plagiochilae* but are unknown in European *Plagiochilae*. In addition, none of the GC–mass spectrometry profiles in a study (Asakawa & Inoue, 1987) of 30 Neotropical *Plagiochilae* (from Peru) is consistent with the presence of methyl everninate (**1**). One specimen of *P. bifaria* was included in that study (Asakawa & Inoue, 1987) and is clearly distinct chemically from *P killarniensis*, in contrast to the recent morphological studies (Heinrichs et al., 1998). Present indications are that the chemosystematic position of *P. killarniensis* lies with *P. spinulosa* in a chemotype characterized by 9,10-dihydrophenanthrenes (Rycroft, 1998), but further work is required in order to determine whether the group description might be refined and described, for example, as a 9,10-dihydrophenanthrene–methyl orsellinate–bibenzyl chemotype. We are currently investigating Bolivian material of *P.* sect. *Arrectae* Carl: initial results (Anton, H., Mues, R., Heinrichs, J, Gradstein, S. R., Cole, W. J. and Rycroft, D. S., work in progress) have demonstrated chemical affinity with both *P. killarniensis* and *P. spinulosa*, in that both the dihydrophenanthrene **6** and the bibenzyl catechol **10** have been identified among the major components. We will also study Neotropical *P. bifaria* (which is also placed in sect. *Arrectae*).

3. Experimental

3.1. General

TLC, GC, GC–MS, HR EI–MS and ^{1}H NMR (360 or 400 MHz, $CDCl_3$) were performed as described previously (Rycroft et al., 1998b).

3.2. Plant material

Specimens of *P. killarniensis* from the Azores were collected in October 1997 by RG from three sites at Terra Brava, Agualva, on Terceira; the other specimens of *P. killarniensis* were collected by DSR in the west of Scotland. Vouchers have been deposited in the University of Glasgow herbarium (GL) except where stated. The following were used to prepare solutions for the NMR and GC/GC–MS studies: (i) No. 96088, collected 3rd August 1996 (in rock cleft, ca. 100 m west of the waterfall Eas Fors, Isle of Mull, v.-c. 103 Mid Ebudes), 54 mg extracted 7th December 1996; (ii) No. 96146, collected 13th August 1996 (on ash tree, Glasdrum N.N.R., Glen Creran, v.-c. 98 Argyll), 84 mg extracted 12th September 1996; (iii) No. 96194, collected 16th August 1996 (tuft on large calcareous schistose boulder below Meall Mór, Glen Coe, v.-c. 98 Argyll), 101 mg extracted 26th August 1996; (iv) No. 96288, collected 27th December 1996 (patch on boulder near the Pollochro Burn, Loch Lomond, 2 km north of Inversnaid, v.-c. 86 Stirling), 43 mg extracted 30th December 1996; (v) No. 97032 (BBSUK), collected 11th May 1997 (growing through mat of *P. spinulosa* covering rock outcrop, Arrochymore Point, Loch Lomond, 1 km northwest of Balmaha, v.-c. 86 Stirling), 75 mg extracted 12th May 1997; (vi) No. 98013, collected 10th April 1998 (on hazel trunk, S. side of Loch Creran, v.-c. 98 Argyll), 55 mg extracted 18th April 1998; (vii) No. RG 3126 (site C, on *Vaccinium cylindraceum*), 40 mg extracted 25th April 1998; (viii) No. RG 3127 (site B, on *V. cylindraceum*), 38 mg extracted 15th November 1997; (ix) No. RG 3129 (site A, on *Laurus azorica*), 107 mg extracted 15th November 1997; (x) No. RG 3130 (site A, on *L. azorica*), 39 mg extracted 25th April 1998.

3.3. Extraction and isolation

Extracts were prepared by triturating dried (except in the case of no. 96288, which was fresh and only partially dry) plant material with sufficient $CDCl_3$ to produce 0.6–0.7 ml of a filtered solution. The concentration of methyl everninate in the extracts (based on NMR integration and comparison with the residual $CHCl_3$ signal) was (i) 3, (ii) 3, (iii) 1, (iv) 5, (v) 5, (vi) 10, (vii) 8, (viii) 5, (ix) 12 and (x) 10 mM. Compounds in the extracts and acetylation derivatives were characterized in situ using GC/GC–MS. The remainder of these solutions was car-

ried over for the TLC studies, which also required extracts of additional material (57 mg of No. 96088 and 140 mg of dried No. 96288). TLC gave a band (R_f 0.68) containing methyl everninate (**1**) (ca. 50 μg), methyl 6-methoxy-2-methyl-3,4-methylenedioxybenzoate (**4**) (ca. 8 μg) and methyl 6-hydroxy-2-methyl-3,4-methylenedioxybenzoate (**5**) (ca. 8 μg). Another TLC band (R_f 0.30) contained killarniensolide (**8**) (ca. 150 μg).

