A New Lignan and two Eudesmanes from *Lepidozia vitrea*

by **Bin Ma**, **Huai-Fang Guo**, and **Hong-Xiang Lou***

School of Pharmaceutical Sciences, Shandong University, Jinan 250012, P. R. China
(phone: +86-531-88382019; fax: +86-531-88382019; e-mail: louhongxiang@sdu.edu.cn)

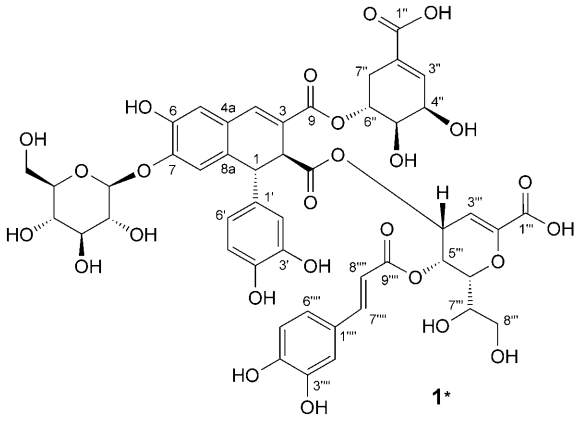
A new lignan, **1**, was isolated from *Lepidozia vitrea*, together with two known eudesmane terpenoids, **2** and **3**, as well as 7,8-dihydroxycoumarin (**4**). Their structures were elucidated on the basis of extensive 1D- and 2D-NMR as well as MS analyses.

Introduction. – *Lepidozia vitrea*, a liverwort belonging to the family of Lepidoziaceae, is a rich source of sesquiterpenoids [1]. Several sesquiterpenoids from the essential oil and ethyl ether extract of this plant have been reported [2]. In the course of our phytochemical investigations on antifungal constituents from liverworts [3], the new lignan derivative **1** was isolated from *L. vitrea*, together with three known compounds: 7α-hydroxyeudesm-3-en-6α-yl acetate (**2**), eudesm-3-ene-6α,7β-diol (**3**), and 7,8-dihydroxycoumarin (=7,8-dihydroxy-1-benzopyran-2(2*H*)-one; **4**). Herein, we report the isolation, structure elucidation, and characterization of these compounds.

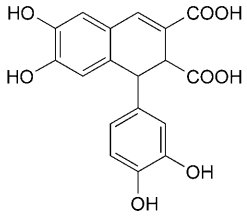
Results and Discussion. – Compound **1**, isolated from the EtOH extract of *L. vitrea*, was obtained as a colorless powder. Its molecular formula, $C_{48}H_{48}O_{26}$, was derived by ESI-MS (M^+ at m/z 1040.012; calc. 1040.243). Five subunits were identified. The first one was an aryl-substituted 1,2-dihydro-6,7-dihydroxynaphthalene-2,3-dicarboxylate moiety, showing 18 signals in the ^{13}C-NMR spectrum (*Table*), including two carboxylic C-atoms (δ(C) 167.5, 170.5), 14 aromatic or olefinic resonances, and two aliphatic CH groups (δ(C) 46.2, 49.6).

We were able to identify the parent structure **1a**, which has been isolated before from *Pellia epiphylla* [4a], and named epiphyllic acid [4c]. The ^{1}H-NMR spectrum of the resonances belonging to its skeleton consisted of two coupled methine H-atoms [δ(H) 3.83 (*d*, J=2.4 Hz, 1 H), 4.40 (br. *s*, 1 H)], three signals of a 3,4-dihydroxyphenyl group [δ(H) 6.35 (*d*, J=7.8 Hz, 1 H), 6.36 (*s*, 1 H), 6.48 (*d*, J=7.8 Hz, 1 H)], and three *singlet*s at δ(H) 6.80, 7.04, and 7.54 (1 H each). These data were identical with those reported for epiphyllic acid proper.

