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

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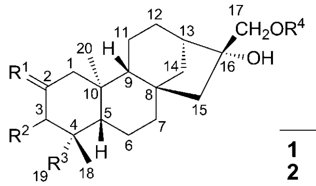
A further chemical investigation of the plant *Rubus corchorifolius* L. f., collected in Hunan Province, afforded three new *ent*-kauranoids **3**–**5**. Their structures were elucidated by various spectroscopic methods.

Introduction. – *Rubus corchorifolius* L. f., also known as raspberry, milk bubble, March bubble *etc.*, is an upright shrub of the 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 diarrhoea, extravasated blood, and alcoholism [3]. Recently, as part of a study on the biologically active constituents from this plant collected in Hunan Province, we have reported two new *ent*-kaurane diterpenoids, *i.e.*, (16α)-16,17-dihydroxy-*ent*-kauran-2-one-17-β-D-glucoside (=(16α)-17-(β-D-glucopyranosyloxy)-16-hydoxykauran-2-one[2]); **1**) and (3α,16α)-*ent*-kauran-3,16,17,19-tetrol (=(3β,4α,16α)-kaurane-3,16,17,18-tetrol[2]); **2**), respectively (*Fig. 1*) [4]. Further investigation on this plant resulted in the isolation of three new *ent*-kaurane diterpenoids (*Fig. 2*). The isolation and structural elucidation of the three compounds are reported in this article.



	R¹	R²	R³	R⁴
1	O	H	Me	β-D-Glc
2	H,H	α-OH	CH_2OH	H

Fig. 1. ent-*Kaurane diterpenoids* **1** *and* **2** *isolated from* Rubus corchorifolius *L. f. [4]*

Results and Discussion. – Compound **3**, obtained as colorless granular crystals and giving quasimolecular-ion peaks at m/z 354 ($[M+H_2O]^+$) and 318 ($[M-H_2O]^+$) in the

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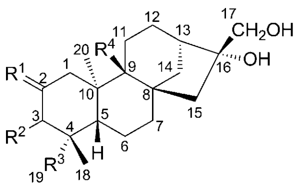
[1]) These two authors contributed equally to this work.

[2]) Name according to *Chem. Abstr.* where "kaurane" is used for "*ent*-kaurane" of IUPAC.



	R^1	R^2	R^3	R^4
3	O	H	CH_2OH	H
4	β-OH,H	α-OH	Me	H
5	O	H	Me	OH

Fig. 2. *Three new compounds isolated from* Rubus corchorifolius *L. f.*

atmospheric-pressure chemical-ionization (APCI) MS, was deduced to have a molecular mass of 336 amu. The ^{1}H-NMR spectrum (*Table*) indicated the presence of two O-bearing CH_2 groups (δ(H) 3.60 and 3.70 (2*d*, $J = 11$, each 1 H); δ(H) 3.37 and 3.49 (2*d*, $J = 11$, each 1 H)), two CH_2 groups attached to a C=O group (δ(H) 2.05 (*d*, $J = 13$, 1 H) and 2.47 (*dd*, $J = 13, 2.0$, 1 H); δ(H) 2.13 (*d*, $J = 14$, 1 H) and 2.53 (*dd*, $J = 14, 2.0$, 1 H)), and two *s* Me groups (δ(H) 1.04 and 1.11). In the ^{13}C-NMR (DEPT) spectrum (*Table*), one C=O group four quaternary C-atoms including an O-bearing one, three CH groups, ten CH_2 groups including two O-bearing ones, and two Me groups were observed. The above spectral evidence revealed the molecular formula $C_{20}H_{32}O_4$; moreover, the ^{13}C-NMR spectrum was similar to those of compounds **1** and **2** which had been isolated from the same plant before. Therefore, the structure of compound **3** was that of 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), H_α–C(1)/C(2), C(3), C(5), C(10), and C(20), H_α–C(3)/C(2), C(4), C(5), C(18), and C(19), Me(18)/C(3), C(4), C(5), and C(19), and CH_2(19)/C(3), C(4), C(5), and C(18). The NOESY plot revealed the NOEs CH_2(19)/Me(20), CH_2(19)/Me(18), CH_2(19)/H_α–C(3), as well as Me(18)/H_β–C(3) and Me(18)/H_β–C(5) (*Fig. 4*). These findings indicated that the C=O C group should be placed at C(2) and three OH groups at C(16) (in α position), C(17), C(19), respectively. Thus, the structure of compound **3** was determined as (4α,16α)-16,17,19-trihydroxy-*ent*-kaur-2-one as shown in *Fig. 2*.

