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NEW SESQUITERPENE LACTONES FROM *HEMISTEPTIA LYRATA* BUNGE

Tae Joung Ha,[a] Ki Hun Park,[a] Dae Sik Jang,[b] Jong Rok Lee,[a] Ki Min Park,[c] and Min Suk Yang*,[a]

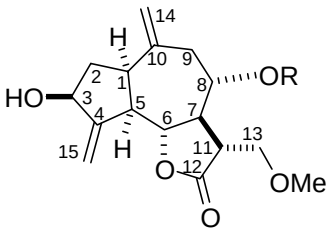
[a]Department of Agricultural Chemistry, Division of Applied Life Science, Gyeongsang National University, Chinju 660-701, Korea, [b]Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, U.S.A, [c]The Research Institute of Natural Science, Gyeongsang National University, Chinju 660-701, Korea

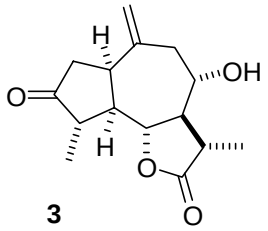
Abstract—From the flowers of *Hemisteptia lyrata*, two new and two known guaianolides were isolated. The structures and relative configuration of the new compounds were established as 8α-*O*-2-hydroxymethyl-2-propenoyl-3β-hydroxy-13-methoxy-4(15),10(14)-guaiadien-12,6-olide (**1**) and 3β,8α-dihydroxy-13-methoxy-4(15),10(14)-guaiadien-12,6-olide (**2**), together with known compounds, isoamberboin (**3**) and 11,13-dihydro-deacylcynaropicrin (**4**). The structures were established by spectral data and X-Ray diffraction analysis.

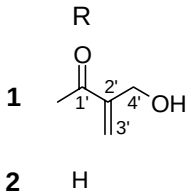
Hemisteptia lyrata B. (Compositae) is wild plant of red-violet flower blooming from May to June at every place in Korea. This species is placed in only one Hemisteptia genus in many compositae. This plant has been used as traditional folk medicine in China and Korea; anti-febrile, anti-bleeding, anti-tumor, anti-bacterial and anti-inflammatory remedy.[1-3] We have been previously reported the isolation of two new guaiane type sesquiterpene lactones, 8-*O*-2-methoxymethyl-2-propenoyl-3-hydroxy-4(15),10(14),11(13)-guaiatrien-12,6-olide and 8-*O*-2-hydroxymethyl-2-propenoyl-3-acetoxy-4(15), 10(14),11(13)-guaiatrien-12,6-olide, together with known compound, 8-hydroxyzaluzanin C from the chloroform extract of *H. lyrata* flowers.[4] Further studies of this plant flowers led to isolation of two new 8α-*O*-2-hydroxymethyl-2-propenoyl-3β-hydroxy-13-methoxy-4(15),10(14)-guaiadien-12,6-olide (**1**) and 3β,8α-dihydroxy-13-methoxy-4(15),10(14)-guaiadiene-12,6-olide (**2**), together with known

compounds, isoamberboin (**3**) and 11,13-dihydro-deacylcynaropicrin (**4**). In this Note, we wish to describe the isolation and structural elucidation of the two new sesquiterpene lactones (**1** and **2**).

Compound (**1**) had the molecular formula $C_{20}H_{26}O_7$ with eight degrees of unsaturation, as deduced from its HREIMS. The IR spectrum of **1** showed absorptions at 3429, 1768 and 1717 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







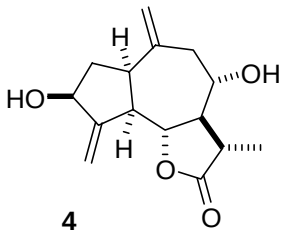
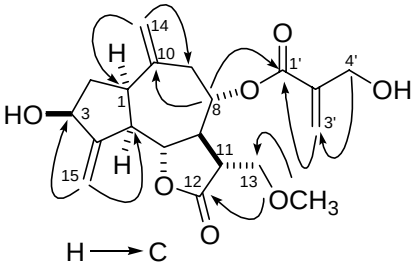


Table 1. ^1H-NMR spectral data for compounds (**1-4**)