3.4. Methyl everninate (*1*)

HREIMS: *m/z* 196.0746 $[M]^+$ ($C_{10}H_{12}O_4$ requires 196.0736). GC–MS *m/z* (rel. int.): 196 (40), 164 (100), 136 (37), 121 (15), 108 (4), 93 (16), 77 (6), 69 (5), 65 (7), 53 (4), 51 (4), 39 (7). ^{1}H NMR (360 MHz): δ 2.50 (s, 6-Me), 3.80 (s, 4-OMe), 3.93 (s, CO_2Me), 6.29 (dq, $J=2.6$, 0.8 Hz, H-5), 6.33 (d, $J=2.6$ Hz, H-3), 11.77 (s, 2-OH).

3.5. Methyl 6-methoxy-2-methyl-3,4-methylenedioxybenzoate (*4*)

HREIMS: *m/z* 224.0679 $[M]^+$ ($C_{11}H_{12}O_5$ requires 224.0685). GC–MS *m/z* (rel. int.): 224 (81), 209 (5), 193 (100), 192 (21), 178 (18), 177 (14), 163 (4), 147 (6), 135 (6), 119 (6), 107 (3), 96 (5), 79 (6), 77 (11), 69 (4), 67 (8), 66 (5), 64 (5), 63 (6), 59 (3), 53 (10), 51 (6), 50 (4), 39 (4). ^{1}H NMR (360 MHz): δ 2.17 (s, 2-Me), 3.77 (s, OMe), 3.89 (s, OMe), 5.93 (s, OCH_2O), 6.41 (s, H-5).

3.6. Methyl 6-hydroxy-2-methyl-3,4-methylenedioxybenzoate (*5*)

HREIMS: *m/z* 210.0536 $[M]^+$ ($C_{10}H_{10}O_5$ requires 210.0528). GC–MS *m/z* (rel. int.): 210 (33), 178 (100), 150 (7), 121 (5), 94 (5), 92 (5), 80 (10), 67 (12), 66 (10), 53 (12), 39 (7). ^{1}H NMR (360 MHz): δ 2.38 (s, 2-Me), 3.92 (s, OMe), 5.93 (s, OCH_2O), 6.36 (s, H-5), 11.71 (s, 2-OH).

3.7. Killarniensolide (*8*) [3-(2-hydroxy-4,5-dimethoxybenzyl)-7-methoxyphthalide]

GC–MS *m/z* (rel. int.): 330 $[M]^+$ (20), 167 (100), 163 (10), 139 (4), 124 (3), 123 (3), 109 (2), 92 (3), 77 (6), 69 (6). ^{1}H NMR (360 MHz): δ 3.10 (dd, $J=14.7$, 6.3 Hz, H-β), 3.28 (dd, $J=14.7$, 5.1 Hz, H-β), 3.72 (s, 5′-OMe), 3.82 (s, 4′-OMe), 3.96 (s, 7-OMe), 5.69 (t, $J=6$ Hz, H-3), 6.45 (s, H-3′), 6.46 (s, H-6′), 6.89 (d, $J=8.2$ Hz, H-6), 6.92 (d, $J=7.6$ Hz, H-4), 7.58 (dd, $J=8.2$, 7.6 Hz, H-5).

3.8. Killarniensolide acetate (*9*)

A sample of killarniensolide that had been isolated by TLC was acetylated (Ac_2O/py) and the *acetate* (ca. 15 μg) isolated by TLC. HREIMS *m/z* (rel. int.): 372.1190 $[M]^+$ (7) ($C_{20}H_{20}O_7$ requires 372.1209); 330.1091 $[M-CH_2CO]^+$ (44) ($C_{18}H_{18}O_6$ requires 330.1103); 167.0705 $[M-CH_2CO-C_6H_3(OMe)CO_2CH]^+$ (100) ($C_9H_{11}O_3$ requires 167.0708); 163.0401 $[M-CH_2CO-C_6H_2(OH)(OMe)_2CH_2]^+$ (20) ($C_9H_7O_3$ requires 163.0395). GC–MS *m/z* (rel. int.): 372 $[M]^+$ (4), 330 (33), 167 (100), 163 (13), 139 (2), 124 (2), 92 (2), 77 (5), 43 (8). ^{1}H NMR (360 MHz): δ 2.32 (s, OAc), 2.96 (dd, $J=14.3$, 6.1 Hz, H-β), 3.10 (dd, $J=14.3$, 6.4 Hz, H-β), 3.83 (s, OMe), 3.85 (s, OMe), 3.98 (s, OMe), 5.55 (t, $J=6$ Hz, H-3), 6.61 (s, H-3′), 6.70 (d, $J=7.8$ Hz, H-6), 6.73 (s, H-6′), 6.91 (d, $J=8.3$ Hz, H-4), 7.54 (t, $J=8$ Hz, H-5).

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