Two *ent*-Kaurane Diterpenoids from *Rubus corchorifolius* L. f.

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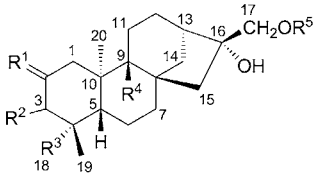
A further chemical investigation of the plant *Rubus corchorifolius* L. f., collected in Hunan Province, afforded two new *ent*-kauranoids **6** and **7**. Their structures were elucidated by various spectroscopic methods.

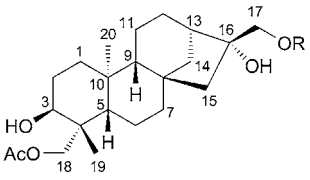
Introduction. – *Rubus corchorifolius* L. f., also known as raspberry, milk bubble, March bubble, *etc.*, is an upright shrub of genus *Rubus* L. It is distributed in the whole country of China, except for the Northeast, Tibet, Gansu, Qinghai, and Xinjiang Provinces [1][2]. It has been used as a Chinese folk medicine to treat diarrhea, extravasated blood, and alcoholism [3]. Recently, as part of a study on the biologically active constituents of this plant collected in Hunan Province, we have reported five new *ent*-kaurane diterpenoids, *i.e.*, (16α)-16,17-dihydroxy-*ent*-kauran-2-one 17-*O*-β-D-glucopyranoside (**1**), (3α,4α,16α)-*ent*-kauran-3,16,17,18-tetraol (**2**), (4α,16α)-16,17,18-trihydroxy-*ent*-kauran-2-one (**3**), (2β,3α,16α)-*ent*-kauran-2,3,16,17-tetraol (**4**), (9β,16α)-9,16,17-trihydroxy-*ent*-kauran-2-one (**5**) (*Fig. 1*) [4][5]. Further investigation on this plant resulted in the isolation of two new *ent*-kaurane diterpenoids (*Fig. 2*). The isolation and structure elucidation of these two compounds are reported in this article.



	R^1	R^2	R^3	R^4	R^5
1	O	H	Me	H	β-D-Glc(p)
2	H,H	α-OH	$HOCH_2$	H	H
3	O	H	$HOCH_2$	H	H
4	β-OH, H	α-OH	Me	H	H
5	O	H	Me	OH	H

Fig. 1. *The* ent-*kaurane diterpenoids* **1**–**5** *isolated from* Rubus corchorifolius *L. f.* [4–5]

[1]) These authors contributed equally to this work.



6 R = H
7 R = β-D-Glc(p)

Fig. 2. *The structures of two new compounds,* **6** *and* **7**, *isolated from* Rubus corchorifolius *L. f.*

Results and Discussion. – Compound **6**, obtained as colorless needle-like crystals and with *quasi*-molecular-ion peaks at *m/z* 381 ($[M+H]^+$), 363 ($[M+H-H_2O]^+$), and 345 ($[M+H-2\,H_2O]^+$) in the atmospheric-pressure chemical-ionization (AP-CI) MS, was deduced to have a molecular mass of 380 amu. The ^{1}H-NMR spectrum (*Table*) indicated the presence of one O-bearing CH group (δ(H) 3.61 (*dd*, $J=3.0$, 2.4 Hz)), two O-bearing CH_2 groups (δ(H) 3.59, 3.69 (2*d*, $J=11.4$, each 1 H); and 3.93, 4.22 (2*d*, $J=10.8$, each 1 H)), one Ac group (δ(H) 2.03 (*s*, 3 H)), two *singlet* Me groups (δ(H) 1.02 and 1.07). In the ^{13}C-NMR (DEPT) spectrum (*Table*), signals of one C=O group, four quaternary C-atoms, including an O-bearing one, four CH groups, including one O-bearing saturated C-atom, ten CH_2 groups, including two O-bearing ones, and three Me groups were observed. The above spectral evidence revealed the molecular formula $C_{22}H_{36}O_5$; moreover, the ^{13}C-NMR spectrum was similar to that of compound **2** (*Table*) which had been isolated from the same plant before. Therefore, compound **6** was determined as an *ent*-kaurane-type diterpenoid [5–7]. The ^{1}H- and ^{13}C-NMR signals were assigned based on the ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY experiments. In the HMBC spectrum, the following long-range correlations were observed (*Fig. 3*): CH_2(17)/C(13), C(15), and C(16); CH_2(18)/C(3), C(4), C(5), C(19), and C(Ac C=O); Me(20)/C(1), C(5), C(9), and C(10). The NOESY plot revealed the NOEs CH_2(18)/Me(20), CH_2(18)/Me(19), CH_2(18)/H_α–C(3), H_β–C(5)/H_β–C(9), H_β–C(5)/Me(19), as well as H–C(15)/H_β–C(9), H_α–C(14)/ Me(20) (*Fig. 4*). These findings indicated that the AcO group should be at C(18) and three OH groups at C(3) (in β position), C(16) (in α position), and C(17), respectively. Thus, the structure of compound **6** was determined as (3β,5β,8α,9β,10α,16β)-3,16,17-trihydroxykauran-18-yl acetate as shown in *Fig. 2*.

