Pergamon

PII: S0031-9422(97)00273-2

Phytochemistry, Vol. 46, No. 2, pp. 293–296, 1997
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Printed in Great Britain
0031 9422/97 $17.00+0.00

BUTENOLIDES FROM *MARCHANTIA PALEACEA* SUBSPECIES *DIPTERA*

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(*Received* 2 *January* 1997)

Key Word Index—*Marchantia paleacea* subsp. *diptera*; Hepaticae; liverwort; butenolide; long-chain lipid.

Abstract—Two new unstable long-chain lipids containing a γ-lactone ring have been isolated from a diethyl ether extract of Japanese *Marchantia paleacea* subsp. *diptera*, in addition to caryophyllene oxide and (+)-7,13-labdadien-15-ol. Their structures were established by extensive 2D NMR techniques. They were shown to be 2-(8′*Z*,11′*Z*-hexadecadienyl)-penta-2,4-dien-4-olide and 2-(8′*Z*-hexadecenyl)-penta-2,4-dien-4-olide. This is the first report of the isolation of long-chain butenolides from liverworts, although they have been isolated previously from the gorgonians *Plexaura flava* and *Euplexaura flava*. © 1997 Elsevier Science Ltd

INTRODUCTION

Our study of Bryophytes has been directed at the search for pharmacologically active substances, including scent and tasting substances and from a chemosystematic point of view [1]. During the chemical investigation of liverworts, it has been found that they occasionally produce their own unique constituents, e.g. marchantin A (**1**), which does not occur in higher plants, fungi or marine organisms. The marchantin series has been isolated from *Marchantia* species and some marchantins possess cytotoxic, muscle-relaxing, antimicrobial, antifungal, and 5-lipoxygenase and calmodulin inhibitory activity, as well as cardiotonic activity [2]. They are also chemical markers of *Marchantia* species. In this genus, six species, *M. emarginata*, *M. polymorpha*, *M. polymorpha* var. *aquatica*, *M. pinnata*, *M. paleacea* and *M. paleacea* subsp. *diptera* are known in Japan. The latter large thalloid liverworts grow on wet rocks and is widely distributed in Japan. Previous work on this species led to the isolation of marchantins A and C–G and terpenoids [3–7]. Further investigation of the diethyl ether extract has resulted in the isolation of two new butenolides.

RESULTS AND DISCUSSION

GC-mass spectral analysis of the diethyl ether extract detected α-pinene, limonene, β-elemene and caryophyllene. This extract was chromatographed on silica gel using an *n*-hexane–EtOAc gradient, giving a mixture containing compounds **2**, **3** and caryophyllene oxide. The mixture was further purified by HPLC on a normal-phase column using *n*-hexane–EtOAc (19:1) to give new very unstable butenolides **2** and **3**, with long-chain alkenyl groups.

The EI-mass spectrum of **2** gave a $[M]^+$ peak at *m/z* 316. The ^1H NMR spectrum (Table 1) showed an equivalent methylene proton signal at δ 1.35 (*br.* 8 H), which is characteristic of a long-chain lipid compound, e.g. fatty acids. Moreover, the triplet proton signal at δ 0.89 (J = 7 Hz, 3H), due to a terminal methyl group, confirmed the long-chain hydrocarbon moiety of **2**. The ^{13}C NMR spectrum (Table 1) indicated the presence of an ester carbonyl (δ 170.5), an exocyclic methylene group (δ 154.0 and 95.4), a trisubstituted (δ 136.2 and 136.6) and two disubstituted double bonds (δ 129.9, 130.2, 127.8 and 128.2). The presence of an α,β-unsaturated γ-lactone was apparent from UV absorption at 262 nm and an intense lactone carbonyl absorption at 1775 cm^{-1} in the IR spectrum. Analysis of the HMQC and HMBC spectra (summarized in Table 2) supported the structural assignment. In particular, the long range ^1H–^{13}C correlation of H_2-1′ (δ 2.37) with C-2 (δ 136.6), C-1 (δ 170.5) and C-3 (δ 136.2) and of H_2-2′ (δ 1.58) with C-2, confirmed the positioning of the side-chain at C-2 of the γ-lactone ring. The presence of two disubstituted double bonds at C-8′ and 11′ was determined by the long-range ^1H–^{13}C correlation of H_3-16′ with C-15′(δ 31.5) and of allylic H_2-13′ with C-15′ in the HMBC spectrum of **1** (Table 2). The *Z*-configuration of the double bonds in the side-chain was established by ^1H–^1H spin