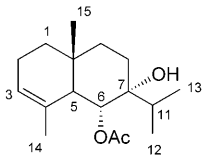
One glucosyl (Glc) moiety was recognized, with an anomeric resonance at δ(C) 103.9 in the ^{13}C-NMR spectrum, as well as five O-bearing aliphatic C-atoms at δ(C) 62.2, 70.2, 74.8, 77.5, and 77.9. The anomeric H-atom resonated at δ(H) 4.85 (*d*, J=7.2 Hz, 1 H), which indicated β-configuration. Also, the anomeric Glc H-atom showed a ^3J correlation to C(7) of epiphyllic acid in the HMBC spectrum. Accordingly, the main skeleton of compound **1** consisted of a 7-*O*-glucosylated epiphyllic acid unit.



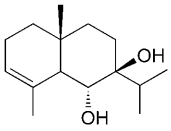
1*



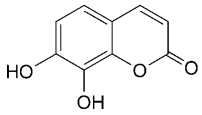
Epiphyllic acid (**1a**)



2



3



4

* Arbitrary atom numbering and relative configuration

Further, a shikimic acid residue, a common subunit in lignans from liverworts [4b], was identified on the basis of a set of ^{1}H,^{1}H-COSY cross-peaks (*Figure*), with signals at δ(H) 6.80 (H–C(3″)), 4.36 (H–C(4″)), 3.91 (H–C(5″)), and 5.18 (H–C(6″)), in combination with a CH_2 group at δ(H) 2.36/2.81 (CH_2(7″))[1]).

An additional C_8 moiety in the ^{13}C-NMR spectrum of **1** was derived from trilobatinoic acid C[2]). This moiety consists of five O-bearing aliphatic C-atoms [δ(C) 68.3, 63.3, 77.4, 71.1, and 63.9 for C(4‴)–C(8‴), resp.], one C=C bond at δ(C) 147.7 (C(2‴) and 107.5 C(3‴), as well as a COOH group at δ(C) 165.8.

The last subunit was recognized as caffeic acid, with characteristic *doublet*s at δ(H) 7.65 (H–C(7⁗)) and 6.38 (H–C(8⁗)), and a common coupling constant of 15.6 Hz, in agreement with an (*E*)-configured C=C bond. In addition, there were three aromatic resonances [δ(H) 7.10 (*s*, H–C(2⁗)), 6.80 (*d*, J = 8.4 Hz, H–C(5⁗)), 6.98 (*d*, J = 8.4 Hz, H–C(6⁗))] belonging to this moiety.

The observed HMBC correlations between H–C(5‴) of trilobatinoic acid C and the carboxy C(9⁗) atom of caffeic acid, between H–C(4‴) of trilobatinoic acid C and the carboxy C(10) atom, and between H–C(6″) of shikimic acid and the carboxy C(9) atom determined the linkages among these subunits.

The relative configuration of **1** was determined by means of NOESY experiments (*Figure*). As there were cross-peaks between H–C(8) and both H–C(1) and the

---

[1]) Arbitrary atom numbering.

[2]) This name was first proposed by *Becker* and co-workers [4b], who had isolated four C_7/C_8 compounds from the liverwort *Bazzania trilobata* [4b].

