Triterpenoids from the Stems of *Schisandra glaucescens*

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Two new lanostane triterpenoids, schiglauzic acid (**1**) and schiglaucyclozic acid (**2**), together with two known ones, anwuweizic acid (**3**) and manwuweizic acid (**4**), were isolated from the stems of *Schisandra glaucescens*. Their structures were determined on the basis of extensive spectroscopic methods, including two-dimensional NMR techniques, and were further confirmed by X-ray crystallographic analysis.

Introduction. – The genus *Schisandra* of the family Schisandraceae has been widely used in Traditional Chinese Medicine for thousands of years. The species of this genus are known to be rich source of lignans with various biological activities [1–3]. In recent years, many triterpenoids with new skeletons were isolated [4], attracting great interest of pharmacologists and phytochemists due to their diverse bioactivities, such as anti-HIV, anticholesteremic, antitumor, and anti-HBV properties [5–9].

Schisandra glaucescens DIELS. is a vine plant mainly distributed in the west of Hubei Province and southeast of Sichuan Province, P. R. China. Its stems were traditionally used for the treatment of contusion, rheumatism, and arthritis [10]. The previous work carried out by our group on *Schisandra glaucescens* led to the isolation of a new tricyclic triterpenoid with a unique 6/7/9-fused skeleton [11]. In a further study, two new triterpenoids **1** and **2**, together with two known ones **3** and **4**, were isolated (*Fig. 1*). The structures of **1** and **2** were determined by extensive NMR spectroscopic experiments, including 1D and 2D (^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY) techniques, and were further confirmed by single-crystal X-ray analysis.

Results and Discussion. – Compound **1** was isolated as white needles. Its HR-ESI-MS showed a $[M-H]^-$ peak at *m/z* 471.3463, corresponding to the molecular formula $C_{30}H_{48}O_4$, indicating seven degrees of unsaturation. The IR spectrum of **1** indicated the presence of a OH (3447 cm^{-1}) and a conjugated COOH group (1688 cm^{-1}). The ^{1}H-NMR spectrum of **1** (*Table 1*) revealed the presence of a terminal angelic acid moiety (δ(H) 6.07 (*t*, *J* = 7, H–C(24)); 2.14 (*s*, Me(27))), and a secondary Me (δ(H) 1.34 (*d*, *J* = 5.6, Me(21))) and five tertiary Me groups (δ(H) 0.78, 0.96, 1.10, 1.24, and 1.24 (each *s*)). These features closely resembled those of anwuweizic acid (**3**) except for the absence of one CH_2 and the presence of one additional CH–O group (δ(H) 4.27 (*d*, *J* = 7.6)), suggesting the OH-substituted anwuweizic acid backbone for **1**. The HMBC correlations (*Fig. 2*) Me(18) (δ(H) 0.78)/C(12) (δ(C) 73.41), H–C(12) (δ(H) 4.27)/