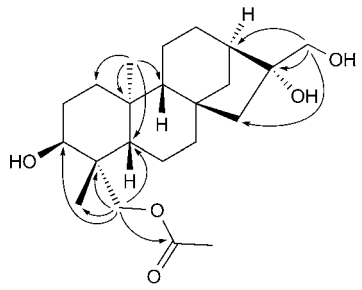


Fig. 3. *Key HMBC correlations of compound* **6**

Compound **7**, obtained as a white powder and with *quasi*-molecular-ion peaks at *m/z* 560 ($[M+H_2O]^+$), 565 ($[M+Na]^+$), and 1107 ($[2\,M+Na]^+$) in the APCI-MS,

Table. *NMR Data of Compounds* **6** *and* **7**, *and the Known Compound* **2**. δ in ppm, J in Hz.

	6[a])		**7**[a])[b])[c])		**2**[d])
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	34.4 (*t*)	1.26–1.32 (*m*), 1.53–1.58 (*m*)	34.4 (*t*)	1.24–1.34 (*m*), 1.50–1.59 (*m*)	38.4 (*t*)
CH_2(2)	26.3 (*t*)	1.52–1.58 (*m*), 1.91–1.99 (*m*)	26.3 (*t*)	1.52–1.59 (*m*), 1.90–2.00 (*m*)	26.0 (*t*)
H–C(3)	71.4 (*d*)	3.61 (*dd*, J = 3.0, 2.4)	71.4 (*d*)	3.61 (br. *s*)	68.6 (*d*)
C(4)	42.7 (*s*)		42.7 (*s*)		42.9 (*s*)
H–C(5)	50.4 (*d*)	1.42–1.47 (*m*)	50.4 (*d*)	1.41–1.48 (*m*)	48.9 (*d*)
CH_2(6)	21.3 (*t*)	1.37–1.45 (*m*), 1.50–1.61 (*m*)	21.3 (*t*)	1.33–1.47 (*m*), 1.49–1.61 (*m*)	20.2 (*t*)
CH_2(7)	43.4 (*t*)	1.48–1.55 (*m*), 1.60–1.66 (*m*)	43.3 (*t*)	1.48–1.57 (*m*), 1.58–1.66 (*m*)	42.6 (*t*)
C(8)	45.7 (*s*)		45.7 (*s*)		44.3 (*s*)
H–C(9)	58.0 (*d*)	1.11–1.16 (*m*)	58.0 (*d*)	1.13 (br. *s*)	56.6 (*d*)
C(10)	40.1 (*s*)		40.1 (*s*)		39.1 (*s*)
CH_2(11)	19.3 (*t*)	1.54–1.61 (*m*), 1.61–1.67 (*m*)	19.3 (*t*)	1.50–1.65 (*m*)	18.2 (*t*)
CH_2(12)	27.2 (*t*)	1.52–1.59 (*m*), 1.60–1.66 (*m*)	27.1 (*t*)	1.46–1.62 (*m*), 1.69–1.77 (*m*)	25.4 (*t*)
H–C(13)	46.4 (*d*)	2.00–2.05 (*m*)	46.8 (*d*)	2.08 (br. *s*)	44.9 (*d*)
CH_2(14)	38.0 (*t*)	1.58–1.64 (*m*), 1.88–1.95 (*m*)	37.9 (*t*)	1.55–1.63 (*m*), 1.88–1.94 (*m*)	36.9 (*t*)
CH_2(15)	53.9 (*t*)	1.36–1.42 (*m*), 1.51–1.57 (*m*)	53.6 (*t*)	1.36–1.43 (*m*), 1.50–1.57 (*m*)	53.0 (*t*)
C(16)	82.8 (*s*)		82.0 (*s*)		80.7 (*s*)
CH_2(17)	66.8 (*t*)	3.59 (*d*, J = 11.4), 3.69 (*d*, J = 11.4)	75.0 (*t*)	3.51 (*d*, J = 10.4), 4.19 (*d*, J = 10.8)	65.4 (*t*)
CH_2(18)	68.9 (*t*)	3.93 (*d*, J = 11.4), 4.22 (*d*, J = 10.8)	68.9 (*t*)	3.93 (*d*, J = 11.2), 4.22 (*d*, J = 11.6)	64.1 (*t*)
Me(19)	23.1 (*q*)	1.02 (*s*)	23.1 (*q*)	1.02 (*s*)	23.0 (*q*)
Me(20)	18.5 (*q*)	1.07 (*s*)	18.5 (*q*)	1.07 (*s*)	18.1 (*q*)
AcO	173.1 (*s*), 20.7 (*q*)	2.03 (*s*)	173.1 (*s*), 20.8 (*q*)	2.03 (*s*)	