Table. *^{1}H- and ^{13}C-NMR Data of* **1**. At 600 and 150 MHz, resp., in CD_3OD; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	δ(H)	δ(C)	HMBC	Position	δ(H)	δ(C)	HMBC
1	4.40 (*s*)	46.2	3, 4a, 10, 2′, 6′	Shikimic acid moiety:			
2	3.83 (*d*, *J* = 2.4)	49.6	10	1″		169.5	
3		124.3		2″		130.5	
4	7.54 (*s*)	139.8	2, 5, 8a, 9, 10	3″	6.79–6.81 (*m*)	138.7	
4a		127.9		4″	4.36 (*s*)	67.3	
5	6.79–6.81 (*m*)	117.7	6, 7	5″	3.91 (*dd*, *J* = 7.2, 3.6)	69.6	3″, 7″
6		147.4		6″	5.18–5.19 (*m*)	72.1	2″, 5″, 9
7		148.7		7″	2.36 (*d*, *J* = 15.6),	28.9	6″
8	7.04 (*s*)	119.3	4a		2.81 (*d*, *J* = 15.6)		
8a		130.8		Trilobatinoic acid moiety:			
9		167.5		1‴		165.8	
10		172.9		2‴		147.7	
1′		135.8		3‴	5.70 (*s*)	107.5	1‴
2′	6.36 (*s*)	115.6	3′, 4′, 6′	4‴	5.70 (*s*)	68.3	3‴, 10
3′		146.0		5‴	5.75 (*s*)	63.3	3‴, 4‴, 9⁗
4′		145.0		6‴	4.16 (*d*, *J* = 8.4)	77.4	7‴, 8‴
5′	6.48 (*d*, *J* = 7.8)	116.4	1′, 3′, 4′, 6′	7‴	3.46–3.49 (*m*)	71.1	6‴, 8‴
6′	6.35 (*d*, *J* = 7.8)	120.0	2′, 4′	8‴	3.46–3.49 (*m*),	63.9	6‴, 7‴
Glucosyl moiety:					3.81–3.83 (*m*)		
1	4.85 (*d*, *J* = 7.2)	103.9	7	Caffeic acid moiety:			
2	3.46–3.49 (*m*)	74.8		1⁗		127.8	
3	3.46–3.49 (*m*)	77.5		2⁗	7.10 (*s*)	115.5	3⁗, 4⁗, 7⁗
4	3.74–3.76 (*m*)	70.2		3⁗		149.7	
5	3.46–3.49 (*m*)	77.9		4⁗		146.7	
6	3.70–3.72 (*m*)	62.2		5⁗	6.80 (*m*)	117.7	
				6⁗	6.98 (*d*, *J* = 8.4)	123.5	4⁗
				7⁗	7.65 (*d*, *J* = 15.6)	148.3	2⁗, 6⁗, 9⁗, 9
				8⁗	6.38 (*d*, *J* = 15.6)	114.9	9⁗
				9⁗		168.5	

anomeric H-atom of Glc, H–C(1) had to be in an equatorial position. Furthermore, H–C(2) was determined to be in axial position, based on the cross-peak between H–C(1) and H–C(2). Similarly, there were cross-peaks between H–C(4‴), H–C(5‴), and H–C(6‴). So, H–C(4‴) and H–C(6‴) had to be in an axial positions, and H–C(5‴) was in equatorial position [5].

From the above data, the structure of the new lignan **1** was elucidated as 'epiphyllic acid-7-*O*-*β*-glucoside-9,6″-*O*-shikimic acid ester-10,4‴-*O*-(5‴,9⁗-*O*-caffeic acid ester)-trilobatinoic acid ester', which corresponds to 2-(6-carboxy-3,4-dihydro-2-(1,2-dihydroxyethyl)-3-{[3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-2*H*-pyran-4-yl) 3-(3-carboxy-5,6-dihydroxycyclohex-3-en-1-yl) 1,2-dihydro-1-(3,4-dihydroxyphenyl)-6-hydroxy-7-[(*β*-glucopyranosyl)oxy]naphthalene-2,3-dicarboxylate[3]).

---

[3]) Correct name based on ephiphyllic acid (**1a**) as parent structure. The fully systematic name of **1** would be 2,6-anhydro-4-*O*-{[3-{[(3-carboxy-5,6-dihydroxycyclohex-3-en-1-yl)oxy]carbonyl}-1,2-dihydro-1-(3,4-dihydroxyphenyl)-7-(*β*-glucopyranosyloxy)-6-hydroxynaphthalen-2-yl]carbonyl}-3-deoxy-5-*O*-[(2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oct-2-enonic acid.

