A New Glycoside and a Novel-Type Diterpene from *Hemerocallis fulva* (L.) L.

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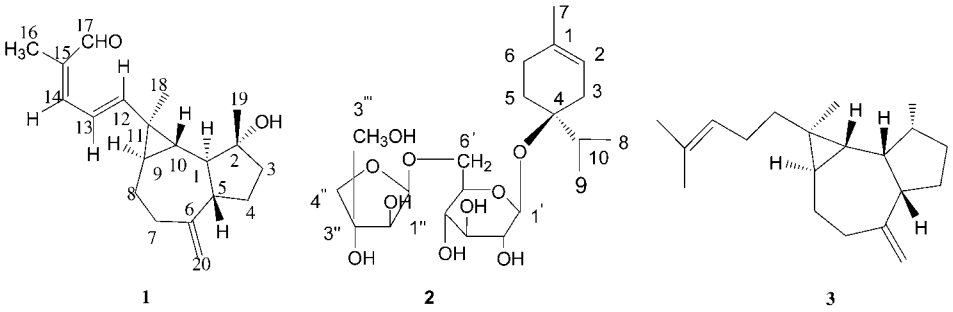
The $CHCl_3$ extract of dried roots of *Hemerocallis fulva* (L.) L. afforded a novel diterpene named hemerocallal A (**1**), which is the second reported naturally occurring diterpene with a *trans*-bicyclo[5.1.0]octane system. The BuOH extract afforded a new glycoside named hemerocalloside (**2**). Their structures were established on the basis of spectroscopic and chemical studies.

1. Introduction. – *Hemerocallis fulva* (L.) L. is a perennial plant native to China and used as antifebrile and diuretic in folk medicine. Some 2,5-dihydrofuryl-γ-lactam derivatives [1], anthraquinones [2], and hemerocallone [2], *etc.*, have been found in this genus. In the present report, we describe the isolation and structure elucidation of a novel-type diterpene named hemerocallal A (=(2*Z*,4*E*)-5-[(1α,1aβ,4aα,7α,7aβ,7bα)-decahydro-7-hydroxy-1,7-dimethyl-4-methylene-1*H*-cycloprop[*e*]azulen-1-yl]-2-methylpenta-2,4-dienal; **1**) and a new glycoside named hemerocalloside (=4-methyl-1-(1-methylethyl)cyclohex-3-en-1-yl] 6-*O*-L-apio-β-D-furanosyl-β-D-glucopyranoside; **2**) from *H. fulva* (L.) L. collected in the Guangxi province. Hemerocallal A (**1**) is a diterpene with a *trans*-bicyclo[5.1.0]octane moiety. In 1995, *Cronan et al.* [3] reported a diterpene named emottene (**3**) (*Fig. 1*) having a similar parent structure as **1**, isolated from the marine organism *Briareum polyanthes*. So far, besides **1** and **3**, no other compounds of this type have been found. Because hemerocallal A (**1**) has more functional groups than emottene (**3**), the isolation of **1** is the starting point of a project aiming at the synthesis of diterpenes of this type by chemical-modification methods, a project that we plan to pursue in our future work.



1 **2** **3**

Fig. 1. *Structures of compounds* **1**–**3**. Numbering arbitrary; for systematic names, see *Exper. Part.*

2. Results and Discussion. – Compound **1**, a colorless oil, had the molecular formula $C_{20}H_{28}O_2$, as determined by HR-EI-MS (*m/z* 300.20909 (M^+, calc. 300.20910) in combination with ^{1}H- and ^{13}C-NMR data (*Table 1*). The IR data suggested the presence of an aldehyde (1656 cm^{-1}) and an OH group (3429 cm^{-1}). The ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMBC (*Fig. 2*), HMQC, and NOE (*Fig. 2*) data, and their comparison with those of emottene (**3**) [3], allowed the assignment of the structure shown in *Fig. 1*.