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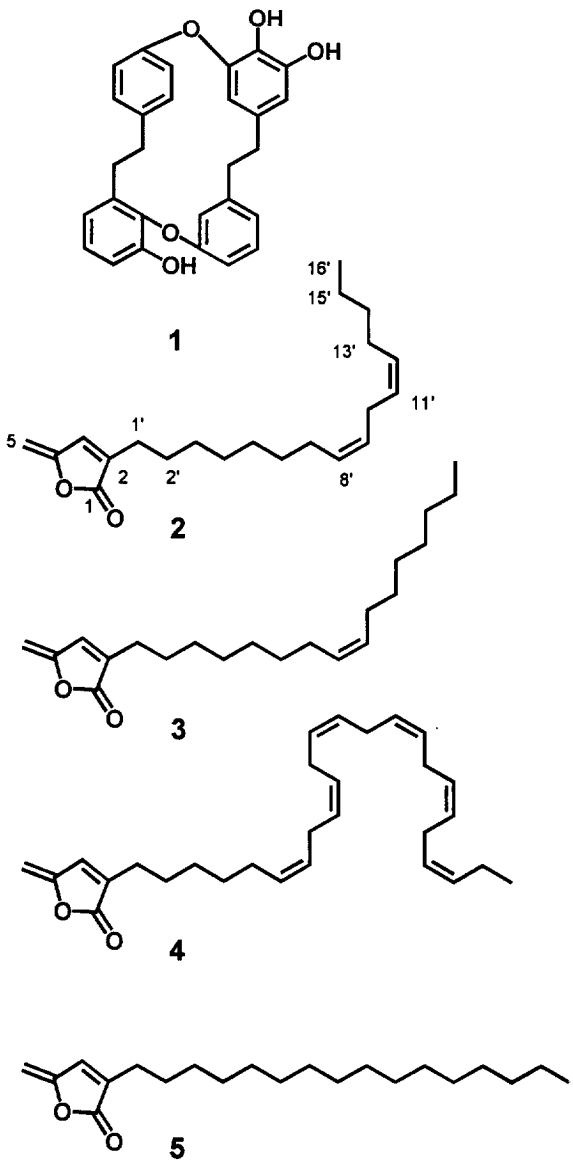

decoupling experiments of **2**. Since the coupling constant between H-8′ and 9′, and H-11′ and H-12′ was 10 Hz, respectively, it was clearly indicated that the two double bonds in the side-chain have the *Z*-configuration. Accordingly, the structure of compound **2** was determined as 2-(8′*Z*,11′*Z*-hexadecadienyl)-penta-2,4-dien-4-olide.

The structure of **3** was deduced by comparing its spectral data with those of **2**. The NMR (Table 1), IR and UV spectral data clearly demonstrated that the γ-lactone portion of the two compounds was identical. Since the EI-mass spectrum of **3** gave a $[M]^+$ at *m/z* 318, it is clear that **3** is the dihydro-derivative of **2**. The ^{13}C NMR spectrum of **3** was similar to that of **2**, except for the absence of two sp^2 carbon signals due to one double bond in the side-chain. Furthermore, ^1H–^{13}C long range correlation between allylic proton at δ 2.01 (*m*) and C-15′ was not observed in the HMBC spectrum of **3**. Consideration of these spectral data led to the conclusion that the structure of **3** was 2-(8′*Z*-hexadecenyl)-penta-2,4-dien-4-olide.

