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Amorphane sesquiterpenoids from the liverwort *Marsupella emarginata* var. *aquatica*

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Abstract—Four oxygenated amorphane derivatives [(−)-(1*R*,2*S*,6*R*,10*S*)-2α-acetoxyamorpha-4,7(11)-dien-8-one, (−)-(1*R*,2*S*,6*R*,10*S*)-2α-acetoxy-11-methoxyamorpha-4,7-diene, (−)-(1*R*,2*R*,3*S*,6*R*,10*S*)-2α,3α-diacetoxyamorpha-4,7(11)-dien-8-one and (−)-(1*R*,2*R*,3*R*,6*R*,9*S*,10*R*)-2α,3α-diacetoxy-9α-hydroxyamorpha-4,7(11)-dien-8-one] have been isolated from the Scottish liverwort *Marsupella emarginata* var. *aquatica*. Their structures were determined following extensive (mainly 1D and 2D NMR) spectroscopic studies.

1. Introduction

Marsupella emarginata (Ehrh.) Dumort. var. *aquatica* (Lindenb.) Dumort. [syn. *M. aquatica* (Lindenb.) Schiffn.] (Hepaticae) is a robust, dark coloured plant, from 1–8 cm in length, which is common in mountain streams in Scotland. Some authorities consider *M. aquatica* to be a variety of *M. emarginata* while others regard it as a distinct species.[1] Previously, four closely related species, namely *M. emarginata* ssp. *tubulosa*,[2] *M. emarginata* var. *patens*,[3,4] *M. emarginata*[5,6,7] and *M. aquatica*[8,9] have been chemically investigated. *ent*-Longipinane sesquiterpenoids were the main components of these species and may be important chemical markers for *Marsupella* species. Only Japanese *M. emarginata* var. *patens*[4] did not contain longipinanes, *ent*-gymnomitrane sesquiterpenoids being isolated instead. French *M. aquatica*[9] also afforded the novel (−)-gymnomitr-3(15)-en-9β-ol in addition to longipinanes and (+)-(4*S*,5*R*,7*S*,8*R*)-eremophila-9,11-dien-8α-ol which was found in French *M. emarginata*.[6] A GC–MS analysis of a French *M. emarginata* indicated the absence of gymnomitrane sesquiterpenoids,[7] whilst highly acetylated longipinanes were found in German *M. emarginata* but not in the Russian species.[5] The present study of *Marsupella emarginata* var. *aquatica* collected in Scotland has failed to give longipinane, gymnomitrane and eremophilane sesquiterpenoids but, instead, four novel amorphanes (**1**, **5**, **6**, **10**).

Keywords: natural products; Hepaticae; *Marsupella*; terpenes and terpenoids.

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2. Results and discussion

2.1. (−)-(1*R*,2*S*,6*R*,10*S*)-2α-Acetoxyamorpha-4,7(11)-dien-8-one (1)

Compound **1** was isolated as a colourless oil, $[\alpha]_D = -155.1$. Its UV spectrum showed a single absorption maximum at 246 nm which suggested the presence of an α,β-unsaturated ketone, whilst its IR spectrum had strong bands for both ester [1727 cm^{-1} (C=O), 1250 and 1032 cm^{-1} (C–O)] and conjugated ketone (1678 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra (see Table 1) contained signals for an α,β-unsaturated ketone group [δ_C 201.0 (C-8), 143.0 (C-11) and 134.2 (C-7)], a trisubstituted olefinic group [δ_H 4.85 (br s, H-5); δ_C 124.4 (C-5) and 132.7 (C-4)], a deshielded methylene group [δ_H 2.39 (dd, J=4.8 and 14.5 Hz, H-9α) and 1.94 (dd, J=8.8 and 14.5 Hz, H-9β); δ_C 49.9 (C-9)], an acetate group [δ_H 1.71 (s, CH_3CO); δ_C 169.5 (CH$_3$*C*O) and 20.9 (*C*H$_3$CO)], three vinyl methyls [δ_H 2.11 (s, H$_3$-12), 1.45 (s, H$_3$-13) and 1.39 (br s, H$_3$-15); δ_C 23.3 (C-12), 21.9 (C-13) and 22.7 (C-15)], one secondary methyl group [δ_H 0.90 (d, J=6.6 Hz, H$_3$-14); δ_C 22.4 (C-14)], four methines, one of which is oxygenated [δ_H 5.19 (ddd, J=3.7, 6.6 and 12.1 Hz, H-2); δ_C 73.4 (C-2), 42.8 (C-1), 41.6 (C-6) and 27.0 (C-10)] and a methylene carbon [δ_C 32.0 (C-3)]. The molecular formula, $C_{17}H_{24}O_3$ (obtained from HR-EIMS and ^{13}C NMR) indicated the presence of six units of unsaturation and hence **1** must be bicarbocyclic.

Analysis of the HMQC and HMBC spectra (Table 1) led to the gross structure of the compound and the ^1H and ^{13}C NMR spectral assignments. The most shielded vinyl methyl protons (H$_3$-15) correlated with the two sp^2 carbons (C-4

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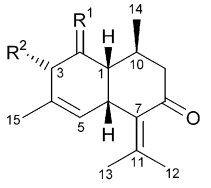
Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data and HMBC correlations for compound **1**

Position	δ_H	δ_C	HMBC correlations
1	1.94 m	42.8	C-14
2	5.19 ddd (3.7, 6.6, 12.1)	73.4	C-10, CH_3CO
3α, 3β	1.97 m	32.0	C-1, C-2, C-4, C-5
4	–	132.7	–
5	4.85 br s	124.4	C-1, C-3, C-4, C-6, C-7, C-15
6	3.53 br s	41.6	–
7	–	134.2	–
8	–	201.0	–
9α	2.39 dd (4.8, 14.5)	49.9	C-1, C-7, C-8, C-10, C-14
9β	1.94 dd (8.8, 14.5)	–	C-1, C-8, C-10, C-14
10	2.02 m	27.0	C-9
11	–	143.0	–
12	2.11s	23.3	C-7, C-11, C-13
13	1.45s	21.9	C-7, C-11, C-12
14	0.90 d (6.6)	22.4	C-1, C-9, C-10
15	1.39 br s	22.7	C-3, C-4, C-5
CH_3CO	1.71 s	20.9	CH_3CO
CH_3CO		169.5	