[a]) Recorded in CD_3OD. [b]) ^{13}C-NMR of Glc: 105.3 (*d*, C(1)); 75.3 (*d*, C(2)); 78.0 (*d*, C(3)); 71.7 (*d*, C(4)); 77.9 (*d*, C(5)); 62.8 (*t*, C(6)). [c]) ^{1}H-NMR of Glc: 4.29 (*d*, J = 7.6, H–C(1)); 3.22 (*dd*, J = 7.6, 9.4, H–C(2)); 3.25–3.30 (*m*, H–C(3)); 3.26–3.30 (*m*, H–C(4)); 3.34–3.40 (*m*, H–C(5)); 3.64–3.69 (*m*, 1 H of CH_2(6)); 3.87(*dd*, J = 1.2, 12.0, 1 H of CH_2(6)). [d]) Recorded in (D_6)DMSO.

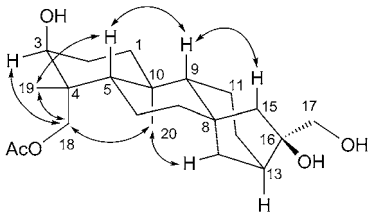


Fig. 4. *Key NOEs of compound* **6**

was deduced to have a molecular mass of 542 amu. The ^{13}C-NMR (DEPT) spectrum (*Table*) exhibited signals of five quaternary C-atoms, including one C=O group, one O-bearing saturated C-atom, nine CH groups, including six O-bearing ones, eleven CH_2 groups, including three O-bearing ones, and three Me groups. Comparing the ^{13}C-NMR data of compound **7** with those of compound **6**, it was evident that compound **6** was the genin of compound **7**; moreover, chemical shifts at δ(H) 4.29 (*d*, *J* = 7.6, 1 H Glc-1) and δ(C) 75.0 (C(17), because of glycosidation shift) revealed that compound **7** was the 17-*O*-β-D-glucopyranoside of **6**. Thus, the structure of compound **7** was established as (3β,5β,8α,9β,10α,13α)-17-[(β-D-glucopyranosyl)oxy]-3,16-dihydroxykauran-18-yl acetate as shown in *Fig. 2*.