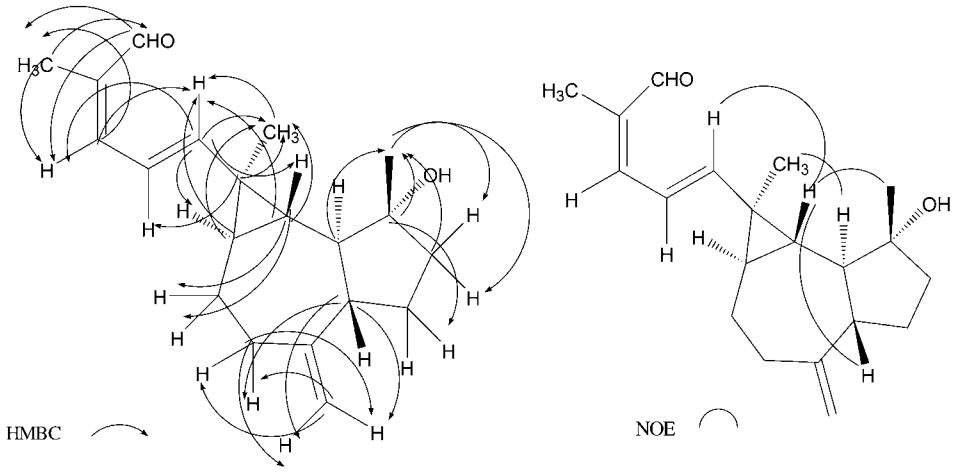


Fig. 2. *HMBC and NOE of compound* **1**

Table 1. *^{1}H- and ^{13}C-NMR Data ($CDCl_3$) of Compound* **1**. Arbitrary numbering. δ in ppm, *J* in Hz.

	δ (H)	δ (C) (DEPT)
CH(1)	1.49 (*t*, *J* = 10.7)	53.31 (CH)
C(2)	–	80.75 (C)
CH_2(3)	1.77 (*ddd*, *J* = 12.7, 6.3, 2.3), 1.58 (*ddd*, *J* = 12.7, 6.0, 1.0)	41.86 (CH_2)
CH_2(4)	1.66 (*dddd*, *J* = 11.7, 6.3, 6.0, 2.0), 1.91 (*dddd*, *J* = 11.7, 9.7, 2.3, 1.0)	26.64 (CH_2)
CH(5)	2.22 (*ddd*, *J* = 10.7, 9.7, 2.0)	52.73 (CH)
C(6)	–	152.40 (C)
CH_2(7)	2.06 (*ddd*, *J* = 13.6, 11.7, 1.4), 2.45 (*dd*, *J* = 13.6, 5.8)	38.13 (CH_2)
CH_2(8)	2.03 (*m*)	24.22 (CH_2)
CH(9)	1.16 (*ddd*, *J* = 10.7, 12.2, 2.0)	30.46 (CH)
CH(10)	1.00 (*t*, *J* = 10.7)	32.86 (CH)
C(11)	–	28.19 (C)
CH(12)	5.79 (*d*, *J* = 15.2)	155.89 (CH)
CH(13)	6.43 (*dd*, *J* = 15.2, 11.3)	120.09 (CH)
CH(14)	6.78 (*d*, *J* = 11.3)	150.00 (CH)
C(15)	–	134.90 (C)
Me(16)	1.82 (*d*, *J* = 1.0)	9.42 (CH_3)
CH(17)	9.37 (*s*)	194.97 (CH)
Me(18)	1.26 (*s*)	12.13 (CH_3)
Me(19)	1.21 (*s*)	26.08 (CH_3)
CH_2(20)	4.72 (*s*), 4.69 (*s*)	107.26 (CH_2)