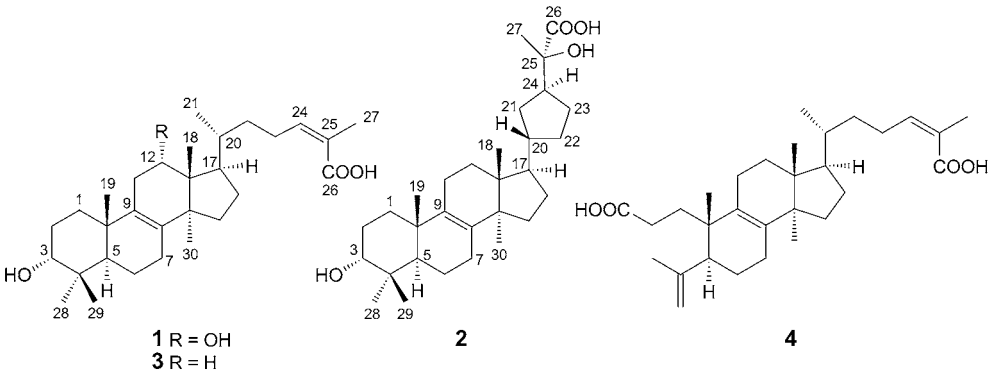

Fig. 1. *Compounds* **1**–**4** *isolated from* Schisandra glaucescens

C(9) (δ(C) 135.5), C(14) (δ(C) 51.22), and C(18) (δ(C) 18.02), and the ^{1}H,^{1}H-COSY correlation (*Fig. 2*) between CH_2(11) (δ(H) 2.5, 2.77) and H–C(12) (δ(H) 4.27) allowed us to determine the C(12)H–O. The OH group at C(12) was α-oriented as indicated by the coupling constant of H–C(12) (δ(H) 4.27 (*d*, $J = 7.6$)) and the significant NOEs (*Fig. 3*) Me(18) (δ(H) 0.78)/H–C(12) (δ(H) 4.27). The OH group at C(3) was established as α-oriented by the coupling constant of H–C(3) (δ(H) 3.64 (br. *s*)) [12]. The NOESY correlation between H–C(24) (δ(H) 6.07) and Me(27) (δ(H) 2.14) implied the (*Z*)-geometry for the C(24)=C(25) bond. Therefore, the structure of **1** was elucidated as 12-hydroxyanwuweizic acid. The proposed structure was further confirmed by single-crystal X-ray-analysis (*Fig. 4*).

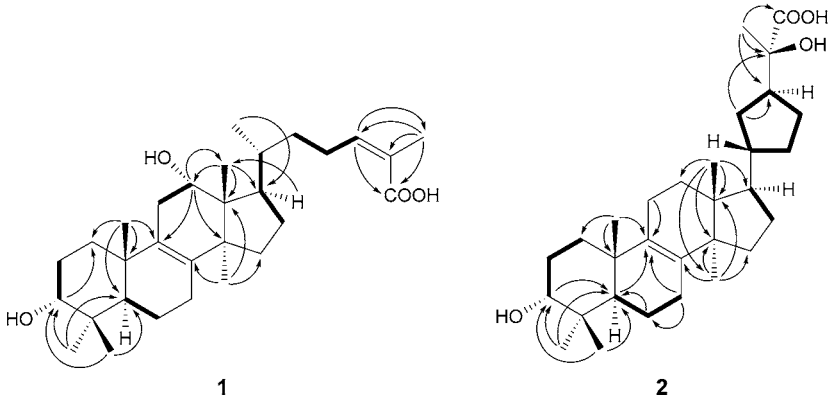

Fig. 2. *Key* ^{1}*H,*^{1}*H-COSY* (—) *and HMBC* (H → C) *correlations of compounds* **1** *and* **2**

Compound **2** was obtained as colorless prismatic crystals. The molecular formula of **2** was determined as $C_{30}H_{48}O_4$, with seven degrees of unsaturation, from the $[M - H]^-$ peak at *m*/*z* 471.3462 in the HR-ESI-MS. The IR spectrum of **2** revealed the presence of