We wish to propose the structures 2-(8′*Z*,11′*Z*-hexadecadienyl)-penta-2,4-dien-4-olide (**2**) and 2-(8′*Z*-hexadecenyl)-penta-2,4-dien-4-olide (**3**) for the new butenolides, although further confirmation of the position of the double bond in the long-chain hydrocarbon moiety is necessary. During this investigation, two other unidentified butenolides were isolated from the same species collected in different location. The EI-mass spectra of the butenolides showed $[M]^+$ at *m/z* 314 and 320, respectively.

Liverwort constituents are often closely related to those of marine organisms [1]. For instance, while higher plants produce (+)-bicyclogermacrene, liverworts and certain species of marine organisms elaborate (−)-*ent*-bicyclogermacrene. The isolation of compounds **2** and **3** from a liverwort for the first time is of phylogenic interest, although the closely related compounds **4** and **5** have already been isolated from the gorgonians, *Plexaura flava* and *Euplexaura flava* [8, 9].

EXPERIMENTAL

General. TLC was carried out on silica gel with *n*-hexane–EtOAc (1:1 and 4:1). Detection was with Godin reagent [10]. For normal phase CC, silica gel 60 (40–63 μm) was used. The eluent for Sephadex LH-20 chromatography was CH_2Cl_2–MeOH (1:1). GC-MS analysis of Et_2O extracts was carried out at 70 eV using a fused-silica capillary column coated with DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm) using He as carrier gas (1 ml min^{-1}). The temp. programming was 50° isothermal for 3 min, then 50–250° at 5° min^{-1} and, finally, isothermal at 250° for 15 min. Injection temp. was 250°.

Spectral data. NMR spectra were recorded at 100 or 150 MHz for ^{13}C and 600, 400 or 200 MHz for ^1H. EIMS were measured at 70 eV.

Plant material. Marchantia paleacea subsp. *diptera* (Nees et Mont.) Inoue (no. 96017) was collected in April 1996 at Kamikatsu-cho, Katsuura-gun (Tokushima, Japan). A voucher specimen is deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and isolation. The liverwort was dried for 1 day, impurities removed and the plant (dry wt. 164.5 g) ground mechanically, then extracted with Et_2O for 3 months. The Et_2O extract (5.63 g) was subjected to CC on silica gel using an *n*-hexane–EtOAc gradient, giving 5 frs (I–V). The first fr. (1.54 g) was rechromatographed on Sephadex LH-20 to give a mixt. containing compounds **2**, **3** and caryophyllene oxide. Further purification of the mixt. by prep. HPLC on silica gel using *n*-hexane–EtOAc (19:1) gave **2** (12.2 mg; 0.22% of total extract) and **3** (7.1 mg; 0.13%). Fr. III (265.8 mg) was subjected to CC on Sephadex LH-20 to give (+)-7,13-labdadien-