	^1H			
position	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**4** (CD_3OD)
1	2.95 ddd(8.5, 8.5, 8.5)	2.93 ddd(8.8, 8.5, 8.5)	3.12 ddd(7.8, 7.8, 2.9)	2.92 ddd(8.5, 8.5, 8.5)
2	1.76 ddd(13.5, 9.0, 7.0)	1.74 ddd(13.3, 9.7, 7.4)	2.48 dd(19.0, 3.0)	1.67 ddd(16.8, 8.4, 8.4)
	2.28 m	2.28 ddd(13.3, 7.3, 7.3)	2.54 dd(19.0, 8.5)	2.23 ddd(15.6, 7.8, 7.8)
3	4.56 br t(1.4)	4.53 m		4.47 m
4			2.23-2.27 m	
5	2.89 m	2.84 br d(7.6)	2.23-2.27 m	2.82 m
6	4.18 dd(10.0, 10.0)	4.13 dd(10.0, 10.0)	3.93 dd(9.1, 9.0)	4.06 dd(9.8, 9.8)
7	2.82 m	2.22 m	2.23-2.27 m	2.05 ddd(10.0, 10.0, 10.0)
8	5.06 ddd(10.2, 6.7, 5.0)	3.68 m	3.75 m	3.65 ddd(8.9, 8.9, 5.1)
9	2.31 m	2.24 dd(6.8, 6.8)	2.23-2.27 m	2.16 dd(12.7, 8.1)
	2.76 dd(13.5, 5.0)	2.69 dd(13.4, 5.0)	2.83 dd(12.5, 5.6)	2.69 dd(12.7, 5.0)
10				
11	2.61 dt(11.0, 2.5)	2.80 m	2.61 dd(10.4, 7.0)	2.61 dd(10.8, 7.0)
12				
13	3.50 dd(9.5, 3.5)	3.49 dd(9.5, 9.5)	1.44 d(7.0)	1.33 d(7.0)
	3.76 dd(9.5, 2.5)	3.99 dd(9.4, 3.4)		
14	5.03 s	5.03 s	4.76 s	4.97 s
	5.16 s	5.08 s	5.06 s	5.02 s
15	5.33 dd(1.5, 1.5)	5.32 dd(1.8, 1.8)	1.22 d(6.3)	5.24 d(0.7)
	5.42 dd(1.5, 1.5)	5.39 dd(1.6, 1.6)		5.27 br s
1′				
2′				
3′	5.92 d(1.0)			
	6.31 s			
4′	4.32 d(14.0)			
	4.37 d(14.0)			
OCH_3	3.45 s	3.45 s		

NOESY). The ^{13}C NMR spectral data showed the presence of twenty carbon atoms as two carbonyl groups, one methoxyl, three sp^2 methylenes, four sp^3 methylenes, seven methines and three quaternary carbons. Hence, the extra degrees of unsaturation were presumed to be due to three rings. The ^1H-NMR spectra data of **1** were very similar to those (Table 1) of **2** and **4** except for the chemical shifts of H-7, H-8 and H-13. A convenient starting point of ^1H-^1H COSY is the H-15a/b vinyl protons resonating at δ 5.33 and 5.42, because nonequivalent methylene protons linked to the same carbon (δ 112.9) from HMQC experiment. The ^1H-^1H COSY spectrum revealed successive connectivities from C-1 to C-15 except between C-9 and C-14. The unassigned connection between C-9 and C-14 was determined on the basis of HMBC correlations. The ester carbonyl resonating at δ 175.8 was assigned to C-12, because it showed HMBC cross peaks with the to H-11 (δ 2.61) and H-13a/b protons (δ 3.50 and 3.76).



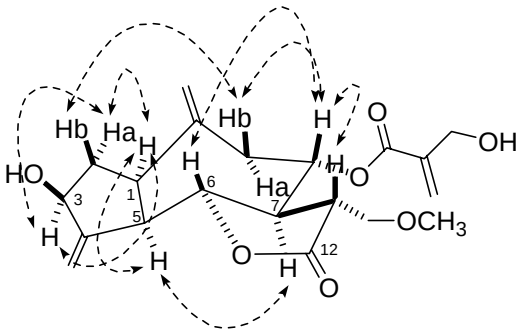


Figure 1. Important HMBC correlations of **1**. **Figure 2.** Selected NOESY correlations of **1**.

A MS fragment at *m/z* 276 (M^+ - 102) indicated that acyl substituent of **1** could be a 2-hydroxymethyl-2-propenoyl group and this was certified by HMBC correlation between H-3′a/b and C-1′, H-4′ and C-3′. MS fragment at *m/z* 346 (M^+ - 32) indicated that the presence of methoxy group and this group correlated with H-13a/b and C-13. This ester group was attached to the C-8 position because the H-8 proton resonating at δ 5.06 displayed HMBC connectivity with C-1′ (Figure 1). The relative stereochemistry of **1** was determined by NOESY experiments. Strong NOE cross peaks were observed between H-1/H-5, H-1/H-3, H-6/H-8 and H-8/H-11, whereas weak NOEs were observed between H-5/H-6, H-6/H-7, and H-7/H-8 (Figure 2).

Compound (**2**) had the molecular formular $C_{16}H_{22}O_5$ as deduced from the HREIMS, suggesting three 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 and MS fragment at *m/z* 262 (M^+ - 32) of **2**, one methoxy group was existed. And this group correlated with H-13a/b and C-13 in HMBC spectrum. The relative stereochemistry of **2** was elucidated to be the same as that of **1** by the NOESY spectrum.

Compound (**3**) gave a molecular ion peak at *m/z* 264, consistent with the molecular formula $C_{15}H_{20}O_4$. The IR spectrum of **3** showed absorptions at 3496, 1750 and 1737 cm^{-1}, suggesting the presence of hydroxyl and two cyclic carbonyl groups. ^1H-^{13}C COSY spectrum have showed that the δ_C 47.3, 51.4, 54.0 and 49.1 signals are overlapped with δ_H 2.23-2.27 broad signals. Therefore, it is very difficult to elucidation of structure and stereochemistry, so that we identified the chemical structure by X-Ray crystallography with give a single crystal (Figure 3), which has not previously been reported.

Compound (**4**) had the molecular formula $C_{15}H_{20}O_4$ with six degrees of unsaturation, as deduced from its HREIMS. The IR spectrum of **4** showed absorptions at 3400 and 1744 cm^{-1}, suggesting the presence of hydroxy and one ester group. The structure of **4** was elucidated with 1 and 2-D NMR experiments. The relative stereochemistry of compound **4** was certified by X-Ray single crystallography experiment (Figure 4), which has not previously been reported.

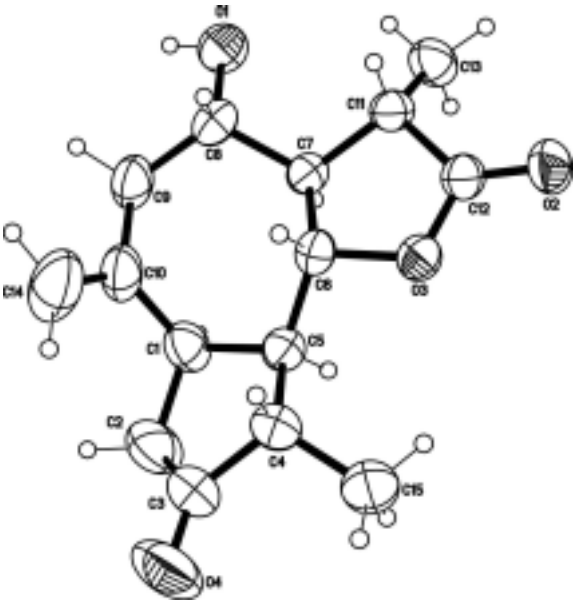


Figure 3. ORTEP view of compound (**3**).

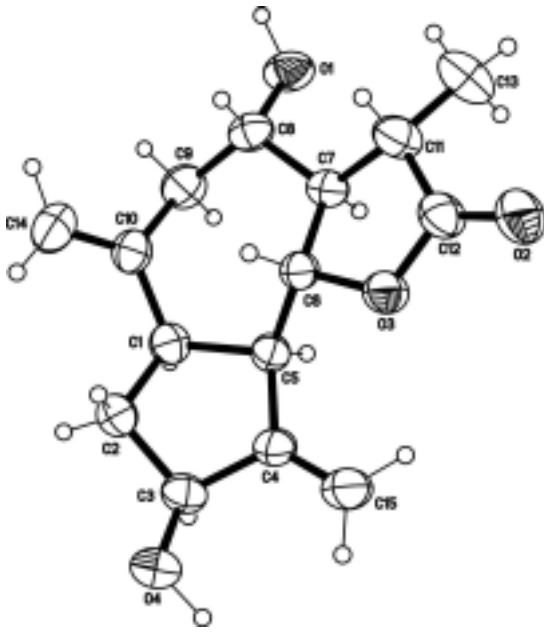


Figure 4. ORTEP view of compound (**4**).