Table 1. *^{1}H- and ^{13}C-NMR* (400 and 100 MHz, resp., in C_5D_5N) *Data of* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	2.09–2.14 (*m*, H_α), 1.52–1.57 (*m*, H_β)	31.89 (*t*)	1.57–1.66 (*m*, H_α), 1.48–1.55 (*m*, H_β)	32.06 (*t*)
CH_2(2)	1.77–1.84 (*m*, H_α), 1.98–2.06 (*m*, H_β)	28.14 (*t*)	1.17–1.21 (*m*, H_α), 1.82–1.85 (*m*, H_β)	28.18 (*t*)
H–C(3)	3.64 (br. *s*)	76.31 (*d*)	3.63 (br. *s*)	76.31 (*d*)
C(4)		39.43 (*s*)		39.42 (*s*)
H–C(5)	2.02–2.07 (*m*)	45.95 (*d*)	1.98 (*dd*, J = 12.9, 2.0)	45.83 (*d*)
CH_2(6)	1.55–1.63 (*m*, H_α), 1.70–1.76 (*m*, H_β)	19.87 (*t*)	1.48–1.59 (*m*, H_α), 1.61–1.69 (*m*, H_β)	19.82 (*t*)
CH_2(7)	2.09–2.21 (*m*)	27.77 (*t*)	1.97–2.09 (*m*)	27.77 (*t*)
C(8)		136.1 (*s*)		135.6 (*s*)
C(9)		135.5 (*s*)		136.7 (*s*)
C(10)		38.57 (*s*)		38.69 (*s*)
CH_2(11)	2.50 (*d*, J = 18.7, H_α), 2.77 (*dd*, J = 18.8, 7.8, H_β)	35.81 (*t*)	2.07–2.15 (*m*, H_α), 1.98–2.03 (*m*, H_β)	22.53 (*t*)
H–C(12) or CH_2(12)	4.27 (*d*, J = 7.6)	73.41 (*d*)	1.66–1.71 (*m*, H_α), 2.01–2.07 (*m*, H_β)	32.01 (*t*)
C(13)		51.34 (*s*)		50.82 (*s*)
C(14)		51.22 (*s*)		46.28 (*s*)
CH_2(15)	1.72–1.78 (*m*, H_α), 1.21–1.27 (*m*, H_β)	33.91 (*t*)	1.12–1.19 (*m*, H_α), 1.56–1.66 (*m*, H_β)	32.74 (*t*)
CH_2(16)	2.12–2.19 (*m*, H_α), 1.43–1.48 (*m*, H_β)	29.59 (*t*)	1.06–1.10 (*m*, H_α), 1.37–1.26 (*m*, H_β)	29.38 (*t*)
H–C(17)	2.67 (*dd*, J = 18.6, 9.7)	44.44 (*d*)	1.49–1.58 (*m*)	52.99 (*d*)
Me(18)	0.78 (*s*)	18.02 (*q*)	0.75 (*s*)	18.14 (*q*)
Me(19)	1.10 (*s*)	20.47 (*q*)	1.06 (*s*)	20.63 (*q*)
H–C(20)	1.58–1.63 (*m*)	38.26 (*d*)	1.87–1.95 (*m*)	45.07 (*d*)
Me(21) or CH_2(21)	1.34 (*d*, J = 5.6)	19.14 (*q*)	1.39–1.49 (*m*, H_α), 2.23–2.37 (*m*, H_β)	33.13 (*t*)
CH_2(22)	1.38–1.44 (*m*, H_a), 1.75–1.81 (*m*, H_b)	37.85 (*t*)	1.80–1.87 (*m*, H_a), 1.06–1.15 (*m*, H_b)	35.99 (*t*)
CH_2(23)	2.78–2.88 (*m*, H_a), 2.95 (*dt*, J = 11.3, 5.8, H_b)	28.56 (*t*)	1.84–1.92 (*m*)	28.8 (*t*)
H–C(24)	6.07 (*t*, J = 6.9)	144 (*d*)	2.79 (*m*)	49.26 (*d*)
C(25)		129.9 (*s*)		77.27 (*s*)
C(26)		172 (*s*)		181.40 (*s*)
Me(27)	2.14 (*s*)	22.75 (*q*)	1.79 (*s*)	27.21 (*q*)
Me(28)	0.96 (*s*)	23.87 (*q*)	0.92 (*s*)	23.87 (*q*)
Me(29)	1.24 (*s*)	30.28 (*q*)	1.21 (*s*)	30.31 (*q*)
Me(30)	1.42 (*s*)	26.43 (*q*)	0.85 (*s*)	25.68 (*q*)

OH (3447 cm^{-1}) and COOH (1726 cm^{-1}) groups. The 1D-NMR (*Table 1*) displayed resonances for six Me, eleven CH_2, and five CH groups (including one CH–O (δ(H) 3.63 (br. *s*), δ(C) 76.31)), eight quaternary C-atoms (including one O-bearing quaternary C-atom (δ(C) 77.27), one COOH group (δ(C) 181.4), and one tetrasubstituted C=C moiety (δ(C) 135.6, 136.7)). These spectral data resembled those of

