Chemical Constituents of *Tripterygium wilfordii*

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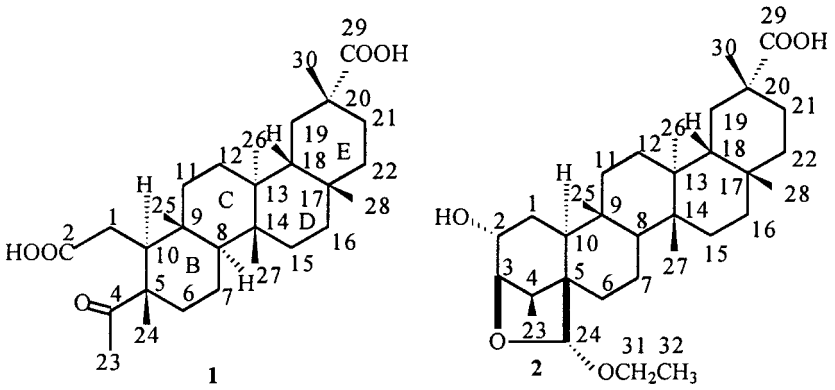
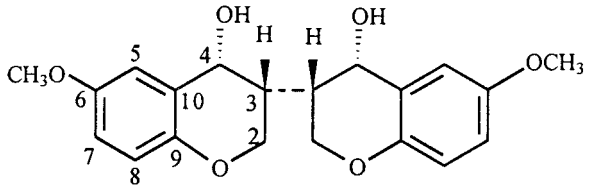
The two new triterpenoids, (5β,8α,9β,10α,13α,14β)-5,9,13-trimethyl-4-oxo-3,24,25,26-tetranor-2,3-secooleanane-2,29-dioic acid (=4-oxo-*D:A*-friedo-3-nor-2,3-secooleanane-2,29-dioic acid; **1**) and (2α,3β,4β,5β,8α,9β,10α,13α,14β,24*R*)-3,24-epoxy-24-ethoxy-2-hydroxy-5,9,13-trimethyl-24,25,26-trinoroleanan-29-oic acid (=3β,24-epoxy-24α-ethoxy-2α-hydroxy-*D:A*-friedooleanan-29-oic acid; **2**) (absolute configurations tentative), together with a novel bi-2*H*-benzopyran, namely (*rel*-(3*R*,3′*S*,4*R*,4′*S*)-3,3′,4,4′-tetrahydro-6,6′-dimethoxy[3,3′-bi-2*H*-benzopyran]-4,4′-diol (**3**), were isolated from *Tripterygium wilfordii* HOOK. F. Their structures were established by chemical and spectroscopic studies and by X-ray crystallography.

1. Introduction. – *Tripterygium wilfordii* HOOK. F. is widely distributed in China and has been used as a traditional plant insecticide and a medicinal plant [1]. Recently, anti-AIDS agents and an anti-HIV principle were isolated from this plant [2], and its triterpenoid constituents were also reported [3]. For an investigation of the active principles, a careful reexamination of this species collected from the Fujian province was carried out in our laboratory. Two novel triterpenoids, *D:A*-friedo-3-nor-2,3-secooleanane-2,29-dioic acid (**1**) and 3β,24-epoxy-24α-ethoxy-2α-hydroxy-*D:A*-friedooleanane-29-oic acid (**2**), and a novel bi-2*H*-benzopyran named [3,3′,4,4′-tetrahydro-6,6′-dimethoxy-3,3′-bi-2*H*-benzopyran]-4,4′-diol (**3**), were isolated from this plant. This paper describes the isolation and the structure elucidation of these three interesting compounds.

2. Results and Discussion. – Compound **1**, obtained as a powder, showed a molecular-ion peak at *m*/*z* 474 in the EI-MS, and the high-resolution EI-MS gave the molecular formula $C_{29}H_{46}O_5$. The IR spectrum indicated the presence of carboxylic-acid and carbonyl groups (2935 and 1701 cm^{-1}). The ^{1}H- and ^{13}C-NMR (*Table*) and HMQC spectra and NOESY experiments, and their comparison with the data of salaspermic acid [4], allowed us to establish the structure of **1** as 4-oxo-*D:A*-friedo-3-nor-2,3-secooleanane-2,29-dioic acid. To date, more than 100 triterpenes have been isolated from members of the Celastraceae. They have been derived from congeners, but the only two examples related to **1** are celastrahydride [5] and regelone [6], which are *D:A*-friedo-24-nor-2,3-secooleanane derivatives. Regelone and celastrahydride are probably formed biogenetically from a quinomethide precursor as result of ring-A oxidation. But compound **1** is not a 24-nor-type derivative, and it is suggested that **1** is formed biogenetically from other precursor. For these reasons, compound **1** is a unique structure in which the pentacyclic triterpenoid skeleton lacks C(3).