Table 1. ^1H and ^{13}C NMR data for compounds **2** and **3***

	2		**3**	
	^1H	^{13}C	^1H	^{13}C
1		170.5		170.5
2		136.6		136.6
3	7.01(*t*, *J* = 1.5)	136.2	7.01(*t*, *J* = 1.5)	136.2
4		154.0		154.0
5	4.77, 5.10 (*dd*, *J* = 2.5, 0.5)	95.4	4.76, 5.10 (*dd*, *J* = 2.5, 0.5)	95.4
1′	2.37 (*br t*, *J* = 7)	25.2	2.39 (*br t*, *J* = 7)	25.2
2′	1.58(*m*)	27.4	1.58(*m*)	27.4
3′	1.35(*m*)	29.1	1.34(*m*)	29.1
4′	1.35(*m*)	28.9	1.34(*m*)	28.9
5′	1.35(*m*)	29.3	1.34(*m*)	29.3
6′	1.35(*m*)	29.5	1.34(*m*)	29.5
7′	2.02(*m*)	27.2[a]	2.01(*m*)	27.1[b]
8′	5.39(*ddddd*, *J* = 10, 7, 7, 1.5, 1.5)[a]	130.2[b]	5.37(*ddddd*, *J* = 10, 7, 7, 1.5, 1.5)[a]	130.1[c]
9′	5.34 (*ddddd*, *J* = 10, 7, 7, 1.5, 1.5)[b]	127.8[c]	5.32(*ddddd*, *J* = 10, 7, 7, 1.5, 1.5)[a]	129.6[c]
10′	2.77 (*br t*, *J* = 7)	25.6	2.01(*m*)	29.3[b]
11′	5.32(*ddddd*, *J* = 10, 7, 7, 1.5, 1.5)[b]	128.2[c]	1.27(*m*)	29.8[a]
12′	5.37(*ddddd*, *J* = 10, 7, 7, 1.5, 1.5)[a]	129.9[b]	1.27(*m*)	29.6[a]
13′	2.02(*m*)	27.1[a]	1.27(*m*)	27.2
14′	1.32(*m*)	22.6	1.27(*m*)	22.7
15′	1.28(*m*)	31.5	1.27(*m*)	31.9
16′	0.89(*t*, *J* = 7)	14.1	0.88(*t*, *J* = 7)	14.1

* Measured in $CDCl_3$ at 600 MHz for ^1H and 150 MHz for ^{13}C.
[a,b,c] May be interchangeable in each vertical column, although assignments were carried out by HMBC and HMQC.

Table 2. ^1H–^{13}C long-range correlation for compound **2***

^1H	^{13}C
H-3 (δ 7.01)	C-1, 2, 4, 1′
H_2-5 (δ 4.77, 5.10)	C-3, 4
H_2-1′ (δ 2.37)	C-1, 2, 2′, 3′
H_2-2′ (δ 1.58)	C-2, 1′, 3′
H_2-7′ (δ 2.02)	C-8′, 9′
H-8′ (δ 5.39)	C-6′, 7′, 10′
H-9′ (δ 5.34)	C-7′, 10′
H_2-10′ (δ 2.77)	C-8′, 9′, 11′, 12′
H-11′ (δ 5.32)	C-10′, 13′
H-12′ (δ 5.37)	C-10′, 13′
H_2-13′ (δ 2.02)	C-11′, 12′, 15′
H_2-14′ (δ 1.32)	C-15′, 16′
H_2-15′ (δ 1.28)	C-16′
H_3-16′ (δ 0.89)	C-14′, 15′

* Observed in HMBC spectrum.

15-ol (42.6 mg; 0.76%) [1, 5]. Fr. V (2.27 g) was rechromatographed on Sephadex LH-20 to give marchantin A (**1**) [1, 2, 5] (1.53 g; 27.2%).

Compound. **2**. Oil. FT-IR ν_{max}^{neat} cm^{-1}: 1775, 1651, 1464, 1290, 1037. EIMS *m/z* (rel. int.): 316[M]$^+$ (82), 123 (40), 110 (56), 109 (57), 95 (96), 81 (100), 67 (97) 55 (46). UV(EtOH) λ_{max}^{neat} nm (log ε): 262 (4.20).

Compound. **3**. Oil. FT-IR ν_{max}^{neat} cm^{-1}: 1773, 1642, 1456, 1290. EIMS *m/z* (rel. int.): 318[M]$^+$ (55), 111 (43), 110 (100), 69 (41), 55 (57). UV λ_{max}^{EtOH} nm (log ε): 262 (4.41).

Acknowledgement—We thank Dr M. Mizutani (Hattori Botanical Laboratory, Miyazaki, Nichinan, Japan) for identification of the liverwort species.

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