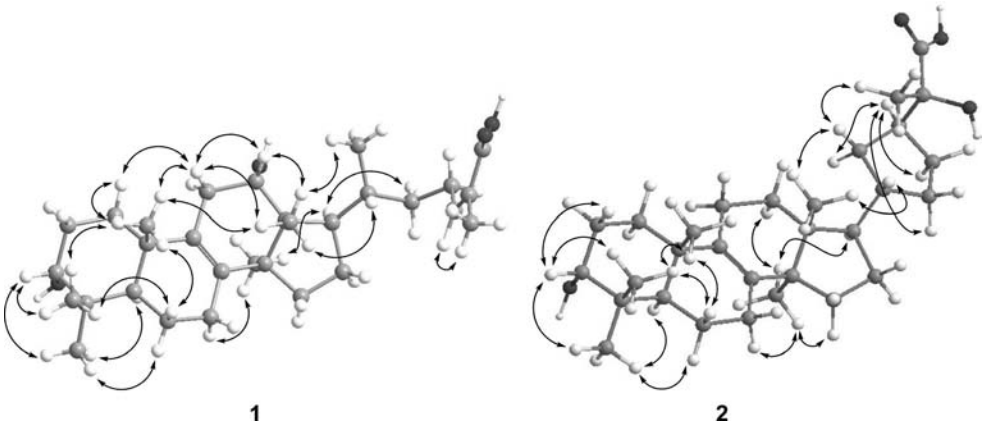

Fig. 3. *Key NOESY correlations of compound* **1** *and* **2**

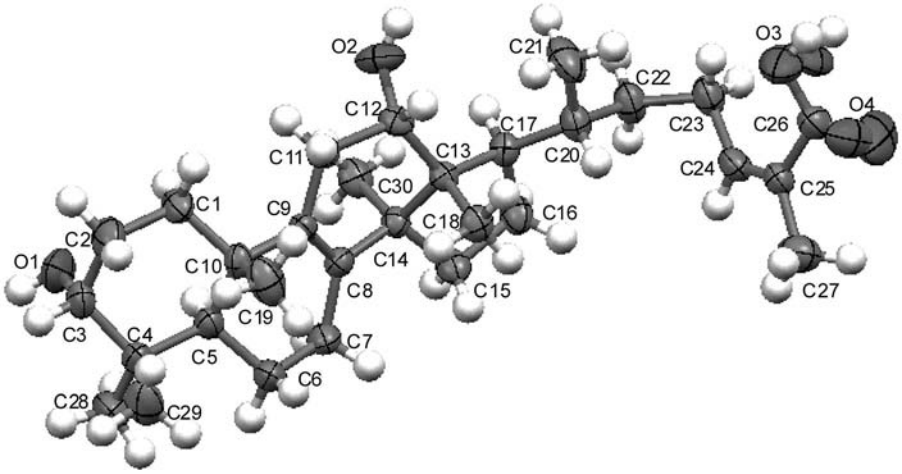

Fig. 4. *Crystallographic structure of compound* **1**. The ellipsoid probability was 30%. Both of the disordered carboxylic acid orientations are shown

anwuweizic acid except for the absence of one secondary Me group (C(21)) and one trisubstituted C(24)=C(25) moiety. Considering degrees of unsaturation and above spectral data, we assumped that C(21) was connected to C(24) forming a new ring at C(20). This assumption was verified by the ^{1}H,^{1}H-COSY correlations (*Fig. 2*) H–C(20) (δ(H) 1.87 – 1.95)/H$_\beta$–C(21) (δ(H) 2.23 – 2.37) and H$_\beta$–C(22) (δ(H) 1.06 – 1.15); H–C(24) (δ(H) 2.79)/H–C(23) (δ(H) 1.84 – 1.92) and H–C(21) (δ(H) 1.39 – 1.49 and 2.23 – 2.37); and H–C(23)/H$_\beta$–C(22). The OH group at C(25) was located with the aid of HMBC correlations (*Fig. 2*) Me(27) (δ(H) 1.79)/C(25) (δ(C) 77.27) and C(26) (δ(C) 181.4). The other OH group was in α-orientation at C(3) as indicated by the small coupling constant of H–C(3) (δ(H) 3.63 (br. *s*)) with H$_\alpha$–C(2) (δ(H) 1.17 – 1.21 (*m*))

and H_β–C(2) (δ(H) 1.82–1.85 (*m*)) [11]. The absolute configuration at C(20), C(24), and C(25) was assigned as (20*S*,24*S*,25*R*) on the basis of the single-crystal X-ray analysis relative to the known configuration of the lanostane skeleton (*Fig. 5*). From the above evidences, the structure of compound **2** was deduced as (3α,20*S*,24*S*,25*R*)-3,25-dihydroxy-21,24-cyclolanost-8-en-26-oic acid, and the proposed structure was verified by the single-crystal X-ray analysis. This type of a triterpenoid with a cyclopentane moiety in the side chain have been reported to be found in fungi, *e.g.*, *Inonotus obliquus* and *Fuscoporia oblique* [13][14], and plants, *e.g.*, *Melia toosendan*, *Melia azedarach*, *Gynostemma pentaphyllum*, *Monocyclanthus vignei*, and *Thalictrum thunbergii DC.* [15–19], and it is the first time that this kind of skeleton was detected in the family Schisandraceae.