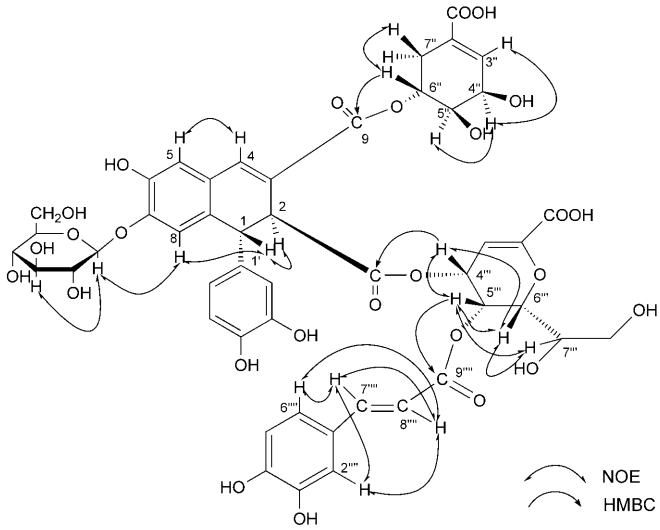


Figure. *Key NOE and HMBC correlations in* **1**

A similar compound, 'epiphyllic acid-7-*O*-β-glucoside-9,1′′′′-*O*-heptitol ester-10,5′′′-*O*-shikimic acid ester', was isolated before from the liverwort *Lepicolea ochroleuca* [6]. There, heptitol, with its unbranched chain including seven O-bearing C-atoms, is linked to C(9) of epiphyllic instead of shikimic acid.

The known compounds **2**–**4** were also obtained from the EtOH extract of *L. vitrea*, and their structures were determined by comparison of their analytical data with those reported in the literature [2b][7]. Note that **4** was isolated from *L. vitrea* for the first time.

The authors express their gratitude to the *Natural Science Foundation of China* (*NSFC*) for supporting this project (Grant No.30271537).

Experimental Part

General. All solvents were of anal. grade. Column chromatography (CC) was performed on silica gel or *Sephadex LH-20* (*Pharmacia*). Melting points (m.p.) were measured on an *X-6* melting-point apparatus (*Beijing TECH Instrument Co., Ltd*). Optical rotations were determined on a *Perkin-Elmer 241 MC* polarimeter. NMR Spectra were recorded on a *Bruker Avance-600* spectrometer at 600 (^{1}H) and 150 MHz (^{13}C); chemical shifts δ in ppm rel. to Me_4Si or rel. to residual solvent peaks [δ(H) 3.30 and δ(C) 49.0 ppm for CD_3OD], coupling constants *J* in Hz. Mass spectra were recorded on an *Apex FT-ICR* apparatus (*Bruker Daltonics, Inc.*); in *m*/*z*.

Plant Material. The plant was collected in Shiwan Mountains, Guang Xi Chuang Municipality, P. R. China, and identified as *Lepidozia vitrea* by Prof. *Ruiliang Zhu*, Huadong Normal University, Shanghai. A voucher specimen was deposited at the School of Pharmaceutical Sciences, Shandong University, China.

Extraction and Isolation. Air-dried *L. vitrea* (1.1 kg) was sequentially extracted with Et_2O, EtOH, and H_2O. The EtOH extract was subjected to CC (SiO_2; $CHCl_3$/MeOH) to yield eleven fractions (Fr.). Further purification of *Fr. 5* by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) afforded **2** (7.0 mg) and **3** (4.2 mg). *Fr. 6* was purified by CC (SiO_2; $CHCl_3$/MeOH) to yield **4** (11.5 mg). Further purification of *Fr. 7* by CC (*Sephadex LH-20*; MeOH) afforded **1** (11 mg).

*2-(6-Carboxy-3,4-dihydro-2-(1,2-dihydroxyethyl)-3-{[3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-2*H*-pyran-4-yl) 3-(3-Carboxy-5,6-dihydroxycyclohex-3-en-1-yl) 1,2-Dihydro-1-(3,4-dihydroxyphenyl)-6-hydroxy-7-[(β-glucopyranosyl)oxy]naphthalene-2,3-dicarboxylate* (**1**)[3]). Colorless powder. $[\alpha]_D^{25} = -0.703$ ($c = 0.8$, MeOH). ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS: 1040.8, 902.6, 699.3, 391.5, 338.6. HR-ESI-MS: 1040.012 (M^+, $C_{48}H_{48}O_{26}$; calc. 1040.243).