The DEPT spectrum indicated that **1** had three Me, five CH_2 (four sp^3-CH_2 and one sp^2-CH_2), eight CH (four sp^3-CH, three sp^2-CH, and one aldehydic CH), four quaternary C-atoms (two sp^2-C, one O–C, and one sp^3-C). The ^{1}H- and ^{13}C-NMR data of **1** showed the presence of a Me group attached to an olefinic C=C bond (δ 1.82 (*d*, *J* = 1.0 Hz), δ 9.42), two *s* Me groups (δ 1.26, 1.21; δ 12.13, 26.08), a penta-1,3-dienal moiety (δ 9.37 (*s*), 6.78 (*d*, *J* = 11.3 Hz), 6.43 (*dd*, *J* = 15.2, 11.3 Hz), 5.79 (*d*, *J* = 15.2 Hz); δ 194.97, 134.90, 150.00, 120.09, 155.89), an exocyclic CH_2=C moiety (δ 4.72 (*s*), 4.69 (*s*); δ 107.26, 152.40), and quaternary C-atom connected to an O-atom (δ 80.75). The HMQC analysis revealed the complete assignment of all C–H bonds in **1**. Sequential ^{1}H,^{1}H correlations from Me(16) to H–C(12) and from H–C(7) to H–C(3) were established. These findings suggested the partial structures **a**–**f** (*Fig. 3*) that could be connected by a HMBC analysis (see *Fig. 2*). The following key HMBC correlations allowed to deduce the gross structure of **1** from **a**–**f**: Me(16)/C(17), C(14); C(12)/Me(18); C(18)/H–C(12); C(9) and C(10)/Me(18); C(6) and C(20)/H–C(7); C(5)/H–C(20); Me(19)/C(1), C(2), and C(3). The relative configuration of **1** was established by the NOE experiment (*Fig. 2*) and ^{1}H,^{1}H coupling constants. The ring junction proton H–C(1) was coupled by 10.7 Hz to H–C(5) and had no observable NOE with that proton, thus suggesting a *trans*-ring junction of the perhydroazulene moiety. A *t* (*J*(9,10) = *J*(1,10), = 10.7 Hz) of H–C(10) (δ 1.00) in the ^{1}H-NMR and NOEs between H–C(10) and H–C(5) (2.9%) and between H–C(9) and H–C(1) (4.0%) was compatible with a *trans*-ring junction of the fused cyclopropane unit. The relative configuration at C(2) was defined by an NOE between Me(19) and H–C(10) (3.4%). The relative configuration at the remaining chiral center C(11) was confirmed by NOEs between H–C(1) and Me(18) (5.2%) and between H–C(10) and H–C(12) (3.9%). The coupling constants (*J*(12,13) = 15.2 Hz, *J*(13,14) = 11.3 Hz) showed that H–C(12) and H–C(13) should be *trans*- and H–C(13) and H–C(14) *s-cis*-positioned to each other. An allylic coupling between Me(16) and H–C(14) (*J*(14,16) = 1.0 Hz) suggested their *cis*-relationship, which was also confirmed by the cross-peak Me(16)/H–C(14) in the ROESY plot.

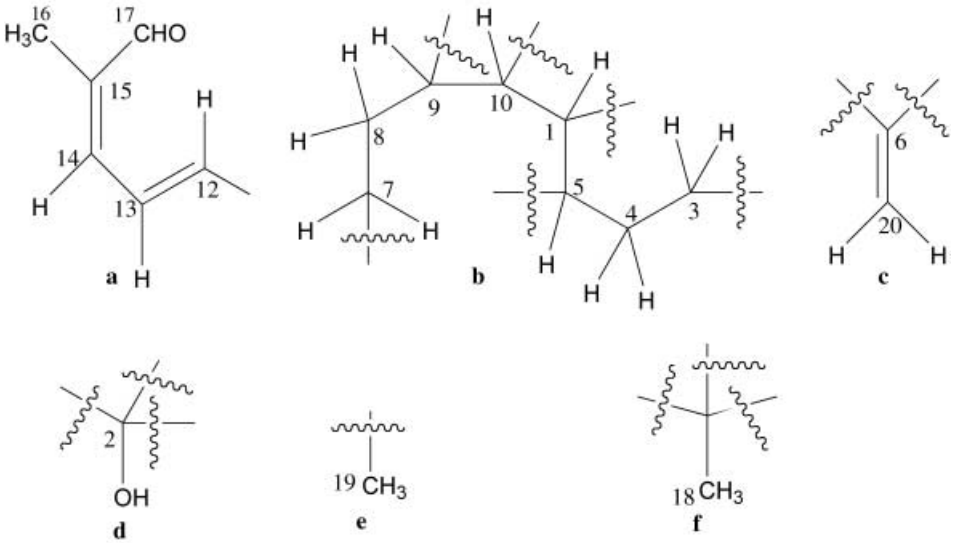


Fig. 3. *Partial structures* **a**–**f** *of* **1**

Compound **2**, an amorphous powder, had the molecular formula $C_{21}H_{36}O_{10}$, as determined by ESI-MS (*m*/*z* 471.3 ($[M+Na]^+$) in combination with its ^{1}H- and ^{13}C-NMR and DEPT data (*Table 2*). The IR spectrum revealed absorptions of OH groups (3420–3430 cm^{-1}), an ^{i}Pr group (1384 cm^{-1}) and a C=C bond (1635 cm^{-1}). The ^{1} H- and ^{13}C-NMR (*Table 2*), HMBC, and HSQC data and their comparison with the data of terpinen-4-ol [4] and *O*-L-apio-β-D-furanosyl [5] and *O*-β-D-glucopyranosyl moieties [6] allowed us to establish the structure of **2** as shown in *Fig. 1*. The presence

Table 2. *^{1}H- and ^{13}C-NMR* (CD_3OD) *Data of Compound* **2**. Arbitrary numbering. δ in ppm, J in Hz. The assignments were based on HSQC, HMBC, and DEPT experiments.

	δ (H)	δ (C) (DEPT)		δ (H)	δ (C) (DEPT)
C(1)	–	135.98 (C)	CH(2′)	3.32[a])	76.83 (CH)
CH(2)	5.29 (br. *s*)	120.19 (CH)	CH(3′)	3.32[a])	78.75 (CH)
CH_2(3)	2.22 (*d*, *J* = 8.5)	32.25 (CH_2)	CH(4′)	3.22 (*t*, *J* = 8.8)	72.45 (CH)
	2.22 (*d*, *J* = 8.5)		CH(5′)	3.15 (*m*)	76.03 (CH)
C(4)	–	81.84 (C)	CH_2(6′)	3.52 (*dd*, *J* = 9.0, 6.3),	69.39 (CH_2)
				3.91 (*dd*, *J* = 9.0, 2.5)	
CH_2(5)	1.64 (*m*), 1.84 (*m*)	30.75 (CH_2)			
			CH(1″)	4.96 (*d*, *J* = 1.8)	111.43 (CH)
CH_2(6)	2.32 (*m*)	29.15 (CH_2)	CH(2″)	3.87 (*d*, *J* = 1.8)	78.57 (CH)
Me(7)	1.64 (*s*)	23.95 (CH_3)	C(3″)	–	81.09 (C)
Me(8)	0.95 (*t*, *J* = 6.6)	18.38 (CH_3)	CH_2(3‴)	3.58 (*s*)	66.33 (CH_2)
Me(9)	0.93 (*t*, *J* = 6.6)	18.14 (CH_3)	CH_2(4″)	3.77 (*d*, *J* = 9.9)	75.49 (CH_2)
CH(10)	1.93 (*m*)	35.82 (CH)		3.94 (*d*, *J* = 9.9)	
CH(1′)	4.40 (*d*, *J* = 7.7)	99.30 (CH)			

[a]) Overlap.

of the sugar units D-glucose and L-apiose were also confirmed by acid hydrolysis of **2** and identification of the formed sugars by TLC comparison with authentic samples.

The ^{1}H- and ^{13}C-NMR data of **2** showed the presence of a *O*-L-apio-β-D-furanosyl (δ 4.96 (*d*, *J* = 1.8 Hz), δ 111.43, 78.57, 81.09, 66.33, 75.49) [5], a *O*-β-D-glucopyranosyl (δ 4.40 (*d*, *J* = 7.7 Hz), δ 99.30, 76.83, 78.75, 72.45, 76.03, 69.39) [6] moiety, and 1 C=C bond (δ 5.29 (br. *s*), δ 135.98, 120.19). The four degrees of unsaturation deduced from the molecular formula $C_{21}H_{36}O_{10}$, two of which were attributed to the *O*-L-apio-β-D-furanosyl and the *O*-β-D-glucopyranosyl moiety, and one of which was caused by the C=C bond, implied the presence of a monocyclic C-skeleton. Except for the ^{1}H- and ^{13}C-NMR signals of the *O*-L-apio-β-D-furanosyl and the *O*-β-D-glucopyranosyl moieties, the remaining signals of **2** closely resembled those of terpinen-4-ol [4]. The cross-peaks in the HMBC plot (see *Table 3*) of compound **2**, *i.e.*, C(4)/H–C(1′) and C(6′)/H–C(1″), showed that the *O*-β-D-glucopyranosyl was located at C(4), and that the *O*-L-apio-β-D-furanosyl and *O*-β-D-glucopyranosyl moieties were connected by a $1'' \rightarrow 6'$ linkage. The cross-peaks H–C(1′)/Me(8) and Me(9) in the ROESY plot suggested the α-orientation of the ^{i}Pr group, thus confirming the structure of **2**.

Table 3. *Selected HMBC and ROESY Data of* **2**

HMBC	C(4)/Me(8), Me(9), and H–C(1′); C(6′)/H–C(1″)
ROESY	H–C(1′)/Me(8) and Me(9); CH_2(6′)/H–C(1″)

Experimental Part

General. Column chromatography (CC): silica gel *60 H* (*Qingdao Haiyang Chemical Group Co.*, China), *MCI* gel *CHP20P* (75–150 μ; *Mitsubishi Chemical Co.*, Japan), and *Diaion HP-20* (*Mitsubishi Chemical Co.*, Japan). Optical rotations: *Jasco DIP-181* polarimeter; 10-cm microcell. IR Spectra: *Perkin-Elmer 599B* IR spectrometer; KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-400* instrument; δ in ppm rel. to $SiMe_4$ as internal standard (= 0 ppm), *J* in Hz, measured at 22°. MS: *MAT 711* spectrometer; in *m*/*z* (rel. %).

Plant Material. The roots of *Hemerocallis fulva* (L.) L. were collected in Guangxi Province, China. The plant material was identified by Prof. *Sheng-Li Pan*, Department of Pharmacognosy, Fudan University.

Extraction and Isolation. The air-dried roots (43 kg) of *Hemerocallis fulva* (L.) L. were powdered and extracted with 95% EtOH. The EtOH extract was extracted with $CHCl_3$ and BuOH. The $CHCl_3$-soluble fraction

(247.5 g) was submitted to CC (silica gel, cyclohexane/acetone 30:1, 20:1, 10:1, 8:1, 6:1, 5:1, 3:1, 2:1, 1:1) to give 13 fractions ($XL_1 - XL_{13}$). *Fr.* XL_5 (20 g) was repeatedly chromatographed (silica gel): **1** (10 mg). The aq. soln. of the BuOH fraction (145.5 g) was passed on a *Dianion-HP-20* column which was then eluted with EtOH. The eluate (30 g) was submitted to CC (silica gel, $CHCl_3$/MeOH 20:1, 10:1, 8:1, 6:1, 5:1, 3:1) to give 8 fractions ($XZ_1 - XZ_8$). *Fr.* XZ_5 (3.4 g) was repeatedly chromatographed (silica gel and *MCI* gel): **2** (13 mg).

Hemerocallal A (*=(2Z,4*E*)-5-[(1α,1aβ,4aα,7α,7aβ,7bα)-Decahydro-7-hydroxy-1,7-dimethyl-4-methylene-1*H*-cycloprop[*e*]azulen-1-yl]-2-methylpenta-2,4-dienal;* **1**). Colorless oil. $[\alpha]_D^{20} = -18.7$ ($c = 0.65$, $CHCl_3$). IR: 3429, 2928, 1656, 1456, 1386, 1205, 1139, 1103, 966, 921, 894, 750, 657. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 300 (40, M^+), 282 (24, $[M - H_2O]^+$), 267 (15), 239 (8), 212 (20), 159 (80), 113 (58), 119 (48), 105 (84), 93 (88), 91 (100), 79 (50), 67 (24), 55 (36). HR-EI-MS: 300.20909 (M^+, $C_{20}H_{28}O_2^+$; calc. 300.20910).

Hemerocalloside (*=4-Methyl-1-(1-methylethyl)cyclohex-3-en-1-yl 6-*O*-*L*-Apio-β-*D*-furanosyl-β-*D*-gluco-pyranoside*; **2**). Amorphous powder. $[\alpha]_D^{20} = -81.0$ ($c = 0.67$, MeOH). IR: 3420–3430, 2927, 1635, 1384, 1060, 577. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 471.3 ($[M + Na]^+$).

Acid Hydrolysis of **2**. A soln. of **2** (2 mg) in 5% H_2SO_4 in 5% MeOH (5 ml) was refluxed for 2 h. The soln. was diluted with H_2O (10 ml) and extracted with AcOEt and the extract washed with H_2O and then evaporated. The sugars were identified as D-glucose and L-apiose by comparison with authentic samples on TLC (BuOH/AcOEt/^{i}PrOH/AcOH/H_2O 7:20:12:7:6). The org. phase showed several spots on TLC that could not be identified.

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Received May 5, 2003