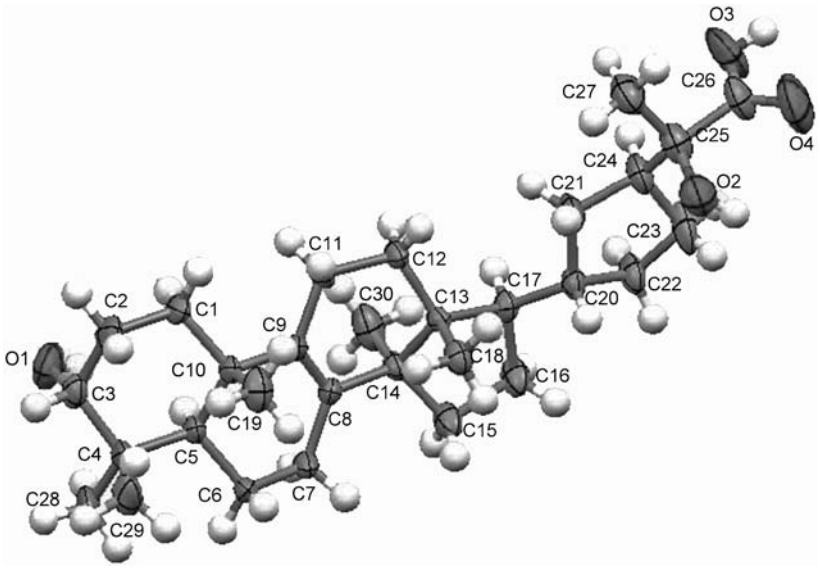

Fig. 5. *Crystallographic structure of compound* **2**. The ellipsoid probability was 30%

It is suggested that compounds **1** and **2** are biosynthesized from anwuweizic acid, which is the main triterpenoid constituent in this plant. This process consists of a series of biochemical reactions such as hydroxylation, protonation, and cyclization (*Scheme*).

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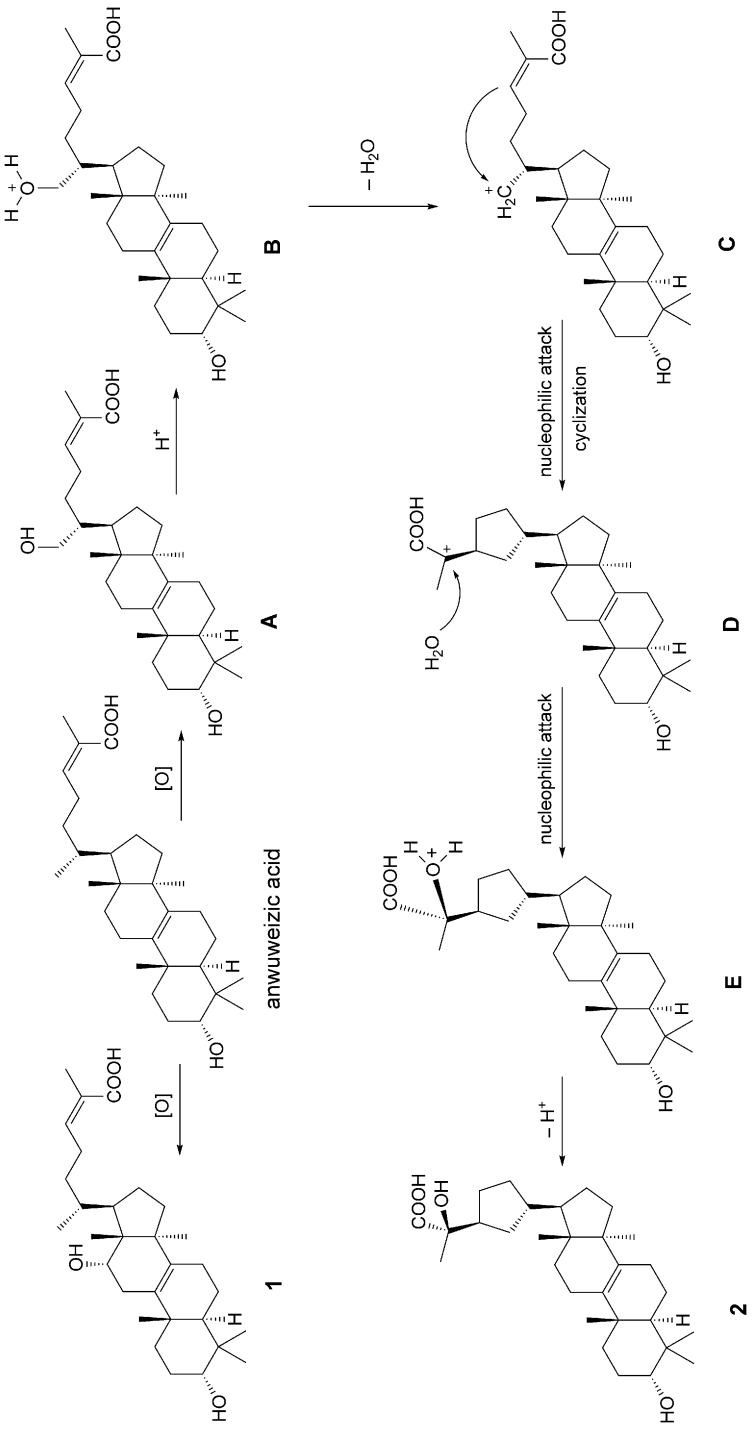
Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 or 300–400 mesh, *Qingdao Marine Chemical Inc.*, Qingdao, China); *Sephadex LH-20* gel (*GE Healthcare*); *MCI*-gel (*CHP20P*, 75–150 mm, *Mitsubishi Chemical Industries Ltd.*). Anal. TLC: SiO_2 plates (*Yantai Institute of Chemical Technology*). MPLC: *Büchi pump module C-605* with *Büchi* plastic-glas column (36 mm × 460 mm). Specific optical rotation: automatic polarimeter (*PE-341LC*, *Perkin-Elmer*). IR Spectra: *Perkin-Elmer Spectrum One* FT-IR spectrometer; KBr pellets; in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR Spectra: *Bruker-AM-*