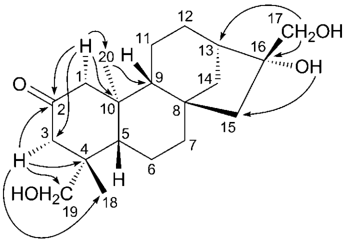


Fig. 3. *Key correlations in the HMBC spectrum of compound* **3**

Compound **4**, obtained as colorless crystals and giving *quasi*molecular-ion peaks at *m*/*z* 356 ($[M + H_2O]^+$) and 320 ($[M - H_2O]^+$) in the APCI-MS, was deduced to have a molecular mass of 338 amu. The ^{1}H-NMR spectrum (*Table*) showed two O-bearing CH groups (δ(H) 3.66–3.75 (*m*, overlap, 1 H); δ(H) 3.52 (*d*, $J = 9$, 1 H)), an O-bearing CH_2 group (δ(H) 3.67–3.73 (*m*, overlap, 1 H) and 3.59 (*d*, $J = 11$, 1 H)), three *s* Me

Table. *NMR Data of Compounds* **3**–**5** *and of the Known Compounds* **1** *and* **2**. δ in ppm, J in Hz.

	3[a])		**4**[a])		**5**[a])		**1**[b])[c])	**2**[b])
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(C)	δ(C)
CH$_2$(1)	2.05 (d, J=13, H$_\beta$), 2.47 (dd, J=13, 2.0, H$_\alpha$)	57.0 (t)	1.48–1.56, 1.64–1.72 (2m)	47.9 (t)	2.20 (dd, J=13, 2.0, H$_\alpha$), 2.86 (d, J=13, H$_\beta$)	51.4 (t)	55.1 (t)	38.4 (t)
C(2) or H–C(2)		214.9 (s)	3.66–3.75 (m)	70.4 (t)		216.9 (s)	211.5 (s)	26.0 (t)
CH$_2$(3) or H–C(3)	2.13 (d, J=14, H$_\beta$), 2.53 (dd, J=14, 2.0, H$_\alpha$)	50.9 (t)	3.52 (d, J=9)	78.9 (d)	2.35 (d, J=13, H$_\beta$), 2.02–2.10 (m, 1 H$_\alpha$)	57.2 (t)	55.9 (t)	68.6 (d)
C(4)		44.9 (s)		38.7 (s)		39.6 (s)	38.6 (s)	42.9 (s)
H–C(5)	1.55–1.62 (m, H$_\beta$)	56.6 (d)	1.14–1.20 (m)	51.6 (d)	2.05–2.12 (m, H$_\beta$)	48.3 (d)	54.3 (d)	48.9 (d)
CH$_2$(6)	1.42–1.58 (m, H$_\alpha$), 1.75–1.81 (m, H$_\beta$)	22.0 (t)	1.41–1.51 (m)	22.6 (t)	1.40–1.55 (m, H$_\alpha$), 1.63–1.73 (m, H$_\beta$)	21.7 (t)	20.6 (t)	20.2 (t)
CH$_2$(7)	1.51–1.62 (m, H$_\beta$), 1.65–1.71 (m, H$_\alpha$)	42.9 (t)	1.44–1.53, 1.57–1.63 (2m)	42.3 (t)	1.34–1.44 (m, H$_\beta$), 1.93–2.03 (m, H$_\alpha$)	37.5 (t)	36.5 (t)	42.6 (t)