Experimental Part

General. Column chromatography (CC): silica gel *H* (SiO_2; 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*). TLC: Normal-phase SiO_2 GF_{254} plates; visualization under UV light (at 254 and 365 nm) and spraying with 0.5% vanillin/H_2SO_4, followed by heating at 110° for 5–10 min. M.p.: *X-4* numeral melting-point instrument (*Beijing Tech Instrument Co., Ltd.*); uncorrected. Optical rotations: *WZZ-2B* polarimeter (cell length, 1.0 dm; *Shanghai Precision Instruments Co., Ltd.*). UV Spectra: *Hitachi-UV-3010* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bruker-Vector-33* spectrometer (KBr discs); $\tilde{\nu}$ in cm^{-1}. NMR (CD_3OD): *Bruker-ARX-600* spectrometer, at 600 (^{1}H) and 150 MHz (^{13}C) for **6**; *Bruker-ARX-400* spectrometer, at 400 (^{1}H) and 100 MHz (^{13}C) for **7**; residual solvent peaks as internal standard; δ in ppm and *J* in Hz; multiplicities of ^{13}C by DEPT. AP-CI-MS: *LCQ-DECA-XP* liquid chromatography/mass spectrometer (*Thermo Finnigan*, vaporizer temp. 450°, mobile phase MeCN/H_2O 1:1); in *m*/*z* (rel. %).

Plant Material. The leaves of *Rubus corchorifolius* L. f. were collected in July 2008 in Zhangjiajie, Hunan Province, P. R. China, and identified by Prof. *Bo-Ru Liao*. A voucher specimen (No. 2008-01) was deposited with the Key Laboratory of Forest Products and Chemical Engineering at Ji-Shou University, Zhangjiajie, P. R. China.

Extraction and Isolation. The air-dried leaves of *Rubus corchorifolius* L. f. (3.0 kg) were crushed and extracted (2 × 35 l) with 80% EtOH at 50° for 48 h. The EtOH extracts were concentrated under vacuum below 55° to give 1.5 l of a liquid residue, which was extracted successively with petroleum ether (b.p. 60–90°; 6 × 1.5 l; 31 g), $CHCl_3$ (6 × 1.5 l; 55 g), AcOEt (10 × 1.5 l; 40 g), and BuOH (6 × 1.5 l; 64 g). The $CHCl_3$ extract (30 g) was subjected to CC (SiO_2 *H*, 8 × 100 cm; $CHCl_3$/MeOH and MeOH/H_2O of increasing polarity): *Frs. 1–11*. Compound **6** (86 mg) was obtained by repeated recrystallization of *Fr. 7* from MeOH. *Fr. 11* (3.1 g) was then subjected to CC (SiO_2 *H*, 3 × 40 cm, $CHCl_3$/MeOH of increasing polarity): *Frs. 11.1–11.15*. *Fr. 11.15* yielded **7** (143 mg) by recrystallization from MeOH.

(3β,5β,8α,9β,10α,16β)-3,16,17-Trihydroxykauran-18-yl Acetate (**6**). Colorless needle-like crystals. M.p. 140–141°. $[\alpha]_D^{24} = -17.3$ ($c = 0.0015$, MeOH). UV (MeOH): 207 (2.28). IR: 3505, 3430 (OH), 2940, 2850 (C–H), 1715 (C=O). NMR: *Table*. AP-CI-MS: 267, 285, 345 (100), 363, 381.

(3β,5β,8α,9β,10α,13α)-17-(β-D-Glucopyranosyloxy)-3,16-dihydroxykauran-18-yl Acetate (**7**). White powder. M.p. 139–141°. $[\alpha]_D^{24} = -25.8$ ($c = 0.0012$, MeOH). UV (MeOH): 205 (1.76), 314 (1.18). IR: 3515, 3480 (OH), 2940, 2860 (C–H), 1730 (C=O). NMR: *Table*. AP-CI-MS: 345, 502, 560 (100), 565, 641, 1049, 1107, 1183.

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