Scheme. *Plausible Biogenetical Pathway to Compounds* **1** *and* **2**



400 spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Thermo Scientific LTQ-Orbitrap XL*; in *m*/*z*.

Plant Material. The stems of *Schisandra glaucescens* were collected in the Shennongjia mountainous areas of Hubei Province, P. R. China, in November 2009, and identified by Mr. *Shigui Shi* at the Shennongjia Institute for Drug Control. A voucher specimen (ID 20091101) has been deposited with Herbarium of Materia Medica, Faculty of Pharmacy, Tongji Medical College of Huazhong University of Science and Technology, P. R. China.

Extraction and Isolation. The air-dried ground stems (6.5 kg) of *Schisandra glaucescens* were extracted exhaustively four times with 70% aq. acetone (4×25 l) at r.t. for three weeks. The acetone extracts were concentrated *in vacuo* to give a crude extract (750 g). Then, the extract was partitioned sequentially with petroleum ether, AcOEt, and BuOH. The AcOEt fraction (126.7 g) was subjected to CC (SiO_2 (2 kg); $CHCl_3$/acetone gradient) to give fourteen fractions, *Frs. 1–14*. *Fr. 5*, eluted with $CHCl_3$/acetone 8:2, was subjected to repeated CC (*MCI* gel; H_2O/MeOH 1:1–0:1; and SiO_2; CH_2Cl_2/MeOH 15:1) to afford **1** (18 mg) and **2** (23 mg). The petroleum ether (PE) fraction (148.9 g) was subjected to CC (SiO_2 (1.5 kg) PE/AcOEt gradient) to give ten fractions, *Frs. 1–10*. *Fr. 7*, eluted with PE/AcOEt 8:2, was applied to CC (SiO_2; petroleum ether/acetone 5:1) to yield **3** (2.3 g). *Fr. 8*, eluted with PE/AcOEt 8:2–7:3, was subjected to CC (*Sephadex LH-20* gel, CH_2Cl_2/MeOH 1:1) to yield **4** (158 mg).