1 **2**



3

The ^{1}H-NMR spectrum of **1** revealed the presence of 7 Me groups at δ 0.95, 0.99, 1.21, 1.27, 1.32, 1.49, and 2.44 (7*s*, each 3 H). In the ^{13}C-NMR spectrum, 3 C=O signals appeared at δ 176.4, 181.3, and 213.8, besides 7 Me, 10 CH_2, 3 CH, and 6 quarternary C signals. The ^{13}C-NMR data of **1** were similar to those of salaspermic acid [4], except for the chemical shifts of C(1), C(2), C(3), C(4), C(5), C(10), C(23), and C(24), suggesting that the B–E ring system of **1** was the same as in salaspermic acid. Unassigned ^{13}C-signals of **1** appeared at δ 18.0 (*q*), 25.6 (*q*), 32.7 (*t*), 50.2 (*d*), 54.0 (*s*), 176.4 (*s*), and 213.8 (*s*). The presence of a Me C=O moiety in **1** was established by signals at δ(C) 25.6 (*q*) and δ (H) 2.44 (3 H, *s*) and by the fragment *m*/*z* 431 ($[M-43]^+$). In the HMBC spectrum, the signal at δ(H) 0.99 (Me(25)) was correlated with δ(C) 34.4 (C(11)), 38.7 (C(9)), 50.7 (C(8)), and 50.2. These observations indicated that the δ(C) 50.2 was attributable to C(10). In the HMQC spectrum, the signal at δ(H) 2.87 (*dd*, J = 6, 4 Hz, 1 H) was correlated with the δ(C) 50.2 (C(10)). The signal at δ(H) 1.27 (*s*, 3 H) correlated with δ(C) 18.0 and showed a long-range correlation with δ(C) 213.8, 54.0, and 50.2 (C(10)). The signal at δ(H) 2.44 (*s*, 3 H) was correlated with δ(C) 25.6 and showed a long-range correlation with δ(C) 213.8. Thus, δ(C) 18.0 (*q*), 25.6 (*q*), 54.0 (*s*), and 213.8 (*s*) were assigned to C(24), C(23), C(5), and C(4), respectively. The signal at δ(H) 2.87 (*dd*, J = 6, 4 Hz, H–C(10)) showed long-range correlations with δ(C) 176.4, 54.0 (C(5)), 38.7 (C(9)), and 32.7, thus allowing the assignment of δ(C) 32.7 and 176.4 to C(1) and C(2). The relative configuration of **1** was established by NOESY experiments: signal at δ(H) 2.87 (H–C(10)) was correlated with δ(H) 1.67 (H–C(8)), and δ(H) 2.44 (Me(23)) was correlated with δ(H) 2.87 (H–C(10)). These findings clearly showed that the orientations of Me(24) and H–C(10) are β and α, respectively.

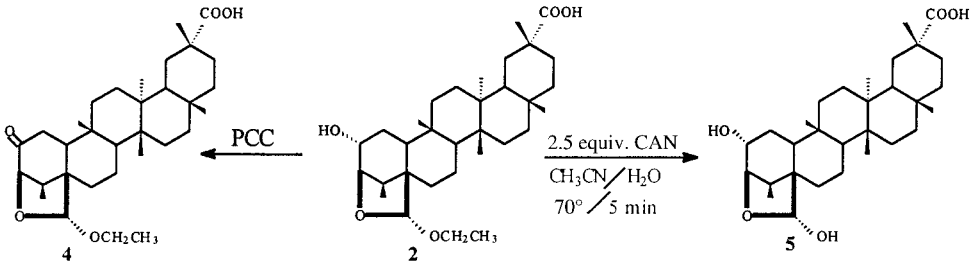
Compound **2** was obtained as colorless crystals (from acetone). Its IR spectrum showed absorptions of OH (3435 cm^{-1}) and C=O (1699 cm^{-1}) groups, and its ^{13}C- and ^{1}H-NMR spectra (*Table*) and elemental analysis were consistent with a molecular formula $C_{32}H_{52}O_5$, but the M^+ of **2** could not be detected in the MS. The ^{1}H- and ^{13}C-NMR, HMQC, HMBC, and mass spectra, COSY experiments, and comparison with the data of salaspermic acid [4], established the structure of **2** to be 3β,24-epoxy-24α-ethoxy-2α-hydroxy-*D:A*-friedooleanan-29-oic acid. The position of the epoxy function was confirmed by pyridinium chlorochromate (PCC) oxidation of **2** to **4**, and

Table. *^{1}H-NMR and ^{13}C-NMR Data* (C_5D_5N) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
CH_2(1)	2.69 (*m*, 1 H); 2.33 (*m*, 1 H)	32.7	1.98 (*m*, 1 H); 1.88 (*m*, 1 H)	28.0
C(2) or CH(2)		176.4	4.49 (*m*, 1 H)	69.2
CH(3)			4.34 (*d*, *J* = 4, 1 H)	85.4
C(4) or CH(4)		213.8	2.54 (*m*, 1 H)	44.7
C(5)		54.0		50.4
CH_2(6)	1.94 (*m*, 1 H); 1.60 (*m*, 1 H)	37.4	1.38 (*m*, 2 H)	30.1
CH_2(7)	1.53 (*m*, 1 H); 1.38 (*m*, 1 H)	18.1	1.56 (*m*, 2 H)	19.7
CH(8)	1.67 (*m*, 1 H)	50.7	1.50 (*m*, 1 H)	51.0
C(9)		38.7		37.8
CH(10)	2.87 (*dd*, *J* = 6, 4, 1 H)	50.2	1.86 (*m*, 1 H)	53.6
CH_2(11)	1.86 (*m*, 1 H); 1.57 (*m*, 1 H)	34.4	1.82 (*m*, 1 H); 1.48 (*m*, 1 H)	37.0
CH_2(12)	1.73 (*m*, 2 H)	29.5	2.54 (*m*, 2 H)	30.3
C(13)		39.8		39.7
C(14)		39.6		39.9
CH_2(15)	1.38 (*m*, 2 H)	29.8	1.74 (*m*, 2 H)	29.8
CH_2(16)	1.77 (*m*, 1 H); 1.47 (*m*, 1 H)	36.7	1.38 (*m*, 2 H)	34.9
C(17)		30.5		30.8
C(18)	1.66 (*m*, 1 H)	44.8	1.68 (*m*, 1 H)	45.1
CH_2(19)	2.71 (*m*, 1 H); 1.75 (*m*, 1 H)	31.1	2.73 (br. *d*, *J* = 15, 1 H); 1.88 (*m*, 1 H)	31.2
C(20)		40.8		41.1
CH_2(21)	2.61 (*m*, 1 H); 1.57 (*m*, 1 H)	30.6	2.62 (*m*, 1 H); 1.57 (*m*, 1 H)	30.8
CH_2(22)	2.33 (*m*, 1 H); 1.08 (*m*, 1 H)	37.5	2.41 (*td*, *J* = 14, 3, 1 H); 1.16 (*m*, 1 H)	37.9
Me(23)	2.44 (*s*, 3 H)	25.6	1.33 (*m*, 3 H)	15.2
Me(24) or CH(24)	1.27 (*s*, 3 H)	18.0	5.34 (*s*, 1 H)	104.0
Me(25)	0.99 (*s*, 3 H)	18.7	1.11 (*s*, 3 H)	17.4
Me(26)	1.32 (*s*, 3 H)	18.3	1.28 (*s*, 3 H)	18.4
Me(27)	0.95 (*s*, 3 H)	16.8	0.99 (*s*, 3 H)	17.2
Me(28)	1.21 (*s*, 3 H)	32.2	1.23 (*s*, 3 H)	32.5
C(29)		181.3		181.8
Me(30)	1.49 (*s*, 3 H)	32.1	1.52 (*s*, 3 H)	32.6
CH_2(31)			4.07 (*m*, 1 H); 3.65 (*m*, 1 H)	64.2
Me(32)			1.33 (*m*, 3 H)	16.1

cerium ammonium nitrate (CAN) mediated hydrolysis of the acetal function [7] of **2** yielded the hemiacetal **5** (see *Scheme* and *Exper. Part*).

Scheme. *Conversion of* **2** to **4** *and* **5**



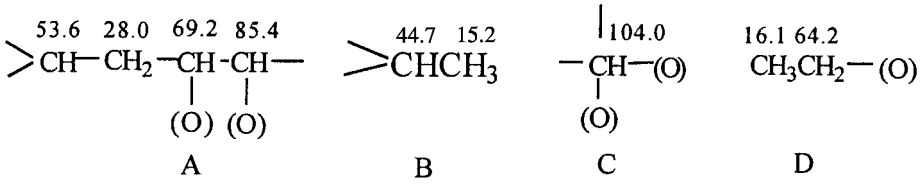


Fig. 1. *Partial structures for ring A of compound* **2**

The ^{1}H- and ^{13}C-NMR spectra of **2** revealed the presence of 7 Me (δ(H) 0.99, 1.11, 1.23, 1.28, 1.52 (*s*, each 3 H), 1.33 (*m*, 3 H), 1.33 (*m*, 3 H)), 2 CH–O (δ(H) 4.49 (*m*, 1 H), 4.34 (*d*, J = 4 Hz, 1 H); δ(C) 69.2, 85.4), 1 O–CH–O (δ(H) 5.34 (*s*, 1 H); (δ(C) 104.0) and 1 CH_2O group (δ(H) 4.07 (*m*, 1 H), 3.65 (*m*, 1 H); (δ(C) 64.2). In addition the ^{13}C-NMR spectrum of **2** showed 1 C=O (δ(C) 181.8), 10 CH_2, 4 CH, and 6 quarternary C signals. The ^{1}H,^{1}H COSY and ^{13}C,^{1}H COSY experiments suggested the presence of a $MeCH_2O$ moiety. The high-resolution MS established that the fragment *m*/*z* 442 ($[M-COOH-C_2H_5]^+$) had the formula $C_{29}H_{46}O_3^+$, in agreement with the molecular formula $C_{32}H_{52}O_5$. The δ(C) of C(6) to C(22) and C(25) to C(30) were found to be similar to those of salaspermic acid [4] suggesting an identical B–E ring system. This was confirmed by very similar HMQC and HMBC data of **2** and salaspermic acid and suggested that ring A of **2** accounted for the remaining two degrees of unsaturation. Analyses of the corresponding signals by ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-COSY experiments established the partial structures **A**–**D** (*Fig. 1*).

In the HMBC spectrum of **2**, the signal at δ(H) 1.11 (Me(25)) was correlated with δ(C) 37.8 (C(9)), 51.0 (C(8)), and 53.6. Thus, the latter was assigned to C(10), and the partial structure **A** could be assigned to C(10), C(1), C(2), and C(3). The signal at δ(H) 1.33 (Me(23)) was correlated with δ(C)15.2 and showed long-range correlations with δ(C)44.7 (*d*) and 50.4 (*s*) and the signal at δ(H) 4.34 (H–C(3)) was correlated with δ(C)85.4 and showed long-range correlations with δ(C)28.0 (*t*, C(1)), 69.2 (*d*, C(2)), 50.4 (*s*), 15.2 (*q*, C(23)), and 104.0 (*d*). These correlations allowed us to connect partial structure **B** to partial structure **A**, and to assign δ(C)50.4 to C(5). The signal at δ(H) 5.34 (Me(24)) was correlated with δ(C)104.0 and showed long-range correlations with δ(C)44.7 (*d*, C(4)), 85.4 (*d*, C(3)), 53.6 (*d*, C(10)), δ(C)64.2 (*t*, CH_2), and 50.4 (*s*, C(5)), establishing the connectivity of partial structures **D** and **C** and the assignment of δ(C)104.0 to C(24).

The remaining unsaturation in A ring of **2** ought to be an epoxy moiety. Its location between C(3) and C(24) was established by the ^{1}H-NMR spectrum of the oxidation product **4**, in which the *m* of H–C(2) (δ(H) 4.49) had disappeared and the *d* of H–C(2) (δ(H) 4.34, J = 4 Hz) become a *s* (δ(H) 3.88), thus excluding an epoxy bridge between C(2) and C(24).