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. ^1H and ^{13}C NMR spectra along with 2D-NMR data were obtained on a Bruker AM 500 (^1H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz) spectrometer in $CDCl_3$ and CD_3OD 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 for 72hr. 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 (F1-F20). Fraction 16 (0.8 g) was eluted with CH_2Cl_2–EtOAc mixtures of increasing polarity (49:1→1/1). Altogether, 100 fractions were collected and combined to give six major subfractions (F16-1 through F16-6), based on the comparison of TLC profiles. F16-5 was further purified in small chromatographic column containing silica gel, eluting with hexane-EtOAc (2:3) to afford the pure compound (**1**) (14 mg, R_f 0.21, *n*-hexane/EtOAc, 1:1). F16-2 was carried out silica gel chromatography with gradient mixture of $CHCl_3$ and EtOH, and gave the crude crystals of **4** (10 mg, R_f 0.46, *n*-hexane/EtOAc, 1:1). Fraction 12 (1.2g) was also carry out the silica gel column chromatography, using mixtures of hexane and EtOAc to the ratio of 19/1 volum percentage. In this column, five major subfractions (F12-1 through F12-5) were obtained. Compound (**3**) (36 mg, R_f 0.4, *n*-hexane/EtOAc, 3:2) was isolated by recrystallization with petroleum ether from F12-3. A part of fraction F12-5 was also carried out silica gel chromatography with gradient mixture of $CHCl_3$ and EtOAc, and purified on a preparative TLC plate (silica gel) developed with *n*-hexane/EtOAc (1:1) to yield **2** (13 mg, R_f 0.4, *n*-hexane/EtOAc, 3:2).

8-*O*-(2-Hydroxymethyl-2-propenoyl)-3-hydroxy-13-methoxy-4(15),10(14)-guaiadien-12,6-olide (1). Oil, $[\alpha]^{20}_D$ +35.42° (*c* 1.66, $CHCl_3$); IR ν_{max} 3429, 2931, 1768, 1717 and 1270 cm^{-1}; UV (MeOH)λ_{max} 210 nm; HREIMS *m/z* 378.1682 (calcd for $C_{20}H_{26}O_7$, 378.1679); EIMS *m/z* 378$[M]^+$ (6), 346 (1), 295 (12), 276 (29), 244 (44), 226 (50), 214 (56), 199 (43), 173 (84), 155 (65), 129 (49), 119 (68), 105 (55), 85 (100), 55 (6); ^{1}H-NMR: see Table 1; ^{13}C NMR ($CDCl_3$, δ): 175.8 (C-12), 165.7 (C-1'), 153.1 (C-4), 142.4 (C-10), 139.8 (C-2'), 127.2 (C-3'), 117.9 (C-14), 112.9 (C-15), 79.3 (C-6), 76.4 (C-8), 74.0 (C-3), 69.3 (C-13), 62.7 (C-4'), 59.7 (O*C*H_3), 50.8 (C-5), 47.9 (C-11), 46.4 (C-7), 44.7 (C-1), 40.6 (C-9), 39.2 (C-2).

3,8-Dihydroxy-13-methoxy-4(15),10(14)-guaiadien-12,6-olide (2). Crystal (pet. ether), mp 152-153°C; $[\alpha]^{20}_D$ +92.2° (*c* 0.55, $CHCl_3$); IR ν_{max} 3414, 3348, 2953, 1768, 1636, 1453 and 1168 cm^{-1}; UV (MeOH) λ_{max} 205 nm; HREIMS *m/z* 294.1470 (calcd for $C_{16}H_{22}O_5$, 294.1467); EIMS *m/z* 294$[M]^+$ (24), 279 (54), 276 (10), 247 (8), 231 (21), 214 (27), 201 (20), 185 (18), 173 (35), 157 (49), 129 (22), 119 (74), 105 (68), 91 (100), 79 (69), 55 (39); ^{1}H-NMR: see Table 1; ^{13}C NMR ($CDCl_3$, δ): 174.2 (C-12), 152.8 (C-4), 143.1 (C-10), 116.4 (C-14), 112.7 (C-15), 79.2 (C-6), 73.7 (C-3), 73.3 (C-8), 72.1 (C-13), 59.2 (O*C*H_3), 56.0 (C-7), 50.4 (C-5), 48.4 (C-11), 44.4 (C-1), 41.8 (C-9), 38.9 (C-2).