C(8)		45.8 (s)		45.7 (s)		50.9 (s)	44.1 (s)	44.3 (s)
H–C(9) or C(9)	1.32 (d, J=8, H$_\beta$)	57.1 (d)	1.10–1.16 (m)	58.4 (d)		78.5 (s)	55.1 (d)	56.6 (d)
C(10)		45.3 (s)		40.5 (s)		50.3 (s)	44.3 (s)	39.1 (s)
CH$_2$(11)	1.46–1.56 (m, H$_\alpha$), 1.58–1.67 (m, H$_\beta$)	19.6 (t)	1.53–1.70 (m)	20.0 (t)	1.18–1.30 (m, H$_\beta$), 1.78–1.92 (m, H$_\alpha$)	30.3 (t)	18.2 (t)	18.2 (t)
CH$_2$(12)	1.47–1.59 (m, H$_\alpha$), 1.60–1.69 (m, H$_\beta$)	26.9 (t)	1.49–1.59, 1.62–1.69 (2m)	27.1 (t)	1.47–1.62 (m, H$_\alpha$), 1.69–1.82 (m, H$_\beta$)	28.6 (t)	25.9 (t)	25.4 (t)
H–C(13)	2.04 (br. s, H$_\alpha$)	46.2 (d)	2.02 (br. s)	46.3 (d)	2.00–2.07 (m, H$_\alpha$)	45.1 (d)	45.0 (d)	44.9 (d)
CH$_2$(14)	1.59–1.67 (m, H$_\beta$), 1.86 (d, J=11.5, H$_\alpha$)	37.7 (t)	1.57–1.64, 1.86 (d, J=11.5)	37.7 (t)	1.73–1.84 (m, H$_\beta$), 2.04–2.13 (m, H$_\alpha$)	39.0 (t)	41.2 (t)	36.9 (t)
CH$_2$(15)	1.43 (d, J=15), 1.53–1.60 (m)	53.5 (t)	1.35–1.42, 1.48–1.54 (2m)	53.7 (t)	1.08–1.15 (m, H$_\beta$), 2.26 (dd, J=15, 2.0, H$_\alpha$)	47.9 (t)	51.9 (t)	53.0 (t)
C(16)		82.7 (s)		82.7 (s)		82.9 (s)	79.6 (s)	80.7 (s)
CH$_2$(17)	3.60, 3.70 (2d, J=11)	66.8 (t)	3.59 (d, J=11), 3.67–3.73 (m,)	66.9 (t)	3.58, 3.69 (2d, J=11)	66.7 (t)	73.9 (t)	65.4 (t)
Me(18)	1.11 (s)	66.1 (t)	0.99 (s)	23.3 (q)	0.89 (s, 3 H)	23.6 (q)	23.2 (q)	64.1 (t)
CH$_2$(19) or Me(19)	3.37, 3.49 (2d, J=11)	27.7 (q)	0.89 (s)	25.3 (q)	1.08 (s)	34.0 (q)	33.2 (q)	23.0 (q)
Me(20)	1.04 (s)	20.0 (q)	1.24 (s)	23.8 (q)	1.16 (s)	21.5 (q)	19.0 (q)	18.1 (q)

[a]) Measured in CD$_3$OD. [b]) Measured in (D$_6$)DMSO. [c]) ^{13}C-NMR((D$_6$)DMSO) Data of Glc: 104.4 (d, C(1)); 73.8 (d, C(2)); 76.3 (d, C(3)); 70.2 (d, C(4)); 77.0 (d, C(5)); 61.2 (t, C(6)).