Schiglauzic Acid (*=12-Hydroxyanwuweizic Acid = (3α,12α,24Z)-3,12-Dihydroxylanosta-8,24-dien-26-oic Acid*; **1**). White needles. M.p. 230–232°. $[\alpha]_D^{20} = +85.7$ ($c = 1.4$, pyridine). UV (MeOH): 210. IR

Table 2. *Crystallographic Data for* **1** *and* **2**

	1	**2**
Empirical formula	$C_{30}H_{48}O_4$	$C_{30}H_{48}O_4$
M_r [g mol^{-1}]	472.68	472.68
Crystal description	Needles	Prismatic
Crystal dimensions [mm]	$0.16 \times 0.12 \times 0.10$	$0.16 \times 0.12 \times 0.10$
Crystal system	Orthorhombic	Orthorhombic
Space group	*P*2(1)2(1)2(1)	*P*2(1)2(1)2(1)
Unit cell parameters:		
a [Å]	6.9193(5)	7.3827(6)
b [Å]	16.2698(11)	11.7625(10)
c [Å]	24.4537(16)	30.600(3)
V [Å^3]	2752.9(3)	2657.2(4)
Z	4	4
D_x [Mg/m^3]	1.140	1.182
μ (MoK_α) [mm^{-1}]	0.073	0.076
F (000)	1040	1040
Θ Range [°]	2.08–26.00	1.85–26.00
Index ranges	$-8 \le h \le 8$, $-18 \le k \le 20$, $-30 \le l \le 27$	$-8 \le h \le 9$, $-14 \le k \le 14$, $-37 \le l \le 34$
Reflections collected	15846	17179
Independent reflections	5387 ($R_{int} = 0.0425$)	5221 ($R_{int} = 0.0627$)
Completeness to $\theta = 26.00°$	99.5%	99.8%
Max. and min. transmission	0.9927; 0.9884	0.9924; 0.9879
Data/restraints/parameters	5387/3/334	5221/0/316
Goodness-of-fit on F^2	1.073	1.087
Final *R* indices ($I > 2\sigma(I)$)	$R_1 = 0.0618$, $wR_2 = 0.1279$	$R_1 = 0.0676$, $wR_2 = 0.1598$
R indices (all data)	$R_1 = 0.0817$, $wR_2 = 0.13623$	$R_1 = 0.0825$, $wR_2 = 0.1675$
Absolute structure parameter	−0.1(18)	2(2)
Largest diff. peak and hole [e Å^{-3}]	0.176; −0.158	0.396; −0.196

(KBr): 3447, 2954, 1688, 1645, 1456, 1374, 1248, 1208, 1160, 1063, 796. ^{1}H- and ^{13}C-NMR (C_5D_5N): *Table 1*. HR-ESI-MS: 471.3463 ($[M-H]^-$, $C_{30}H_{47}O_4^-$; calc. 471.3474).

Schiglaucyclozic Acid (=*(3α,24*S*,25*R*)-3,25-Dihydroxy-21,24-cyclolanost-8-en-26-oic Acid*; **2**). Colorless prismatic crystal. M.p. 237–239°. $[\alpha]_D^{20} = +45.2$ ($c = 1.55$, pyridine). UV (MeOH): 205. IR (KBr): 3455, 2948, 2872, 2830, 1727, 1456, 1372, 1279, 1196, 1131, 1061, 977. ^{1}H- and ^{13}C-NMR (C_5D_5N): *Table 1*. HR-ESI-MS: 471.3462 ($[M-H]^-$, $C_{30}H_{47}O_4^-$; calc. 471.3474).

X-Ray Crystal Analysis of **1** *and* **2**[1]). Single crystals suitable for X-ray-analysis of **1** and **2** were both obtained from MeOH. All measurements were conducted on a *Bruker SMART APEX CCD* apparatus, empolying graphite-monochromated MoK_α radiation ($\lambda = 0.71073$ Å) at 298 K and operating in the $\omega/2\theta$ mode. The crystal structures were solved by direct methods and refined by full-matrix least-squares on F^2, using SHELXTL-97 program on a PC. In the refinement, all the C-bound H-atoms were located at the geometrical positions with C–H distances of 0.96 Å (Me), 0.97 Å (CH_2), 0.98 Å (CH), and 0.93 Å (alkylene), and their displacement parameters were set to 1.2 (CH_2 and alkylene C) times or 1.5 times (Me and CH C-atoms) of their parent atoms. H-Atoms bonded to O-atoms were positioned at their ideal positions with O–H distance of 0.82 Å and *Uiso*(H) = 1.5 *Ueq*(O) in complex **1**. H-Atoms bonded to O(1)- and O(4)/O(4′)-atoms in complex **2** were also positioned at their ideal positions with O–H distance of 0.82 Å and *Uiso*(H) = 1.5 *Ueq*(O). H-Atom bonded to O(2) was found from the difference map and the O–H distance was refined freely with *Uiso*(H) = 1.5 *Ueq*(O). The COOH group is disordered over two sites with the final satisfactory occupancies being 0.59 : 0.1 for the major and minor components, resp. Command 'DFIX' was used to restrain the COOH C–O bond distances. All calculations were carried out using the Wingx-32 crystallographic software package. The crystallographic data for **1** and **2** are given in *Table 2*.