In the ^{1}H-NMR spectrum of the hydrolysis product **5**, the δ(H) 1.33 (*m*, 3 H), 4.07 (*m*, 1 H), and 3.65 (*m*, 1 H) had disappeared, and H–C(24) appeared at δ(H) 5.95. In the ^{13}C-NMR spectrum of **5**, δ(C)64.2 (*t*) and 16.1 (*q*) had disappeared, and C(24) appeared at δ(C)98.0. These observations were consistent with the removal of $MeCH_2$ from **2** to give the hemiacetal **5**.

The structure, relative configuration, and probable absolute configuration of **2** were finally established by X-ray analysis (*Fig. 2*)[1]). Compound **2** was also simultaneously isolated from *Tripterygium wilfordii* by *Takaishi* and co-workers [8].

Compound **3**, obtained as colorless oil, exhibited IR absorptions for OH (3365 cm^{-1}) and aromatic-ring moieties (1605, 1514, and 1463 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra, including ^{1}H,^{1}HCOSY, HMQC, and HMBC experiments, suggested that **3** was a 3,3′-bi-2*H*-benzopyrane derivative, and the high-resolution MS gave the molecular formula $C_{20}H_{22}O_6$, consistent with a symmetric structure. Many terpenoids and alkaloids were isolated from *Tripterygium wilfordii*, but compound **3** with its 3,3′-bi-2*H*-benzopyrane structure was isolated from this plant for the first time.

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[1]) The structure of **2** was reported by us at the *UNESCO* Regional Symposium on Drug Development from Medicinal Plant (Proceedings), March, 2000, Kunming, P.R. China.

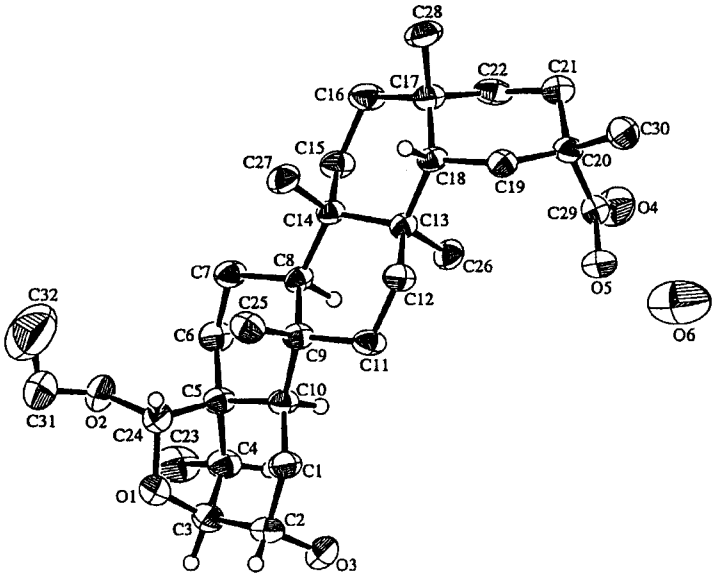

Fig. 2. *X-Ray crystal structure of compound* **2**

The ^{1}H-NMR spectrum of **3** showed a pair of *ortho*-coupled aromatic protons at δ(H) 6.81 (*dd*, $J = 8.2$, 1.8 Hz) and 6.88 (*d*, $J = 8.2$ Hz), a pair of *meta*-coupled aromatic protons at δ(H) 6.81 (*dd*, $J = 8.2$, 1.8 Hz) and 6.89 (*d*, $J = 1.8$ Hz), 1 CH–O at δ(H) 4.73 (*d*, $J = 4.1$ Hz), 1 CH at δ(H) 3.10 (*m*), 1 CH_2O at δ(H) 4.24 (*dd*, $J =$ 10, 6.9 Hz) and 3.87 (*dd*, $J = 10$, 3.7 Hz), and 1 MeO at δ(H) 3.90 (*s*). The ^{13}C-NMR spectrum revealed three substituted benzene-ring C-atoms (108.7 (*d*), 146.8 (*s*), 119.0 (*d*), 114.4 (*d*), 145.3 (*s*), 133.3 (*s*)), 1 CH–O, 1 CH_2O, 1 CH_2, and 1 MeO signal.

The relative configuration of **3** was deduced from ^{1}H,^{1}H-coupling constants. Thus, the J(2,3) values of 6.9 and 3.7 Hz suggested that H–C(3) was an axial proton with the C(3)–C(3′) linkage having *a* orientation, and J(3,4) = 4.1 Hz was consistent with H–C(4) being equatorial and the OH group having the *a* orientation.

Experimental Part

General. Column chromatography (CC): silica gel *60 H* from *Qingdao Haiyang Chemical Group Co.*, China. M.p.: *Fisher-John*, no correction. $[\alpha]_D$: *Jasco DIP-181* polarimeter; 10-cm microcell. IR Spectra: *Perkin-Elmer 599B* IR spectrometer, KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-400* instrument, $SiMe_4$ as internal standard. EI-MS: *MAT-711* spectrometer; 70 eV; *m*/*z* (rel. int. in %).

Plant Material. The roots of *Tripterygium wilfordii* were collected in Fujian Province, P.R. China. The plant material was identified by Pharmacognosy Associate Professor *Guan-Yuan Gu*, Vice Chairman of the Scientific and Technical Archives of the Shanghai Medical University, Shanghai, China.

Extraction and Isolation. The air-dried roots (200 kg) of *Tripterygium wilfordii* were powdered and extracted with 95% EtOH. The EtOH extract was extracted with $CHCl_3$ and the $CHCl_3$-soluble fraction (500 g) submitted to CC (silica gel, $CHCl_3$/MeOH 95 : 5 → 8 : 2): *Fractions A – E*. *Fr. A* was repeatedly chromatographed (silica gel, cyclohexane/acetone 5 : 1): **1** (15 mg), **2** (330 mg), and **3** (20 mg).

(5β,8α,9β,10α,13α,14β)-5,9,13-Trimethyl-4-oxo-3,24,25,26-tetranor-2,3-secooleanane-2,29-dioic Acid[2]) (**1**). Amorphous powder. $[\alpha]_D^{20.0} = -11.8$ ($c = 0.5$, C_5H_5N). IR: 2935, 1701, 1466, 1234, 939, 752. ^{1}H- and ^{13}C-NMR: D *Table*. EI-MS: 474 (10, M^+), 431 (20), 413 (25), 385 (100), 235 (60), 189 (38), 121 (27), 95 (30). HR-EI-MS: 474.3226 ($C_{29}H_{46}O_5^+$; calc. 474.3245).

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[2]) Absolute configurations tentative.

*(2α,3β,4β,5β,8α,9β,10α,13α,14β,24*R*)-3,24-Epoxy-24-ethoxy-2-hydroxy-5,9,13-trimethyl-24,25,26-trinoroleanan-29-oic Acid*[2]) (**2**). Colorless crystals. M.p. 254–256°. $[\alpha]_D^{20.0} = -49.9$ ($c = 0.5$, C_5H_5N). IR: 3435, 1699, 1458, 1379, 1115, 1051, 962. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 442 (60, $[M - COOH - C_2H_5]^+$), 424 (32), 409 (17), 289 (16), 235 (25), 147 (63), 121 (70), 109 (100). HR-EI-MS: 442.3467 ($[M - COOH - C_2H_5]^+$, $C_{29}H_{46}O_3^+$; calc. 442.3447). Anal. calc. for $C_{32}H_{52}O_5 \cdot H_2O$: C 71.91, H 10.11; found: C 71.32, H 10.30.

rel-*(3*R*,3′*S*,4*R*,4′*S*)-3,3′,4,4′-Tetrahydro-6,6′-dimethoxy[3,3′-bi-2*H*-benzopyran]-4,4′-diol* (**3**). Colorless oil. IR: 3365, 1605, 1514, 1464, 1124, 854, 819, 752. ^{1}H-NMR ($CDCl_3$, 400 MHz): 3.87 (*dd*, $J = 10$, 3.7, 1 H–C(2)); 4.24 (*dd*, $J = 10$, 6.9, 1 H–C(2)); 3.10 (*m*, H–C(3)); 4.73 (*d*, $J = 4.1$, H–C(4)); 6.89 (*d*, $J = 1.8$, H–C(5)); 6.88 (*d*, $J = 8.2$, H–C(8)); 6.81 (*dd*, $J = 8.2$, 1.8, H–C(7)). ^{13}C-NMR ($CDCl_3$, 400 MHz): 71.7 (*t*, C(2)); 54.2 (*d*, C(3)); 85.9 (*d*, C(4)); 108.7 (*d*, C(5)); 146.8 (*s*, C(6)); 119.7 (*d*, C(7)); 114.4 (*d*, C(8)); 145.3 (*s*, C(9)); 133.0 (*s*, C(10)); 56.0 (*q*, MeO). EI-MS: 358 (53, M^+), 327 (12), 205 (21), 151 (100), 180 (11), 137 (43). HR-EI-MS: 358.1412 ($C_{20}H_{22}O_6^+$; calc. 358.1416.

*(3β,4β,5β,8α,9β,10α,13α,14β,24*R*)-3,24-Epoxy-24-ethoxy-5,9,13-trimethyl-2-oxo-24,25,26-trinoroleanan-29-oic Acid*[2]) (**4**). Excess pyridinium chlorochromate was added to a stirred soln. of **2** (100 mg) in CH_2Cl_2 (10 ml). The mixture was stirred at r.t. for 40 min and then extracted with Et_2O (3×10 ml). The combined extract was evaporated and the crude product purified by CC (silica gel, cyclohexane/acetone 5:1): **4** (75 mg). Amorphous powder. $[\alpha]_D^{20.0} = -101.9$ ($c = 1.30$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.84, 0.89, 0.95, 1.05, 1.20 (*s*, each 3 H); 1.41 (*d*, $J = 6.9$, Me(23)); 1.16 (*m*, Me(32)); 3.43 (*m*, 1 H, CH_2(31); 3.77 (*m*, 1 H, CH_2 (31)); 3.88 (*s*, H–C(3)); 5.23 (*s*, H–C(24)). ^{13}C-NMR ($CDCl_3$, 400 MHz): 29.6 (*t*, C(1)); 211.1 (*s*, C(2)); 87.1 (*d*, C(3)); 50.6 (*d*, C(4)); 50.5 (*s*, C(5)); 30.1 (*t*, C(6)); 18.9 (*t*, C(7)); 50.9 (*d*, C(8)); 38.0 (*s*, C(9)); 56.8 (*d*, C(10)); 36.7 (*t*, C(11)); 29.6 (*t*, C(12)); 39.0 (*s*, C(13)); 39.1 (*s*, C(14)); 29.4 (*t*, C(15)); 35.5 (*t*, C(16)); 30.0 (*s*, C(17)); 44.0 (*d*, C(18)); 34.1 (*t*, C(19)); 40.3 (*s*, C(20)); 29.0 (*t*, C(21)); 36.0 (*t*, C(22)); 15.3 (*q*, C(23)); 103.7 (*d*, C(24)); 17.0 (*q*, C(25)); 17.8 (*q*, C(26)); 16.6 (*q*, C(27)); 31.4 (*q*, C(28)); 185.1 (*s*, C(29)); 31.8 (*q*, C(30)); 64.0 (*t*, C(31)); 14.0 (*q*, C(32). EI-MS: 514 (32, M^+), 469 (12), 440 (100), 235 (85), 189 (70), 163 (46), 109 (78), 95 (76).

*(2α,3β,4β,5β,8α,9β,10α,13α,14β,24*R*)-3,24-Epoxy-24-ethoxy-2,24-dihydroxy-5,9,13-trimethyl-24,25,26-trinoroleanan-29-oic Acid*[2]) (**5**). To a stirred soln. of **2** (50 mg, 0.096 mmol) in MeCN (5 ml) at 70°, a soln. of CAN (142 mg, 0.25 mmol) in H_2O (2 ml) was added in one portion. After 5 min, the crude mixture was poured into H_2O and extracted with Et_2O (3×20 ml). The extract was dried ($MgSO_4$) and evaporated and the crude product purified by CC (silica gel, cyclohexane/acetone 4:1) **5** (24 mg). Amorphous powder. $[\alpha]_D^{20.0} -4.2$ ($c =$ 1.30, EtOH). ^{1}H-NMR (C_5D_5N, 400 MHz) 0.83, 0.98, 1.10, 1.20, 1.40 (5*s*, each 3 H); 1.46 (*d*, $J = 6.9$, Me(23)); 5.94 (*s*, H–C(24)); 4.42 (*m*, H–C(2)); 4.36 (*d*, $J = 4$, H–C(3)). ^{13}C-NMR (C_5D_5N, 400 MHz): 27.9 (*t*, C(1)); 69.2 (*d*, C(2)); 84.9 (*d*, C(3)); 44.8 (*d*, C(4)); 50.0 (*s*, C(5)); 30.5 (*t*, C(6)); 19.8 (*t*, C(7)); 50.8 (*d*, C(8)); 37.7 (*s*, C(9)); 53.5 (*d*, C(10)); 37.5 (*t*, C(11)); 30.5 (*t*, C(12)); 39.4 (*s*, C(13)); 39.6 (*s*, C(14)); 29.8 (*t*, C(15)); 34.7 (*t*, C(16)); 30.9 (*s*, C(17)); 44.8 (*d*, C(18)); 29.6 (*t*, C(19)); 40.7 (*s*, C(20)); 30.5 (*t*, C(21)); 36.7 (*t*, C(22)); 15.2 (*q*, C(23)); 98.0 (*d*, C(24)); 17.1 (*q*, C(25)); 18.1 (*q*, C(26)); 16.9 (*q*, C(27)); 32.1 (*q*, C(28)); 181.3 (*s*, C(29)); 32.2 (*q*, C(30)). EI-MS: 488 (5, M^+), 473 (8), 384 (100), 369 (82), 355 (62), 329 (25), 255 (41), 185 (26), 55 (95).

Single-Crystal X-Ray Analysis of **2**. Data were acquired with a *Rigaku AFC7R* diffractometer, MoK_α radiation (λ 0.71069 Å), graphite monochromator. M_r 534.78 ($C_{32}H_{52}O_5 \cdot H_2O$); crystal size $0.20 \times 0.20 \times 0.30$ mm; tetragonal, space group $P2_12_12(19)$, 293 K; $a = 12.864(3)$, $b = 30.706(3)$, $c = 7.805(4)$ Å, $V =$ 3082(1) Å^3, $D_C = 1.152$ g/cm^3, $Z = 4$, $F_{000} = 1176.00$, $\mu = 0.77$ cm^{-1}. The data were collected at $20 \pm 1°$ by the ω-2θ scan technique to a maximum 2θ value of 55.0°. A total of 4033 reflections were collected. The intensities of three representative reflections were measured after every 200 reflection. Over the course of data collection, the standards decreased by −0.1%. The structure was solved by direct methods [9] and expanded by the *Fourier* technique [10]. The non-H atoms were refined anisotropically. H-Atoms were included but not refined.

REFERENCES

[1] 'Zhong-yao-da-ci-dian', Jiangsu New Medical Academy, Shanghai Science and Technology Publisher, Shanghai, 1986, p. 2469.

[2] K. Chen, Q. Shi, T. Fujioka, D. C. Zhang, C. Q. Hu, J. Q. Jin, R. Kilkuskie, K. H. Lee, *J. Nat. Prod*. **1992**, *55*, 88.

[3] T. Morota, C. X. Yang, H. Sasaki, W. Z. Qin, K. Sugama, K. L. Miao, T. Yoshino, L. H. Xu, M. Maruno, B. H. Yang, *Phytochemistry* **1995**, *39*, 1153; K. H. Li, H. Q. Duan, K. Kawazoe, Y. Takaishi, *Phytochemistry* **1997**, *45*, 791.

[4] H. Q. Duan, K. Kawazoe, M. Bando, M. Kido, Y. Takaishi, *Phytochemistry* **1997**, *46*, 535.

[5] C. B. Gamalath, A. A. L. L. Gunatilaka, Y. Tezuka, T. Kikuchi, S. Balasubramaniam, *Phytochemistry* **1990**, *29*, 3189.
[6] Y. Takaishi, K. Miyagi, K. Kawazoe, K. Nakano, K. H. Li, H. Q. Duan, *Phytochemistry* **1997**, *45*, 975.
[7] A. Ates, A. Gautier, B. Leroy, J. M. Plancher, Y. Quesnel, I. E. Marko, *Tetrahedron Lett*. **1999**, *40*, 1799.
[8] H. Q. Duan, Y. Takaishi, H. Momota, Y. Ohmoto, T. Taki, Y. F. Jia, D. Li, *Phytochemistry* **2000**, *53*, 805.
[9] 'SHELXS86', G. M. Sheldrick, in: 'Crystallographic Computing 3', Eds. G. M. Sheldrick, C. Kruger, and R. Goddard, Oxford University Press, Oxford, 1985, p. 175.
[10] P. T. Beuskens, G. Admiral, G. Beurskens, W. P. Bosman, S. Garcia-Granda, Gould, R. O. Gould, J. M. M. Smits, C. Smykalla, 'DIRDIF 92', Technical Report of the Crystallography Laboratory, University of Nijmegen, The Netherlands, 1992.

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