7α-Hydroxyeudesm-3-en-6α-yl Acetate (**2**) [2b]. Colorless needles. M.p. 141.0–142.0° (petroleum ether/acetone). ^{1}H-NMR (600 MHz, $CDCl_3$): 0.86 (*s*, Me(15)); 0.97 (2*d*, $J = 7.0$ each, Me(12), Me(13)); 1.35, 1.48 (*m*, CH_2(1)); 1.24, 1.46 (*m*, CH_2(9)); 1.58, 1.78 (*m*, CH_2(8)); 1.62 (*s*, Me(14)); 1.99 (*m*, H–C(11)); 1.92, 2.10 (*m*, CH_2(2)); 2.44 (*t*, $J = 1.0$, H–C(5)); 3.87 (*s*, H–C(6)); 5.47 (*s*, H–C(3)). ^{13}C-NMR (150 MHz, $CDCl_3$): 15.9 (C(12)); 16.3 (C(13)); 17.9 (C(15)); 21.9 (C(14)); 22.9 (*Me*CO); 23.0 (C(8)); 23.2 (C(2)); 34.1 (C(11)); 34.2 (C(9)); 34.4 (C(12)); 38.0 (C(1)); 45.1 (C(5)); 73.9 (C(7)); 76.8 (C(6)); 124.6 (C(3)); 133.3 (C(4)); 170.5 (C=O).

Eudesm-3-ene-6α,7β-diol (**3**) [2b]. Yellowish oil. ^{1}H-NMR (600 MHz, CD_3OD): 0.90, 0.93 (2*d*, $J = 7.0$ each, Me(12), Me(13)); 0.99 (*s*, Me(15)); 1.26, 1.36 (*m*, CH_2(1)); 1.29, 1.45 (*m*, CH_2(9)); 1.58, 1.78 (*m*, CH_2(8)); 1.99 (*m*, H–C(11)); 1.92, 2.10 (*m*, CH_2(2)); 2.44 (*t*, $J = 1.0$, H–C(5)); 3.87 (*s*, H–C(6)); 5.47 (*s*, H–C(3)). ^{13}C-NMR (150 MHz, CD_3OD): 15.9 (C(12), C(13)); 18.1 (C(15)); 20.5 (C(14)); 22.9 (C(2)); 27.8 (C(8)); 31.5 (C(10)); 32.8 (C(11)); 35.3 (C(9)); 39.3 (C(1)); 45.3 (C(5)); 71.7 (C(6)); 74.8 (C(7)); 124.3 (C(3)); 132.7 (C(4)).

7,8-Dihydroxycoumarin (*=7,8-Dihydroxy-1-benzopyran-2(2*H*)-one*; **4**) [7]. Colorless needles. M.p. 250–252° (petroleum ether/acetone). ^{1}H-NMR (500 MHz, CD_3OD): 7.82 (*d*, $J = 9.5$, H–C(4)); 6.99 (*d*, $J = 8.5$, H–C(5)); 6.80 (*d*, $J = 8.5$, H–C(6)); 6.17 (*d*, $J = 9.5$, H–C(3)). ^{13}C-NMR (125 MHz, CD_3OD): 163.4 (C(2)); 151.1 (C(7)); 146.7 (C(4)); 145.0 (C(9)); 133.5 (C(8)); 120.2 (C(5)); 113.9 (C(10)); 113.7 (C(6)), 112.2 (C(3)).

REFERENCES

[1] Y. Asakawa. *Phytochemistry* **2004**, *65*, 623.
[2] a) Y. F. Shu, H. C.Wei, C. L. Wu, *Phytochemistry* **1994**, *37*, 773; b) M. Toyota, E. Nakaishi, Y. Asakawa, *Phytochemistry* **1996**, *41*, 833.
[3] G. Y. Li, B. Ma, H. X. Lou, *Nat. Prod. Res. Dev.* **2002**, *14*, 5; W. Zhang, H. X. Lou, G. Y. Li, H. M. Wu, *J. Asian Nat. Prod. Res.* **2003**, *5*, 189.
[4] a) F. Cullmann, K-P. Adam, H. Becker, *Phytochemistry* **1993**, *34*, 831; b) J. M. Scher, J. Zapp, H. Becker, *Phytochemistry* **2003**, *62*, **7**69; c) F. Cullmann, A. Schmidt, F. Schuld, M. L. Trennheuser, H. Becker, *Phytochemistry* **1999**, *52*, 1647.
[5] F. Cullmann, K-P. Adam, J. Zapp, H. Becker, *Phytochemistry* **1996**, *41*, 611.
[6] F. Cullmann, H. Becker, *Phytochemistry* **1999**, *52*, 1651.
[7] G. F. Liu, Y. Q. Fu, F. F. Hou, *China J. Chin. Materia Med.* **1995**, *20*, 738 [in Chinese].

Received August 30, 2006