In C_6D_6 (J in Hz is given in parentheses).

and C-5) of the trisubstituted double bond. Both the olefinic proton (H-5) and H_3-15 correlated to a methylene carbon (C-3) indicating the existence of a bond between C-3 and C-4. The two most deshielded vinyl methyl groups [δ_H 2.11 and 1.45 (H_3-12 and H_3-13)] were observed to correlate with each other as well as to two substituted sp^2 carbons [δ_C 143.0 (C-11) and 134.2 (C-7)]. From the UV and IR data and the chemical shifts of C-7 and C-11, this fully substituted olefinic group must be conjugated with the ketone group [δ_C 201.0 (C-8)]. The correlation from H-5 to C-7 must be ^{3}J since the alkenes were not conjugated. The remaining correlations of H-5 to two methine carbons identified these as C-1 and C-6 and established their positions within the molecule. The correlations of the secondary methyl protons (H_3-14) were only consistent with the bonded pair C-9 and C-10 being attached to the carbonyl and the C-1 methine, respectively, and with the secondary methyl itself being attached to C-10. The remaining methine, C-2, is, by default, between C-1 and C-3. The chemical shift of C-2 was sufficient to determine that it bears the acetoxy group but confirmation was available from the correlation from H-2 to the acetate carbonyl. Other correlations were in accord with the proposed structure.



1 R^1 = β-H, α-OAc; R^2 = H
2 R^1 = H_2; R^2 = OAc
3 R^1 = β-H, α-OH; R^2 = H
4 R^1 = O; R^2 = H

The relative stereochemistry at the chiral carbons as well as the molecular conformation was determined using difference NOE spectroscopy (see Fig. 1). When the H-2 proton was irradiated, the signals due to H-1 and H-6 were enhanced indicating that all three protons were *cis* to each other and that the molecule had a *cis* ring junction. Therefore, the compound was an amorphane or a muurolane. Irradiation of the secondary methyl protons (H_3-14) resulted in the expected enhancement at H-10 and also H-1 which indicated that the methyl group was *cis* to H-1, H-2 and H-6. The observation of NOEs between the secondary methyl group and both H-9α and H-9β suggested a chair-like conformation for the other ring. Both rings were, of course, flattened around the sp^2 carbons. The C-9 methylene protons had rather different chemical shifts (δ_H 1.94 and 2.39) and their assignments were made on the basis of the magnitude of their couplings with H-10. The observation of an NOE at H-10 upon saturation of the more deshielded proton confirmed the assignments. Similarly, the two vinyl methyls (H_3-12 and H_3-13) could be differentiated by NOE experiments. When H-6 was irradiated, it was the more shielded vinyl methyl signal (H_3-13) that was enhanced. The singlet at δ_H 2.11 was then assigned to H_3-12 and its deshielded nature could be attributed to the fact that it is in the deshielding cone of the ketone. The compound was therefore (−)-(1*R*,2*S*,6*R*,10*S*)-2α-acetoxyamorpha-4,7(11)-dien-8-one (**1**). The other NOEs observed (see Section 3) were in agreement with the proposed structure. The ^{1}H NMR shifts of **1**, except for those of H-1, H-2 and H-3, compare well with those of a similar amorphane (**2**) isolated from a *Eupatorium* species (Compositae).[10] Arguments for the stereochemistry of the acetate, however, were not presented. Compound **1** is novel and this is the first report of amorphanes from *Marsupella*.

Gentle methanolysis of the ester (with CH_3OH–K_2CO_3) afforded a secondary alcohol **3** [ν_{max} 3471 cm^{-1} (OH); δ_H 4.15 (ddd, J=3.9, 6.1 and 10.0 Hz, H-2)] which showed the expected increased shielding of H-2. Oxidation of **3** with PCC gave a diketone **4** [ν_{max} 1716 and 1686 (C=O) cm^{-1};

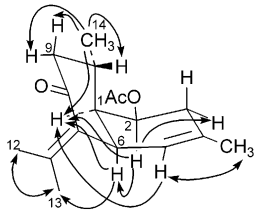


Figure 1. Selected NOEs for **1**.

Table 2. ^{1}H NMR (360 MHz) and ^{13}C NMR (90 MHz) data for compound **5**

Position	δ_H	δ_C
1	1.98 dt (9.6, 3.5)	42.7
2	5.47 dt (3.5, 8.5)	74.5
3α	2.11 m	32.5
3β	2.26 m	
4	–	130.1
5	5.57 br s	124.9[a]
6	3.37 m	38.3
7	–	141.8
8	5.77 t (3.9)	124.2[a]
9	2.24 m and 1.84 m	35.3
10	2.17 m	25.4
11	–	77.0
12	1.36[a] s	25.9[a]
13	1.39[a] s	26.3[a]
14	1.19 d (6.4)	23.1[a]
15	1.64 br s	21.8[a]
CH$_3$CO	1.86 s	21.0
CH$_3$*CO*	–	169.5
OMe	3.09 s	49.6

In C_6D_6 (*J* in Hz is given in parentheses).
[a] Interchangeable.

δ_C 210.8 and 202.2 (C-2 and C-8)]. In the ^{1}H NMR spectrum, the oxygenated methine proton was no longer present and H-1 [δ_H 2.39 (m)] and H$_2$-3 [δ_H 2.84 and 2.66 (br ABq, J_{AB}=21.5 Hz)] were significantly more deshielded than in **1**. The methylene protons appeared as a broadened AB quartet, presumably due to long range coupling through the π system of the non-conjugated double bond. The diketone still possessed a *cis* ring junction since irradiation of H-6 resulted in the enhancement of the H-1 signal. Therefore, epimerisation did not occur during the formation of the diketone.

2.2. (−)-(1*R*,2*S*,6*R*,10*S*)-2α-Acetoxy-11-methoxy-amorpha-4,7-diene (5)

This minor compound of the extract was isolated as a colourless oil, $[\alpha]_D$=−36.9. The ^{13}C NMR spectrum indicated a molecular formula of $C_{18}H_{28}O_3$. The molecular ion, however, was not observed in the mass spectrum. Instead, it shows a peak at *m*/*z* 277, which is indicative of the ion, $[M-CH_3]^+$. The IR spectrum showed characteristic bands for an ester group [ν_{max} 1732 cm^{-1} (C=O), 1246 and 1029 cm^{-1} (C–O)].

The ^{1}H NMR spectrum (see Table 2) had signals for two olefinic protons [δ_H 5.77 (t, *J*=3.9 Hz, H-8) and 5.57 (br s, H-5)], four methine protons, one of which was oxygenated [δ_H 5.47 (dt, *J*=3.5 and 8.5 Hz, H-2), 3.37 (m, H-6), 2.17 (m, H-10) and 1.98 (dt, *J*=9.6 and 3.5 Hz, H-1)], two methylene groups [δ_H 2.26 (m, H-3β), 2.24 (m, H-9α or H-9β), 1.84 (m, H-9β or H-9α) and 1.69 (m, H-3α)], a methoxy group [δ_H 3.09 (s, 11-OCH$_3$)], an acetate group [δ_H 1.86 (s, 2-CH$_3$CO)], a vinyl methyl group [δ_H 1.64 (br s, H$_3$-15)], and three methyl groups, one of which was secondary [δ_H 1.39 and 1.36 (each s, H$_3$-12 and H$_3$-13) and 1.19 (d, *J*=6.5 Hz, H$_3$-14)]. The ^{13}C NMR spectrum (see Table 2) showed the presence of an ester carbonyl [δ_C 169.5 (CH$_3$*CO*)], four olefinic carbons [δ_C 141.8 (C-7), 130.1 (C-4), 124.9 (C-5 or C-8) and 124.2 (C-8 or C-5)], two oxygenated carbons [δ_C 77.0 (C-11) and 74.5 (C-2)], a methoxyl carbon [δ_C 49.6 (OCH$_3$)], five methyls, two methylenes and three methine carbons indicating the presence of five units of unsaturation and, thus, a bicarbocyclic compound.

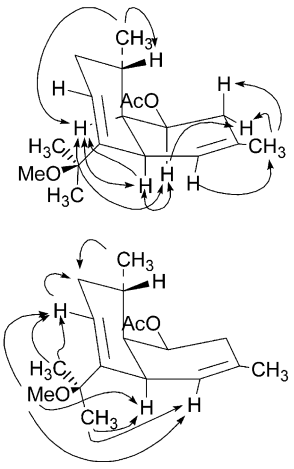
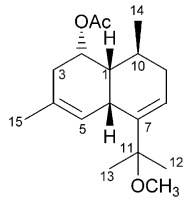


Figure 2. NOEs for **5**.



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The ^{13}C NMR spectra of **1** and **5** were very similar and the ^{13}C shifts differed only in ring B, in particular the shifts of carbons 7, 8, 9, 11, 12 and 13. The α,β-unsaturated ketone, the fully substituted double bond and two vinyl methyls were no longer present in compound **5**. Instead, signals characteristic of a methoxyl group, a pair of methyl groups and a trisubstituted double bond were observed. This double bond must be located at carbons 7 and 8 to account for H-8 being a triplet due to couplings with the C-9 methylene. As for the side chain, the methoxyl group was placed at C-11 as both the methyl groups were deshielded (δ_H 1.39 and 1.36). The similarity in the ^{13}C shifts of both **1** and **5** also suggested that the relative stereochemistry at the chiral centres was the same as in **1**. The structure and the stereochemistry of **5** were supported by the results of difference NOE experiments (Fig. 2).

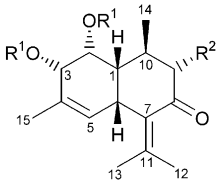
When the methoxyl group and both the vinyl methyls (H$_3$-12 and H$_3$-13) were irradiated, the signals due to H-5, H-6 and H-8 were enhanced. These observations supported the attachment of the side chain at C-7. Saturation of H-8 enhanced the signals for the C-9 methylene protons indicating that C-9 was adjacent to C-8. As in compound **1**, NOEs observed between the secondary methyl (H$_3$-14) and the C-9 methylene suggested a chair conformation for ring B while the enhancement of H-1 when the secondary methyl was irradiated indicated that they were *cis*. Irradiation of H-5 results in an NOE at the vinyl methyl signal (H$_3$-15). This methyl group and the H-2 proton enhanced the signal for one of the C-3 methylene protons (H-3β) when they were irradiated. The molecule therefore had an A ring which was

Table 3. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data and HMBC correlations for compound **6**

Position	δ_H	δ_C	HMBC correlations
1	2.02 m	42.3	C-9, C-10, C-14
2	5.19 m	72.8	C-1, C-3, C-10, 2-CH_3CO
3	5.43 br d (3.5)	68.0	C-1, C-2, C-4, C-5, C-15, 3-CH_3CO
4	–	131.3	–
5	5.19 m	129.4	C-1, C-3, C-7, C-15
6	3.66 ddq (2.0, 6.0, 2.0)	41.8	C-1, C-4, C-5, C-7, C-8, C-10, C-11
7	–	133.6	–
8	–	203.1	–
9α	2.37 dd (4.4, 14.9)	–	C-1, C-7, C-8, C-10, C-14
9β	2.01 dd (10.7, 14.9)	50.3	C-1, C-7, C-8, C-10, C-14
10	2.51 dddq (4.4, 10.0, 10.7, 6.6)	28.2	C-1, C-8, C-9, C-14
11	–	143.7	–
12	1.95 s	23.1	C-7, C-11, C-13
13	1.78 s	22.0	C-7, C-11, C-12
14	1.00 d (6.6)	21.9	C-1, C-9, C-10
15	1.59 br s	19.8	C-3, C-4, C-5
2-CH_3CO	1.98 s	21.0	2-CH_3CO
3-CH_3CO	2.05 s	20.8	3-CH_3CO
2-CH_3CO	–	170.0	–
3-CH_3CO	–	170.6	–

In $CDCl_3$ (J in Hz is given in parentheses).

identical to that of **1**. Since the saturation of H-2 enhanced the signals due to H-1 and H-6, the two rings must be *cis*-fused and compound **5** was therefore also an amorphane. With the observation of NOEs between H-2 and H-6, it could also be concluded that they were both axial and the molecule had a similar ring A conformation to **1**. Other NOE enhancements observed for the compound (see Section 3) were in agreement with the structure proposed. Compound **5** was therefore (−)-(1*R*,2*S*,6*R*,10*S*)-2α-acetoxy-11-methoxyamorpha-4,7-diene, a novel natural product. The possibility that it was an artefact of the isolation procedure was discounted since MeOH was not employed.



6 R^1 = Ac; R^2 = H
7 R^1 = R^2 = H
9 R^1 = Bz; R^2 = H
10 R^1 = Ac; R^2 = OH

2.3. (−)-(1*R*,2*R*,3*S*,6*R*,10*S*)-2α,3α-Diacetoxyamorpha-4,7(11)-dien-8-one (6)

Compound **6**, the major compound of the extract, was obtained as an oil, $[\alpha]_D$=−93.8, $C_{19}H_{26}O_5$ (*m/z* 334.1769). The UV (λ_{max} 248 nm) and IR spectra [1746 cm^{-1} (C=O), 1240 and 1040 cm^{-1} (C–O); 1686 cm^{-1} (α,β-unsaturated ketone)] were very similar to those of **1**. The ^{1}H and ^{13}C NMR spectra (see Table 3) contained almost identical resonances to those of **1** with the exception of a second acetate group [δ_H 2.05 s, (3-OAc); δ_C 20.8 (3-OAc)] and an oxygenated methine [δ_H 5.43 (br d, J=3.5 Hz, H-3); δ_C 68.0 (C-3)] which replaced a methylene of **1**. Again, the structure of the compound and the assignments of the NMR spectra were determined using HMQC and HMBC spectra. A set of HMBC correlations (see Table 3) that were essentially identical to those of **1** was observed. The major difference was the correlations of the new methine hydrogen (H-3), which determined that the second acetoxy group was attached to C-3. Correlations from H-2 and H-3 through the ester oxygens enabled the acetate resonances to be differentiated.

The relative stereochemistry and the conformation of the molecule were determined by consideration of the ^{1}H–^{1}H couplings and difference NOE spectral data (see Fig. 3). A 6.0 Hz coupling between H-1 and H-6 suggested that the rings were *cis*-fused and this was supported by the observation of an NOE at H-1 upon irradiation of H-6. Compound **6** was therefore another amorphane. Both H-2 and H-6 were axial since the saturation of H-2 enhanced the H-6 signal. It followed that **6** had a ring A conformation which was similar to that of both **1** and **5**. The magnitude of $J_{2,3}$ (3.7 Hz) indicated the *cis*-disposition of the acetoxy groups and this was confirmed by the observation of NOEs between H-2 and H-3. The other methine proton, H-10, was *trans* to H-1 as a

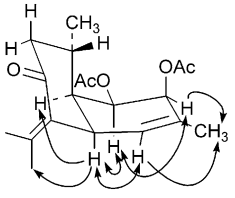


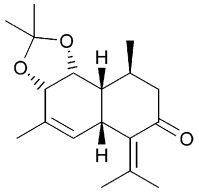
Figure 3. Selected NOEs for **6**.

Table 4. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data for compound **10**

Position	δ_H	δ_C
1	2.23 dt (11.4, 4.2)	42.7
2	5.22 dd (3.6, 4.2)	73.0
3	5.48 d (3.6)	67.8
4	–	132.0
5	5.16 br s	128.5
6	3.69 m	40.1
7	–	132.2
8	–	203.1
9	2.58 dd (2.9, 11.4)	80.1
10	2.36 tq (11.4, 6.1)	37.0
11	–	145.0
12	1.97 s	22.8
13	1.85 s	21.6
14	1.29 d (6.1)	17.0
15	1.63 br s	19.7
2-*CH*$_3$CO	2.05 s	21.1[a]
3-*CH*$_3$CO	2.11 s	20.9[a]
2-CH$_3$*CO*	–	170.0[a]
3-CH$_3$*CO*	–	170.6[a]
9-OH	3.93 d (2.9)	–

In $CDCl_3$ (J in Hz is given in parentheses).
[a] Interchangeable.

large value of $J_{1,10}$ (10.0 Hz) was observed. The secondary methyl group (H_3-14) must be equatorial and this was supported both by the couplings between H-10 and the C-9 methylene protons ($J_{9\beta,10}$=10.7 Hz and $J_{9\alpha,10}$=4.4 Hz) and by the enhancement of H-1 and both C-9 protons when the secondary methyl was irradiated. Thus the compound was the novel (−)-(1*R*,2*R*,3*S*,6*R*,10*S*)-2α,3α-diacetoxyamorpha-4,7(11)-dien-8-one (**6**). The other NOE enhancements observed (see Section 3) were in agreement with the proposed structure.



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Methanolysis of the ester groups afforded a diol (**7**) [ν_{max} 3427 cm^{-1} (OH); δ_H 4.05 (br d, J=4.2 Hz, H-3) and 3.97 (t, J=4.2 Hz, H-2)], which showed the expected shielding of the two oxygenated methine protons, H-2 and H-3. The diol (**7**) readily formed an acetonide **8** and a dibenzoate **9**. When taken in conjunction with the conformational information from the NOE experiments, the CD spectrum of the dibenzoate ($\Delta\varepsilon_{219}$=−4.5 and $\Delta\varepsilon_{238}$=+14) indicated a positive chirality[11] for the dibenzoate system and the absolute configuration is therefore as depicted. The co-metabolites are assumed to belong to the same series of absolute configuration.

2.4. (−)-(1*R*,2*R*,3*R*,6*R*,9*S*,10*R*)-2α,3α-Diacetoxy-9α-hydroxyamorpha-4,7(11)-dien-8-one (10)

The final compound, **10**, was obtained as a white solid, mp 126–127°C, $[\alpha]_D$=−117.0, $C_{19}H_{26}O_6$ (*m/z* 350.1740). Its UV spectrum, which exhibited a single absorption band at 250 nm, was similar to those of **1** and **6**, as was the IR spectrum, apart from the presence of an additional hydroxyl band at 3470 cm^{-1}. A comparison of the ^{1}H and ^{13}C NMR spectra (see Table 4) with those of compound **6** showed a great similarity between them. The only major difference was the replacement of the methylene resonances by those of a secondary hydroxyl group [δ_H 2.58 (dd, J=2.9 and 11.4 Hz, H-9) and 3.93 (d, J=2.9 Hz, 9-OH, exchangeable with D_2O); δ_C 80.1 (C-9)]. This suggested that **10** possessed a hydroxyl group at C-9 which must be equatorial due to the large value of $J_{9,10}$ (11.4 Hz). The results of difference NOE experiments supported the proposed structure and relative stereochemistry. As in the case of **6**, irradiation of H-2 enhanced the signals due to H-1, H-3 and H-6, whilst the hydroxyl group signal was enhanced when H-10 was irradiated. The compound was therefore (−)-(1*R*,2*R*,3*R*,6*R*,9*S*,10*R*)-2α,3α-diacetoxy-9α-hydroxyamorpha-4,7(11)-dien-8-one (**10**). Other NOEs observed for the compound, all of which supported the proposed structure, are given below (see Section 3).

3. Experimental

3.1. General

Mps are uncorr; $[\alpha]_D$: $CHCl_3$; IR: $CHCl_3$ unless otherwise specified; UV: EtOH; EIMS: 70 eV; column chromatography: silica gel (Baker, 40 μm).

3.2. NMR spectroscopy

1D: 300 MHz (^{1}H) and 75 MHz (^{13}C) in $CDCl_3$ (unless otherwise specified) relative to TMS at δ 0.0. 2D and NOE experiments were run at 500 MHz (^{1}H) and 125 MHz (^{13}C). Difference NOE spectra were run using the NOEMULT programme. The relaxation delay was 2.5 s and the total irradiation time was 3–4 s. Difference NOE results are recorded below in the following manner: H irradiated [H enhanced (% enhancement)]. Proton-detected HMQC experiments were optimised for a $^1J_{CH}$ value of 140 Hz. The relaxation delay was 2.5 s. Five hundred and twelve increments, each of 32 scans, were used in t_1 and zero filled to 1K prior to Fourier transformation. In t_2, 2K points were used prior to Fourier transformation. Sine multiplication was used in both dimensions to improve the signal to noise ratio and suppress truncation errors. Proton-detected HMBC experiments were performed under the same conditions as in the HMQC experiment except for modulation tuning which was optimised for $^nJ_{CH}$=7 Hz and a composite pulse which was used to eliminate $^1J_{CH}$.

3.3. Extraction and isolation

The plant material was collected from the River Croe (Argyll, Scotland) in September 1980 and on subsequent occasions. It was identified by Dr D. S. Rycroft, Department of Chemistry, University of Glasgow. The air-dried material (1 kg) was ground and extracted with Et_2O. The crude extract (5.0 g) was chromatographed on silica gel using a gradient of 0–40% EtOAc in hexane and subjected to gel permeation chromatography [Sephadex LH-20 (CH_2Cl_2/hexane 4:1)] to give three fractions. Fraction 1 (200 mg)

was flash chromatographed (silica gel, 5% EtOAc–hexane) to give (−)-(1*R*,2*S*,6*R*,10*S*)-2α-acetoxyamorpha-4,7(11)-dien-8-one (**1**) (42 mg) and (−)-(1*R*,2*S*,6*R*,10*S*)-2α-acetoxy-11-methoxyamorpha-4,7-diene (**5**) (9.5 mg). Fraction 2 (1.5 g) was subjected to flash chromatography (silica gel, 25% EtOAc–hexane) to give the major compound of the extract, (−)-(1*R*,2*R*,3*S*,6*R*,10*S*)-2α,3α-diacetoxyamorpha-4,7(11)-dien-8-one (**6**) (1 g). Fraction 3 (20 mg) was identified as (−)-(1*R*,2*R*,3*R*,6*R*,9*S*,10*R*)-2α,3α-diacetoxy-9α-hydroxyamorpha-4,7(11)-dien-8-one (**10**).

3.3.1. (−)-(1*R*,2*S*,6*R*,10*S*)-2α-Acetoxyamorpha-4,7(11)-dien-8-one (1). Colourless oil; $[\alpha]_D=-155.1$ (c=4.10); UV λ_{max} (nm) (log ε): 246 (3.77); FT-IR ν_{max} ($CHCl_3$) (cm^{-1}): 1727 (ester C=O), 1678 (α,β-unsaturated ketone C=O), 1377, 1250 and 1032 (C–O); EI-MS *m/z* (rel. int.): 276 $[M]^+$ (14), 216 $[M-AcOH]^+$ (65), 201 (72), 173 (58), 159 (62), 145 (60), 105 (43), 91 (52), 43 (100); HREI-MS: *m/z* 276.1702 ($C_{17}H_{24}O_3$ requires *m/z* 276.1725); ^{1}H NMR: δ 5.14 (m, H-2), 4.94 (br s, H-5), 3.71 (br s, H-6), 2.38 (dd, *J*=4.9 and 14.6 Hz, H-9α), 2.17 and 2.03 (3H and 1H, resp., both m, H-1, H-3α, H-3β, H-10), 2.04 (dd, *J*=9.0 and 14.6 Hz, H-9β), 2.03 (s, H_3-12), 2.00 (s, CH_3CO), 1.80 (s, H_3-13), 1.61 (s, H_3-15), 1.00 (d, *J*=6.7 Hz, H_3-14); ^{13}C NMR (100 MHz): δ 202.7 (s), 170.1 (s), 143.5 (s), 133.8 (s), 132.6 (s), 123.7 (d), 73.2 (d), 49.6 (t), 42.4 (d), 41.1 (d), 31.7 (t), 26.7 (d), 22.9 (q), 22.6 (q), 22.1 (q), 22.0 (q), 21.1 (q); ^{1}H and ^{13}C NMR (C_6D_6): see Table 1. Difference NOE (C_6D_6): H-2 [H-1 (5), H-3β (7), H-6 (6)]; H-5 [H-6 (3), H_3-15 (1)]; H-6 [H-1 (6), H-2 (8), H-5 (4), H_3-13 (3)]; H-9α [H-9β (21), H-10 (5), H_3-14 (1)]; H_3-12 [H_3-13 (1)]; H_3-13 [H-5 (3), H-6 (11), H_3-12 (2)]; H_3-14 [H-1 and H-9β (11), H-9α (3), H-10 (10)].

3.3.2. Deacetylation of 1. The acetate **1** (9 mg) was deacetylated with K_2CO_3 and CH_3OH. After workup, the crude product was flash chromatographed (silica gel, 18% EtOAc–hexane) to afford the alcohol **3** (4 mg) as a gum; UV λ_{max} (nm) (log ε): 252 (3.87); FT-IR ν_{max} (cm^{-1}): 3471 (OH), 1685 (α,β-unsaturated ketone C=O); EI-MS *m/z* (rel. int.): 234 $[M]^+$ (65), 216 $[M-H_2O]^+$ (47), 201 (100), 161 (83), 147 (57), 105 (51), 91 (43), 41 (70); HREI-MS: *m/z* 234.1604 ($C_{15}H_{22}O_2$ requires *m/z* 234.1620); ^{1}H NMR (400 MHz): δ 4.94 (br s, H-5), 4.15 (ddd, *J*=3.9, 6.1 and 10.0 Hz, H-2), 3.63 (m, H-6), 2.42 (dd, *J*=5.4 and 14.7 Hz, H-9α), 2.16 (2H, m, H-1 and H-10), 2.05 (dd, *J*=6.8 and 14.7 Hz, H-9β), 2.04 (s, H_3-12), 1.92 (2H, m, H-3), 1.81 (s, H_3-13), 1.62 (br s, H_3-15), 1.06 (d, *J*=6.9 Hz, H_3-14); ^{13}C NMR (100 MHz): δ 203.6 (C-8), 143.6 (C-11), 134.3 (C-7), 133.3 (C-4), 123.7 (C-5), 71.3 (C-2), 49.3 (C-9), 45.8 (C-1), 41.3 (C-6), 35.2 (C-3), 26.2 (C-10), 23.4 (C-12), 23.2 (C-15), 22.9 (C-14), 22.4 (C-13).

3.3.3. Oxidation of 3. A sixfold excess of PCC was added to the alcohol **3** (4 mg) in CH_2Cl_2 (1 ml) and the reaction mixture was stirred at room temperature until the alcohol had disappeared upon TLC. After workup, the crude product was purified by flash chromatography (silica gel, 10% EtOAc–hexane) to give the diketone **4** (3.5 mg) as a colourless solid, mp 105–106°C (hexane); UV λ_{max} (nm) (log ε); 248 (3.69); FT-IR ν_{max} (cm^{-1}) 1716 (ketone C=O), 1686 (α,β-unsaturated ketone C=O), 1612 (C=O); EI-MS *m/z* (rel. int.): 232 $[M]^+$ (100), 217 (28), 189 (66), 161 (92), 17 (90); HREI-MS: *m/z* 232.1455 ($C_{15}H_{20}O_2$ requires *m/z* 232.1463); ^{1}H NMR (400 MHz): δ 5.12 (br s, H-5), 3.92 (m, H-6), 2.84 and 2.66 (2H, br ABq, J_{AB}=21.5 Hz, H-3β and H-3α), 2.47 (dd, *J*=3.9 and 14.5 Hz, H-9α), 2.39 (2H, m, H-1 and H-10), 2.06 (dd, *J*=12.0 and 14.5 Hz, H-9β), 1.96 (s, H_3-12), 1.78 (s, H_3-13), 1.67 (s, H_3-15), 0.91 (d, *J*=6.1 Hz, H_3-14); ^{13}C NMR (100 MHz): δ 210.8 (s), 202.2 (s), 143.5 (s), 133.1 (s), 132.4 (s), 123.8 (d), 57.3 (d), 50.1 (t), 42.4 (t), 41.7 (d), 29.9 (d), 23.2 (q), 22.4 (q), 21.8 (q), 19.8 (q). Difference NOE: H-6 [H-5 (4), H-1 (7), H_3-13 (5)]; H-3β [H-3α (20), H-1 (5)]; H-3α [H-3β (20), H_3-15 (1)]; H-9α [H-9β (21), H_3-14 (0.5)]; H-9β [H-9α (19), H-1 (5), H_3-14 (0.5)]; H_3-14 [H-3β (4), H-9α (4), H-1 and H-10 (17), H-9β (6)].

3.3.4. (−)-(1*R*,2*S*,6*R*,10*S*)-2α-Acetoxy-11-methoxyamorpha-4,7-diene (5). Colourless oil; $[\alpha]_D=-36.9$ (c=0.36); FT-IR ν_{max} (cm^{-1}): 1732 (ester C=O), 1246 and 1029 (C–O); EI-MS *m/z* (rel. int.): 277 $[M-CH_3]^+$ (0.4), 260 $[M-CH_3OH]^+$ (3), 232 $[M-AcOH]^+$ (12), 200 $[M-CH_3OH-AcOH]^+$ (58), 185 (100); HREI-MS: *m/z* 277.1832 ($C_{17}H_{25}O_3$ requires *m/z* 277.1804); ^{1}H and ^{13}C NMR (C_6D_6): see Table 2. ^{1}H NMR: δ 5.74 (br t, *J*=4.0 Hz, H-8), 5.28 (br s, H-5), 5.16 (dt, *J*=3.1 and 8.8 Hz, H-2), 3.11 (br s, H-6), 3.05 (s, OCH_3), 2.17 (m), 2.04 (s, CH_3CO), 1.96 (m), 1.74 (2H, m), 1.65 (br s, H_3-15), 1.32 and 1.31 (each s, H_3-12 and H_3-13), 0.98 (d, *J*=6.3 Hz, H_3-14); ^{13}C NMR: δ 170.6 (s), 140.9 (s), 130.3 (s), 124.22 (d), 124.16 (d), 77.2 (s), 74.4 (d), 49.9 (q), 42.3 (d), 37.8 (d), 35.0 (t), 32.3 (t), 26.4 (q), 25.8 (q), 24.9 (d), 23.0 (q), 21.5 (q), 21.5 (q). Difference NOE (360 MHz, C_6D_6): H-1 [H-2 (3), H-6 (4)]; H-2 [H-1 (3), H-3β (3), H-6 (5)]; H-5 [H-6 (3), H_3-15 (1), 11-OCH_3 (1)]; H-6 [H-1 (5), H-2 (7), H-5 (3)]; H-8 [H-9^a (2), H-9^b (3), H_3-12 (1), H_3-13 (1)]; H_3-12 [H-5 (20), H-6 (15), H-8 (22), 11-OCH_3 (1)]; H_3-13 [H-5 (3), H-6 (17), H-8 (23), 11-OCH_3 (2)]; H_3-14 [H-1 (8), H-9^a (8), H-9^b (4), H-10 (15)]; H_3-15 [H-3β (11), H-5 (17)]; 11-OCH_3 [H-1 (2), H-5 (4), H-6 (1), H-8 (4), H_3-12 (1), H_3-13 (2)]. [a] δ_H 2.24, [b] δ_H 1.84.

3.3.5. (−)-(1*R*,2*R*,3*S*,6*R*,10*S*)-2α,3α-Diacetoxyamorpha-4,7(11)-dien-8-one (6). Colourless oil; $[\alpha]_D=-93.8$ (c = 1.23); UV λ_{max} (nm) (log ε): 248 (3.84); FT-IR ν_{max} (cm^{-1}): 1746 (ester C=O), 1686 (α,β-unsaturated ketone C=O), 1613 (C=O), 1369, 1240 and 1040 (C–O); EI-MS *m/z* (rel. int.): 334 $[M]^+$ (0.4), 274 $[M-AcOH]^+$ (17), 231 (50), 214 $[M-2AcOH]^+$ (91), 199 (78), 173 (87), 145 (25), 118 (56), 91 (48), 43 (100); HREI-MS: *m/z* 334.1769 ($C_{19}H_{26}O_5$ requires *m/z* 334.1780). ^{1}H and ^{13}C NMR: see Table 3. Difference NOE (C_6D_6): H-2 [H-3 (12), H-6 (10)]; H-3 [H-2 (8), H_3-15 (1)]; H-5 [H-6 (4), H_3-15 (2)]; H-6 [H-1 (7), H-2 (11), H-5 (5), H_3-13 (4)].

3.3.6. Deacetylation of 6. Excess K_2CO_3 was added to a solution of **6** (11 mg) in CH_3OH (1 ml). The reaction mixture was stirred at room temperature until no more starting material was present (by TLC). Workup as above afforded the diol (**7**) (8 mg) as a white solid, mp 103–104°C (EtOAc–hexane); $[\alpha]_D=-157.9$ (c=2.03); UV λ_{max} (nm) (log ε): 250 (3.89); FT-IR ν_{max} (cm^{-1}): 3427 (OH), 1686 (α,β-unsaturated ketone C=O), 1606 (C=O); EI-MS *m/z* (rel. int.): 250 $[M]^+$ (4), 232 $[M-H_2O]^+$ (70), 214 $[M-2H_2O]^+$ (62), 189 (37), 161 (100), 135 (52), 91 (59), 77 (74), 41 (84); HREI-MS: *m/z* 250.1578 ($C_{15}H_{22}O_3$

requires *m/z* 250.1569); ^{1}H NMR: δ 5.16 (br s, H-5), 4.05 (br d, *J*=4.2 Hz, H-3), 3.97 (t, *J*=4.2 Hz, H-2), 3.54 (m, H-6), 2.77 (2H, br s, OH), 2.52 (dd, *J*=4.9 and 16.7 Hz, H-9α), 2.52 (m, H-10), 2.01 (dd, *J*=7.3 and 16.7 Hz, H-9β), 2.04 (s, H_3-12), 1.98 (m, H-1), 1.83 (s, H_3-13), 1.79 (br s, H_3-15), 1.07 (d, *J*=6.6 Hz, H_3-14); ^{13}C NMR (100 MHz): δ 204.1 (s), 143.8 (s), 134.5 (s), 134.2 (s), 127.3 (d), 72.8 (d), 69.6 (d), 49.6 (t), 44.6 (d), 41.1 (d), 27.5 (d), 23.3 (q), 23.2 (q), 22.3 (q), 20.3 (q).

3.3.7. Formation of the acetonide of 7. Anhydrous $CuSO_4$ (30 mg) was added to a solution of the diol (**7**) (15 mg) in acetone (1 ml) and the mixture was refluxed for 24 h. Acetone was removed and the crude product was redissolved in $CHCl_3$. $CuSO_4$ was filtered off and the crude product was purified by flash chromatography (silica gel, 25% EtOAc–hexane) to afford the acetonide **8** (12 mg) as a gum. UV λ_{max} (nm) (log ε): 244 (3.66); FT-IR ν_{max} ($CHCl_3$) (cm^{-1}): 1678 (α,β-unsaturated ketone C=O), 1607 (C=C), 1382 (*gem*-dimethyl); EI-MS *m/z* (rel. int.): 290 $[M]^+$ (1), 232 $[M-C_3H_6O]^+$ (31), 214 $[M-C_3H_6O-H_2O]^+$ (83), 161 (35), 145 (32), 105 (18), 91 (28), 43 (100); HREI-MS: *m/z* 290.1858 ($C_{18}H_{26}O_3$ requires *m/z* 290.1882); ^{1}H NMR: δ 5.38 (br s, H-5), 4.45 (dd, *J*=5.1 and 7.2 Hz, H-2), 4.39 (br d, *J*=7.2 Hz, H-3), 3.31 (m, H-6), 2.76 (dd, *J*=9.1 and 14.3 Hz, H-9β), 2.29 (m, H-10), 2.11 (s, H_3-12), 1.88 (dd, *J*=1.1 and 14.3 Hz, H-9α), 1.83 (s, H_3-13), 1.77 (dt, *J*=6.9 and 5.1 Hz, H-1), 1.44 and 1.41 (each s, CH_3), 1.06 (d, *J*=7.0 Hz, H_3-14); ^{13}C NMR: 203.3 (s), 144.2 (s), 134.0 (s), 133.7 (s), 127.0 (d), 108.7 (s), 77.0 (d), 74.1 (d), 46.6 (t), 40.5 (d), 37.9 (d), 27.9 (d), 26.2 (q), 25.0 (q), 23.9 (q), 22.8 (q), 22.6 (q), 19.6 (q).

3.3.8. Formation of the dibenzoate 9. The diol (**7**) (10 mg) and benzoyl chloride (0.1 ml) were dissolved in pyridine (0.5 ml). After 24 h, the mixture was diluted with $CHCl_3$ and was washed sequentially with 0.5 M H_2SO_4, 5% Na_2CO_3, and H_2O, and dried with Na_2SO_4. The $CHCl_3$ layer was concentrated in vacuo to give a residue which was purified by flash chromatography (silica gel, $CHCl_3$) to give the dibenzoate **9** (4 mg) as a gum. $[\alpha]_D$=+44.1 (*c*=0.37); UV λ_{max} (nm) (log ε): 232 (4.42); FT-IR ν_{max} (cm^{-1}): 1726 (ester C=O), 1687 (α,β-unsaturated C=O), 1602, 1492, 1452 (benzene ring), 1274, 1105; EI-MS *m/z* (rel. int.): 458 $[M]^+$ (1), 336 $[M-C_6H_5COOH]^+$ (15), 231 (68), 214 (81), 105 (100), 77 (83); HREI-MS: *m/z* 458.2112 ($C_{29}H_{30}O_5$ requires *m/z* 458.2093); ^{1}H NMR (300 MHz): δ 8.01 and 7.85 (each 2H, d, *J*=7.9 Hz, H-2, H-2′, H-6 and H-6′), 7.60 and 7.49 (each t, *J*=7.9 Hz, H-4 and H-4′), 7.46 and 7.31 (each 2H, t, *J*=7.9 Hz, H-3, H-3′, H-5 and H-5′), 5.94 (br d, *J*=4.0 Hz, H-3), 5.61 (dd, *J*=4.0 and 4.4 Hz, H-2), 5.37 (br s, H-5), 3.89 (m, H-6), 2.86 (m, H-10), 2.59 (dd, *J*=4.3 and 14.8 Hz, H-9α), 2.33 (dt, *J*=9.2 and 4.6 Hz, H-1), 2.17 (dd, *J*=10.8 and 14.8 Hz, H-9β), 2.06 (s, H_3-12), 1.90 (s, H_3-13), 1.74 (br s, H_3-15), 1.07 (d, *J*=6.6 Hz, H_3-14).

3.3.9. (−)-(1*R*,2*R*,3*R*,6*R*,9*S*,10*R*)-2α,3α-Diacetoxy-9α-hydroxyamorpha-4,7(11)-dien-8-one (10). White solid; mp 126–127°C (EtOAc–hexane); $[\alpha]_D$=−117.0 (*c*=1.80); UV λ_{max} (nm) (log ε): 250 (3.87); FT-IR ν_{max} (cm^{-1}): 3470 (OH), 1747 (ester C=O), 1682 (α,β-unsaturated ketone C=O), 1620 (C=C), 1242, 1021 (C–O); EI-MS *m/z* (rel. int.): 350 $[M]^+$ (1), 290 (18), 230 (24), 202 (44), 187 (49), 173 (38), 159 (53), 83 (82), 43 (100); HREI-MS: *m/z* 350.1740 ($C_{19}H_{26}O_6$ requires *m/z* 350.1729); ^{1}H and ^{13}C NMR: see Table 4. Difference NOE (400 MHz): H-1 [H-2 (9), H-6 (7), H-9 (11), H_3-14 (2)]; H-2 [H-1 (9), H-3 (22), H-6 (13)]; H-3 [H-2 (13), H-5 (3), H_3-15 (2)]; H-6 [H-1 (9), H-2 (15), H-5 (7), H_3-13 (5)]; H-9 [H-1 (10), H-10 (4), 9-OH (10), H_3-14 (1)]; H-10 [9-OH (3), H_3-14 (4)]; H_3-14 [H-1 (18), H-9 (8), H-10 (29)].

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References

1. Paton, J. A. *The Liverwort Flora of the British Isles*; Harley Books: Colchester, 1999; p 303.
2. Matsuo, A.; Uto, S.; Sakuda, K.; Uchio, Y.; Nakayama, M.; Hayashi, S. *Chem. Lett.* **1979**, 73–76.
3. Matsuo, A.; Takaoka, D.; Kawahara, H. *Phytochemistry* **1986**, *25*, 2335–2337.
4. Matsuo, A.; Nozaki, H.; Yano, K.; Uto, S.; Nakayama, M.; Huneck, S. *Phytochemistry* **1990**, *29*, 1921–1924.
5. Nagashima, F.; Ohi, Y.; Nagai, T.; Tori, M.; Asakawa, Y.; Huneck, S. *Phytochemistry* **1993**, *33*, 1445–1448.
6. Harrison, L. J.; Becker, H.; Connolly, J. D.; Rycroft, D. S. *Phytochemistry* **1992**, *31*, 4027–4028.
7. Nagashima, F.; Ishimaru, A.; Asakawa, Y. *Phytochemistry* **1994**, *37*, 1767–1768.
8. Huneck, S.; Connolly, J. D.; Rycroft, D. S.; Matsuo, A. *Phytochemistry* **1982**, *21*, 143–145.
9. Nagashima, F.; Ishimaru, A.; Asakawa, Y. *Phytochemistry* **1994**, *37*, 777–779.
10. Bohlmann, F.; Jakupovic, J.; Lonitz, M. *Chem. Ber.* **1977**, *110*, 301–314.
11. Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.