Isoamberboin (3). White needle crystal (pet. ether); mp 179°C; $[\alpha]^{20}_{D}$ +139.2° (*c* 1.59, $CHCl_3$); IR ν_{max} 3496, 2923, 1750, 1737 and 1172 cm^{-1}; UV (MeOH) λ_{max} 208 nm; HREIMS *m/z* 264.1365 (calcd for $C_{15}H_{20}O_4$, 264.3169); EIMS *m/z* 264$[M]^+$ (8), 246 (7), 168 (43), 139 (31), 83 (44), 71 (100), 69 (73), 53 (44); ^{1}H-NMR: see Table 1; ^{13}C NMR ($CDCl_3$, δ): 219.1 (C-3), 178.6 (C-12), 143.9 (C-10), 114.9 (C-14), 83.2 (C-6), 75.7 (C-8), 54.1 (C-7), 51.4 (C-5), 49.2 (C-9), 47.3 (C-4), 43.6 (C-2), 41.1 (C-11), 39.6 (C-1), 16.4 (C-13), 14.4 (C-15).

11,13-Dihydro-deacylcynaropicrin (4). White needle crystal (pet. ether); mp 159°C; $[\alpha]^{20}_{D}$ +64.1° (*c* 0.12, $CHCl_3$); IR ν_{max} 3402, 2932, 1744 and 12852 cm^{-1}; UV (MeOH) λ_{max} 205 nm; HREIMS *m/z* 264.1361 (calcd for $C_{15}H_{20}O_4$, 264.3169); EIMS *m/z* 264$[M]^+$ (8), 246 (7), 229 (3), 218 (7), 200 (7), 173 (25), 155 (14), 119 (56), 105 (50), 91 (96), 71 (100), 69 (55), 53 (55); ^{1}H-NMR: see Table 1; ^{13}C NMR (CD_3OD, δ): 181.5 (C-12), 154.6 (C-4), 145.6 (C-10), 115.7 (C-14), 110.2 (C-15), 81.5 (C-6), 76.0 (C-8), 73.8 (C-3), 56.9 (C-7), 51.0 (C-5), 46.8 (C-9), 44.4 (C-1), 42.8 (C-11), 39.5 (C-11), 16.4 (C-13).

X-Ray analysis. Molecular formula of compound (**3**), $C_{15}H_{20}O_4$, MW, 264.31; monoclinic; P2(1); a = 8.1470(7) Å, b = 7.6097(7) Å, c = 11.5763(11) Å, V = 711.68(11) $Å^3$, Z = 2; D_{calc} = 1.233Mg/cm^3; T = 298(2)K. A colorless fragment with dimensions of 0.30×0.30(0.40 mm^3 was used for data collection by a Siemens Smart diffractometer equipped with graphite monochromated MoKa radiation (λ=0.71073 Å) and a CCD detector. Molecular formula of compound (4), $C_{15}H_{20}O_4$, MW, 264.31; monoclinic; P2(1); a = 5.5528(7) Å, b = 16.535(2) Å, c = 7.5545(9) Å, V = 683.89(14) Å3, Z = 2; D_{calc} = 1.284Mg/cm^3; T = 298(2)K. A colorless fragment with dimensions of 0.30×0.50×0.50 mm^3 was used for data collection by a Siemens Smart diffractometer equipped with graphite monochromated MoKa radiation (λ=0.71073 Å) and a CCD detector. The frame data were processed to give structural factors by the SAINT program.[5] The intensity data were corrected for Lorentz and polarization effects. The first 50 frames were retaken after complete data collection. The crystal showed no significant decay and no correction was applied for absorption or decay. The structure was solved by a direct method and refined by full-matrix least squares against F^2 for all data by using the SHELXTL program package.

ACKNOWLEDGEMENT

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