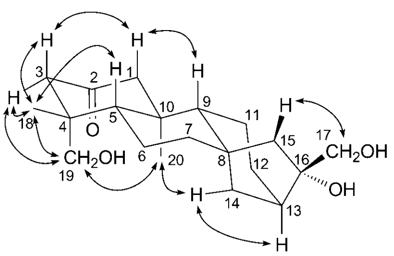


Fig. 4. *Key NOEs of compound* **3**

groups (δ(H) 0.89, 0.99, and 1.24). The ^{13}C-NMR (DEPT) spectrum (*Table*), which manifested four quaternary C-atoms including an O-bearing one, five CH groups including two O-bearing ones, eight CH_2 groups including an O-bearing one, and three Me groups, was similar to that of compound **3**, except for C(2), C(3), and C(19). Based on the above evidences, the structure of compound **4** was also an *ent*-kaurane-type diterpenoid including four OH groups, and its molecular formula should be $C_{20}H_{34}O_4$. The ^{1}H- and ^{13}C-NMR signals were assigned according to the ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments. The following long-range correlations in the HMBC spectrum were observed (*Fig. 5*): CH_2(17)/C(13), C(15), and C(16), H_β–C(3)/C(1), C(2), C(4), C(18), and C(19), Me(18)/C(3), C(4), C(5), and C(19), and Me(19)/C(3), C(4), C(5), and C(18). The correlation H–C(3)/H–C(2) in the ^{1}H,^{1}H-COSY plot revealed that the four OH groups should be placed at C(2), C(3), C(16), and C(17). Moreover, the signal at δ(H) 3.52 (*d*, $J = 9$) of H–C(3) suggested that both H–C(2) and H–C(3) should be axial. Thus, the structure of compound **4** was established to be (2β,3α,16α)-*ent*-kaurane-2,3,16,17-tetrol, as shown in *Fig. 2*.

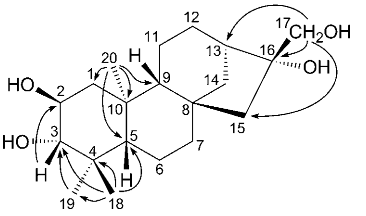


Fig. 5. *Key correlations in the HMBC spectrum of compound* **4**

Compound **5**, obtained as colorless crystals and giving *quasi*molecular-ion peaks at *m*/*z* 354 ([*M* + $H_2O]^+$) and 318 ([*M* − $H_2O]^+$) in the APCI-MS, was deduced to have a molecular mass of 336 amu. In the ^{1}H-NMR spectrum (*Table*), it showed one O-bearing CH_2 group (δ(H) 3.69 and 3.58 (2*d*, $J = 11$, each 1 H)), two CH_2 groups attached to one CO group (δ(H) 2.86 (*d*, $J = 13$, 1 H) and 2.20 (*dd*, $J = 13$, 2.0, 1 H); δ(H) 2.35 (*d*, $J = 13$, 1 H) and 2.02–2.10 (*m*, overlap, 1 H)), and three *s* Me groups (δ(H) 0.89, 1.08, and 1.16). The ^{13}C-NMR (DEPT) spectrum (*Table*) indicated one C=O group, five quaternary C-atoms including two O-bearing ones, two CH groups, nine CH_2 groups including an O-bearing one, and three Me groups. According to this evidence, both compound **3** and **5** had the same molecular formula $C_{20}H_{30}O_4$, the same skeleton and groups including one C=O and three OH groups. The ^{1}H- and ^{13}C-NMR signals of compound **5** were assigned based on the ^{1}H,^{1}H-COSY, HSQC, HMBC, and

NOESY experiments. In the HMBC spectrum, the following correlations were observed (*Fig. 6*): CH_2(17)/C(13), C(15), and C(16), Me(18)/C(3), C(4), C(5), and C(19), Me(19)/C(3), C(4), C(5), and C(18), H_α–C(1)/C(2), C(3), C(4), C(5), C(10), and C(20), H_α–C(3)/C(1), C(2), C(4), C(18), and C(19), and Me(20)/C(5) and C(9). Therefore, the C=O group should be placed at C(2) and the three OH groups at C(9), C(16), and C(17). Comparing the ^{13}C-NMR of compound **5** with that of compound **3**, the *syn*-γ-effect exerted by the 9β-OH group (*Fig. 7*) made C(1), C(5), C(7), and C(15) to shift to higher field in **5** ($\Delta\delta = -5.6$, -8.3, -5.4, and -5.6, resp.), while C(12) and C(14) were shifted to slightly lower field ($\Delta\delta$: $+1.7$ and $+1.3$, resp.) thus confirming the relative configuration of these *ent*-kaurane diterpenoids. Thus, the structure of compound **5** was determined as (9β,16α)-9,16,17-trihydroxy-*ent*-kauran-2-one as shown in *Fig. 2*.

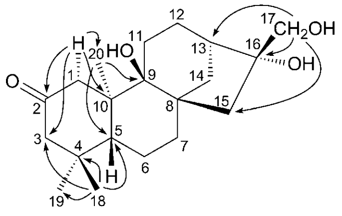


Fig. 6. *Key correlations in the HMBC spectrum of compound* **5**

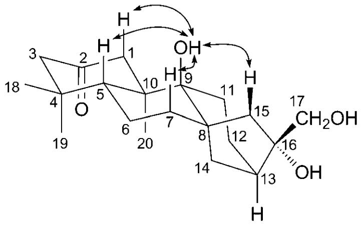


Fig. 7. syn-γ-*Effect of the 9β-OH group of* **5**

Experimental Part

General. Column chromatography (CC): silica gel *H* (200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*): TLC: normal-phase silica gel *GF254* on plates; visualization under UV light (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.*). 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 (KBr discs): *Bruker-Vector-33* spectrometer; in cm^{-1}. NMR (CD_3OD): *Bruker-ARX-400* spectrometer; at 400 (^{1}H) and 100 MHz (^{13}C); residual solvent peaks as internal standard; multiplicities of ^{13}C by DEPT. APCI-MS: *LCQ-DECA-XP* liquid chromatography/mass spectrometer (*Thermo Finnigan*, vaporizer temp. 450°, mobile phase MeCN/H_2O 1:1).

Plant Material. The leaves of *Rubus corchorifolius* L. f. were collected in July 2007 in Zhangjiajie, Hunan Province, P. R. China, and identified by associate professor *Bo-Ru Liao*. A voucher specimen 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. (3.5 kg) were crushed and extracted (2 × 35 l) with 80% EtOH, first for 0.5 h by treatment with ultrasonic wave, then at r.t. for 48 h. The EtOH extracts were concentrated under vacuum below 55° to give 2.5 l of a liquid residue which was extracted successively with petroleum ether (b.p. 60–90°; $6 \times 2.5\,l \rightarrow 35$ g), $CHCl_3$ ($6 \times 2.5\,l \rightarrow 20$ g), AcOEt ($10 \times 2.5\,l \rightarrow 45$ g), and BuOH ($6 \times 2.5\,l \rightarrow 58.4$ g). The AcOEt extract was subjected to CC (SiO_2 *H*, hexane/AcOEt and AcOEt/MeOH of increasing polarity: *Fractions 1–16*. *Fr. 6* (3.1 g) was then subjected to CC (SiO_2 *H*, $CHCl_3$/MeOH of increasing polarity: *Frs. 6.1–6.17.* Compounds **3** (15 mg) and **5** (25 mg) were separated by repeated recrystallization of *Fr. 6.7* from AcOEt, while *Fr. 6.8* yielded **4** (20 mg) by recrystallization from AcOEt/MeOH.

*(4α,16α)-16,17,19-Trihydroxy-*ent*-kauran-2-one* (= *(4α,16α)-16,17,19-Trihydroxykauran-2-one*[2]); **3**): Colorless granular crystals. M.p. 170–172°. $[\alpha]_D = +170.18$ ($c = 0.0006$, MeOH). UV (MeOH): 224 (3.23). IR (KBr): 3540, 3440 (OH), 2943, 2921 (C−H), 1692 (C=O). NMR: *Table.* APCI-MS: 354 (100), 336, 318, 301, 283, 253.

*(2β,3α,16α)-*ent*-kaurane-2,3,16,17-tetrol* (= *(2β,3α,16α)-Kauran-2,3,16,17-tetrol*[2]); **4**): Colorless needle crystals. M.p. 223–225°. $[\alpha]_D = +200$ ($c = 0.0004$, MeOH). UV (MeOH): 207 (1.672). IR (KBr): 3180 (OH), 2925, 2857 (C−H). NMR: *Table.* APCI-MS: 356, 320, 303, 285 (100).

*(9β,16α)-9,16,17-Trihydroxy-*ent*-kauran-2-one* (= *(9β,16α)-9,16,17-Trihydroxykauran-2-one*[2]); **5**): Colorless needle crystals. M.p. 169–170°. $[\alpha]_D = -16.13$ ($c = 0.003$, MeOH). UV (MeOH): 217 (2.48), 278 (0.70). IR (KBr): 3540, 3330 (OH), 2943, 2926 (C−H), 1692 (C=O). NMR: *Table.* APCI-MS: 354, 336, 318 (100), 301, 283.

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