REFERENCES

[1] Y.-H. Kuo, S.-Y. Li, M.-D. Wu, R.-L. Huang, L.-M. Yang Kuo, C.-F. Chen, *Chem. Pharm. Bull.* **1999**, *47*, 1047.
[2] S.-Y. Li, M.-D. Wu, C.-W. Wang, Y.-H. Kuo, R.-L. Huang, K.-H. Lee, *Chem. Pharm. Bull.* **2000**, *48*, 1992.
[3] M. Chen, N. Kilgore, K.-H. Lee, D.-F. Chen, *J. Nat. Prod*. **2006**, *69*, 1697.
[4] W.-L. Xiao, R.-T. Li, S.-X. Huang, J.-X. Pu, H.-D. Sun, *Nat. Prod. Rep.* **2008**, *25*, 871.
[5] R.-T. Li, Q.-B. Han, Y.-T. Zheng, R.-R. Wang, L.-M. Yang, Y. Lu, S.-Q. Sang, Q.-T. Zheng, Q.-S. Zhao, H.-D. Sun, *Chem. Commun*. **2005**, 2936.
[6] W.-L. Xiao, R.-T. Li, S.-H. Li, X.-L. Li, H.-D. Sun, Y.-T. Zheng, R.-R. Wang, Y. Lu, C. Wang, Q.-T. Zheng, *Org. Lett.* **2005**, *7*, 1263.
[7] L.-N. Li, H. Xue, D.-L. Ge, K. Kunio, M. Toshio, O. Sadafumi, *Planta Med*. **1989**, *55*, 300.
[8] J.-X. Pu, W.-L. Xiao, Y. Lu, R.-T. Li, H.-M. Li, L. Zhang, S.-X. Huang, X. Li, Q.-S. Zhao, Q.-T. Zheng, H.-D. Sun, *Org. Lett*. **2005**, *7*, 5079.
[9] Y.-C. Shen, Y.-C. Lin, M. Y. Chiang, S. F. Yeh, Y.-B. Cheng, C.-C. Liao, *Org. Lett.* **2005**, *7*, 3307.
[10] Y.-H. Zhan, 'Shennongjia TCM Resources', Hubei Science & Technology Press, 1994.
[11] F.-Y. Meng, J.-X. Sun, X. Li, H.-Y. Yu, S.-M. Li, H.-L. Ruan, *Org. Lett.* **2011**, *13*, 1502.
[12] J.-S. Liu, M.-F. Huang, *Acta Chim. Sin.* **1984**, *42*, 264.
[13] S. Taji, T. Yamada, R. Tanaka, *Helv. Chim. Acta* **2008**, *91*, 1513.
[14] J. He, Y.-Z. Feng, Y. Lu, B. Zhao, *J. Asian Nat. Prod. Res.* **2001**, *3*, 55.
[15] Y. S. Sang, C. Y. Zhou, A. J. Lu, X. J. Yin, Z. D. Min, R. X. Tan, *J. Nat. Prod*. **2009**, *72*, 917.
[16] Q.-G. Tan, X.-N. Li, H. Chen, T. Feng, X.-H. Cai, X.-D. Luo, *J. Nat. Prod*. **2010**, *73*, 693.
[17] J.-X. Wei, Y.-G. Chen, *Acta Chim. Sin.* **1991**, *49*, 933.
[18] H. Achenbach, D. Frey, *Phytochemistry* **1992**, *31*, 4263.
[19] H. Yoshimitsu, M. Nishida, T. Nohara, *Tetrahedron* **2001**, *57*, 10247.

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[1]) CCDC-806153 and -806154 contain the supplementary crystallographic data for this article. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif.