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NEW GUAIANE-TYPE SESQUITERPENE LACTONES FROM *HEMISTEPTIA LYRATA* BUNGE

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Abstract—Two new guaiane-type sesquiterpene lactones (**1**) and (**2**) together with 8-hydroxyzaluzanin C (**3**) were isolated from the flower of *Hemisteptia lyrata*. Compounds (**1-3**) were examined for their cytotoxic activity against LOX-IMVI, MCF-7, PC-3 and HCT-15 human cell line.

More than twenty 3,8-dihydroxy-4(15),10(14),11(13)-guaiatriene-12,6-olide (**3**) derivatives with different ester groups at the C-3 and/or C-8 position have been isolated from several Compositae plants.[1-3] Here, we describe the isolation of two new sesquiterpene lactones (**1**, **2**) from the flower of *Hemisteptia lyrata* Bunge which is the only species of the *Hemisteptia* genus and a well-known Chinese herb to cure sore throat and treat tumor.[4-6] Extract of flower of this plant showed strong cytotoxic activity as determined by the sulforhodamine B assay.[7] A bioactivity-guided fractionation of the extracts of flower of *Hemisteptia lyrata* B. resulted in the isolation of three compounds (**1**–**3**). The spectroscopic data of compound (**3**) agreed with 8-hydroxyzaluzanin C, previously isolated from *Amberboa muricata*.[8]

Compound (**1**) had the molecular formula $C_{20}H_{24}O_6$ with nine degrees of unsaturation, as deduced from its HREIMS. The IR spectrum of **1** showed absorptions at 3500, 1765 and 1715 cm^{-1}, suggesting the presence of hydroxy and two ester groups. The structure of **1** was inferred from the ^1H and ^{13}C NMR spectral data together with DEPT and 2D NMR experiments (^1H-^1H COSY, HMQC, HMBC, and NOESY). The ^{13}C NMR spectral data showed the presence of twenty carbon atoms as two carbonyl groups, one hydroxymethyl, four sp^2 methylenes, three sp^3 methylenes, six methines and four

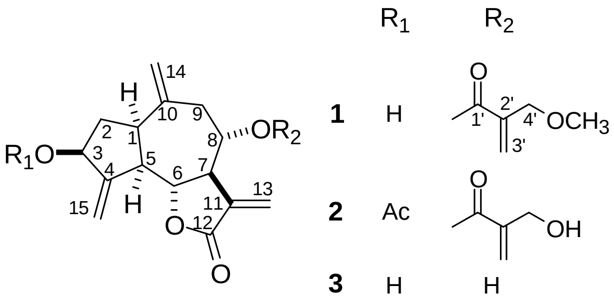
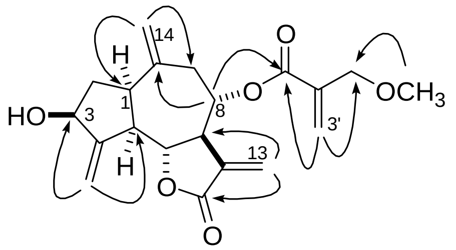


Table 1. ^{1}H and ^{13}C NMR spectral data for compounds (**1**-**3**)

	^{1}H^{a}			^{13}C^{b}		
position	**1**	**2**	**3**	**1**	**2**	**3**
1	2.98 m	3.03 m	2.97 m	45.7 d	45.6 d	45.3 d
2	1.74 m	1.80 m	1.73 m	39.5 t	36.3 t	39.4 t
	2.25 m	2.37 m	2.23 m			
3	4.55 m	5.57 m	4.55 m	74.1 d	74.6 d	73.8 d
4				152.6 s	147.0 s	152.6 s
5	2.85 dd(9.0,10.2)	2.85 dd(8.8,10.3)	2.81 m	51.7 d	51.7 d	51.5 d
6	4.24 dd(9.1,10.5)	4.18 dd(9.0,10.5)	4.15 dd(9.1,10.5)	78.9 d	77.9 d	78.7 d
7	3.29 m	3.12 m	2.78 m	48.0 d	47.5 d	51.1 d
8	5.12 m	5.16 m	3.96 m	74.6 d	74.2 d	72.1 d
9	2.40 dd(3.9,15.0)	2.41 dd(3.9,14.6)	2.29 dd(3.9,14.0)	37.5 t	37.2 t	41.4 t
	2.71 dd(5.2,15.0)	2.70 dd(5.2,14.6)	2.70 dd(5.1,14.0)			
10				142.1 s	141.2 s	142.8 s
11				137.7 s	137.2 s	138.1 s
12				169.4 s	168.8 s	169.7 s
13	5.65 d(3.1)	5.63 d(3.0)	6.14 dd(0.7, 3.1)	123.1 t	122.8 t	123.1 t
	6.22 d(3.4)	6.24 d(3.4)	6.26 dd(0.7, 3.4)			
14	4.95 d(0.9)	4.97 s	4.98 s	118.5 t	118.5 t	117.1 t
	5.15 d(0.9)	5.15 s	5.13 s			
15	5.36 dd(1.7,1.7)	5.36 s	5.34 dd(1.6,1.7)	113.9 t	116.2 t	113.2 t
	5.50 dd(1.5,1.5)	5.54 s	5.48 dd(1.6,1.7)			
1′				165.3 s	165.2 s	
2′				137.4 s	139.2 s	
3′	5.95 d(1.4)	5.94 s		127.6 t	126.7 t	
	6.37 d(0.9)	6.34 s				
4′	4.15 d(13.5)	4.39 s		71.1 t	62.3 t	
	4.18 d(13.5)					
OCH_3	3.41 s			58.9 q		
CH_3*CO*					170.7 s	
CH_3CO		2.09 s			21.1 q	

^{a} Recorded at 500 MHz in $CDCl_3$. ^{b} Recorded at 125 MHz in $CDCl_3$; multiplicity by DEPT.

quaternary carbons. The ^{1}H and ^{13}C NMR spectra data of **1** were very similar to those (Table 1) of **3** except for the chemical shifts of H-1, C-7, C-8 and C-9. Based on ^{13}C-NMR spectrum, two esters and four exomethylenes double bonds have been characterized, and these account for six degrees of unsaturation. Hence, the extra degrees of unsaturation were presumed to be due to three rings. A convenient starting point of ^{1}H-^{1}H COSY is the H-13a/b vinyl protons resonating at δ 5.65 and 6.22, because nonequivalent methylene protons linked to the same carbon (δ 123) from HMQC experiment. The ^{1}H-^{1}H COSY spectrum revealed successive connectivities from C-13 to C-14 and from C-7 to C-1.



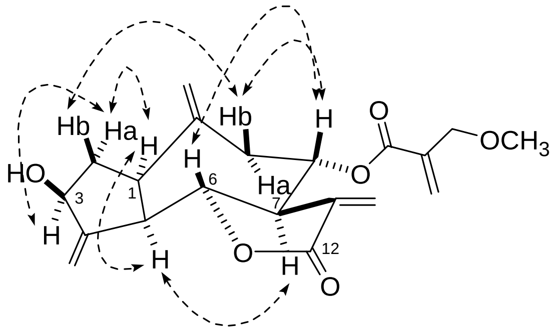


Figure 1. Important HMBC correlations of **1**. **Figure 2.** Selected NOESY correlations of **1**.

The connectivity between C-1 and C-14 was determined on the basis of HMBC correlations. The ester carbonyl resonating at δ 169 was assigned to C-12, because it showed HMBC cross peaks with the to H-13a/b vinyl protons resonating at δ 6.22 and 5.65. A MS fragment at *m/z* 99 (M^+ - 261) indicated that acyl substituent of **1** could be a 2-methoxy- methyl-2-propenoyl group and this was certified by HMBC correlation between H-3′a/b and C-1′, H-4′ and C-3′, and methoxy carbon and C-4′. This ester group was attached to the C-8 position because the H-8 proton resonating at δ 5.12 displayed HMBC connectivity with C-1′ (Figure 1). The relative stereochemistry of **1** was determined by 2D-NOESY experiments. Strong NOE cross peaks were observed between H1-H5, H1-H2a, H2a-H3 and H6-H8, whereas weak NOEs were observed between H5-H6, H6-H7, and H7-H8 (Figure 2).

Compound (**2**) had the molecular formular $C_{21}H_{24}O_7$ as deduced from the HREIMS, suggesting seven olefinic double bonds and three rings. The ^1H-^1H COSY spectrum of **2** revealed good connectivities to infer the same skeleton with **1**. Based on the ^{13}C NMR spectrum of **2**, two of three esters are come from acyl groups (Table 1). The first one was elucidated as 2-hydroxymethyl-2-propenoyl group by the MS fragment at *m/z* 85 (M^+ - 303) and the observed HMBC correlation between H-3′a/b and C-1′ and between H-4′ and C-3′. The other acyl substituent was an acetyl group by the mass fragment at *m/z* 345 (M^+ - 43) and the HMBC correlation of the C-3 proton (δ 5.57) with the carbonyl group of the acetate (δ 170.7). Since the H-8 proton resonating at δ 5.16 displayed HMBC correlation with the C-1′, the 2-hydroxymethyl-2-propenoyl group is placed at C-8, therefore, acetoxyl group must be at C-3. The relative stereochemistry of **2** was elucidated to be the same as that of **1** by the NOESY spectrum.

The cytotoxicities of **1-3** were examined for their *in vitro* cytotoxic activity against LOX-IMVI (human melanoma cell), MCF-7 (human breast adenocarcinoma), PC-3 (human prostate adenocarcinoma cell) and HCT-15 (human colorectal adenocarcinoma cell) cell lines. The IC_{50} value for **1-3** are shown in Table 2, and it is apparent that esterfied compounds (**1**) and (**2**) are more potent than **3**.

Table 2. *In vitro* cytotoxicity of the compounds (**1-3**) on human cell lines.

compound	cell lines IC_{50} (μg/mL)			
	LOX-IMVI	MCF-7	PC-3	HCT-15
1	3.4 ± 0.4	1.3 ± 0.1	3.3±0.2	1.0 ± 0.1
2	13.2 ± 0.3	10.9±0.5	14.5±0.4	6.2 ± 0.2
3	> 30	> 30	> 30	17.8 ± 0.4

EXPERIMENTAL

General Experimental Procedures. Optical rotations were recorded on a PERKIN-ELMER polarimeter. IR spectra were recorded on a Bruker IFS66 infrared Fourier transform spectrophotometer (KBr) and UV spectra were measured in MeOH on a Beckman DU650 spectrophotometer. Low-resolution EIMS and HREIMS were obtained on JEOL JMS-700 spectrometer. ^{1}H and ^{13}C NMR spectra along with 2D-NMR data were obtained on a Bruker AM 500 (^{1}H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz) spectrometer in $CDCl_3$ solution.

Plant Material. The sample of *Hemisteptia lyrata* Bunge was collected at Parkjeon, Hamyang, Korea in June 1998, and identified by prof. Myong Gi Chung. A voucher specimen (Park, K. H. 103) of this raw material has been deposited at the Herbarium of the Gyeongsang National University (GNUC).

Extraction and Isolation. The dry flowers (1 kg) were extracted with $CHCl_3$ (10 L x 3) at rt. The extracts were washed with brine, dried over anhydrous Na_2SO_4, and then concentrated to give a thickish residue (120 g). The residue was chromatographed on a Silica gel (1.2 kg) column eluted with a gradient of 100 % hexane to 100 % EtOAc and then to 20% MeOH to afford twenty fractions. Fraction 12 (1.6 g) possessing promising activity was subjected to flash chromatography on a Silica gel (100 g) using a gradient of 100 % hexane to 80 % EtOAc to yield fraction A (containing **1** and **2**) and fraction B (containing **3**). A part of fraction A (130 mg) was applied on a preparative TLC plate (Silica gel) developed with hexane/EtOAc (1:1) to yield **2** (15 mg, R_f 0.37, *n*-hexane/EtOAc, 1:1) and **1** (32 mg, R_f 0.3, *n*-hexane/EtOAc, 1:1). A part of fraction B (90 mg) was purified on a preparative TLC plate (Silica gel) developed with *n*-hexane/EtOAc (2:3) to yield 8-hydroxyzaluzanin C (**3**) (23 mg, R_f 0.4, *n*-hexane/EtOAc, 2:3).

8-*O*-(2-Methoxymethyl-2-propenoyl)-3-hydroxy-4(15),10(14),11(13)-guaiatrien-12,6-olide (1). Oil, $[\alpha]^{20}_{D}$ +80.2° (*c* 1.0, $CHCl_3$); IR ν_{max} 3438, 2927, 1765, 1716, 1269 cm^{-1}; UV (MeOH)λ_{max} 240 nm; HREIMS *m/z* 360.1573 (calcd for $C_{20}H_{24}O_6$, 360.1580); EIMS *m/z* 360$[M]^+$ (6), 304 (1), 290 (8), 275 (2), 262 (3), 244 (57), 226 (28), 216 (23), 198 (17), 173 (25), 159 (15), 148 (24), 129 (19), 119 (25), 99 (58), 91 (39), 69 (100), 55 (16); ^{1}H and ^{13}C NMR: see Table 1

8-*O*-(2-Hydroxymethyl-2-propenoyl)-3-acetoxy-4(15),10(14),11(13)-guaiatrien-12,6-olide (2). Oil, $[\alpha]^{20}_D$ +38.3° (*c* 0.67, $CHCl_3$); IR ν_{max} 3461, 2927, 1765, 1728, 1716, 1238 cm^{-1}; UV (MeOH) λ_{max} 224 nm; HREIMS *m/z* 388.1522 (calcd for $C_{21}H_{24}O_7$, 388.1505); EIMS *m/z* 388$[M]^+$ (0.5), 345 (43), 286 (1), 261 (3), 244 (37), 226 (56), 198 (30), 181 (22), 169 (21), 129 (22), 119 (21), 91 (37), 85 (100), 57 (18); ^1H and ^{13}C NMR: see Table 1

Sulforhodamin B Assay. Human cancer cell lines were cultivated in humidified incubators (37 °C, 5% CO_2). The cells were grown in RPMI 1640 with additional glutamine (300 mg/L), 1% penicillin/streptomycin, and 10% fetal calf serum. The cells were free from mycoplasm contamination as tested routinely; cells were seeded in 24-well plates and allowed to grow 24 h before treatment. Cytotoxicity was determined as described previously,[7] and calculated as survival of treated cells over control cells x 100 [% T/C].

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