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Note

Studies on chemical constituents of *Sanguisorba longifolia Bertol*

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Three new triterpenoids named as Changyediyuine **I** (**1**), Changyediyuine **II** (**2**) and Changyediyuine **III** (**3**) have been isolated from the root of *Sanguisorba longifolia Bertol* (Chinese name Changyediyu berberidaceae), together with ten known compounds and their structures are deduced from spectroscopic methods and 2D NMR techniques.

Keywords: Triterpenoids, Changyediyuine, *Sanguisorba,* berberidaceae

Sanguisorba longifolia Bertol is distributed mainly in Neimenggu of China. It shows a strong effect on stopping bleeding, antineoplastic, antiophidica, antinociceptive used in traditional Chinese folk drug for cure dysentery, rheumatic, arthritis etc[1]. However, very little information is known about its chemical constituents. A detailed chemical investigation on this plant has been carried out and three new triterpenoids along with ten known compounds are isolated.

The known compounds were identified by comparing their spectral data with those of authentic samples or with data reported in the literature as 2α,3β-dihydroxy-28-norurs-12,17,19(20),21(22)-tetra-en-23-oic-acid **4** (ref. 2), 3β-angeloyl-2β,23-dihydroxyolean-12-en-28-oic acid **5** (ref. 3), caffeic acid **6** (ref. 4), ferulogl glucosie **7**(ref. 5), ursolic acid **8** (ref. 6), kaempferol-3-*O*-*β*-*D*-glucoside **9**(ref. 7), morin-7-O-β-D-glucopranoside **10** (ref. 8), qurcetin **11** (ref. 9), gallic acid **12** (ref. 9), procyanidin B-2 monogallate **13** (ref. 10). The present paper reports three new compounds.

Results and Discussion

Compound 1 was obtained as a white amorphous powder. It was shown by HR-EIMS to have the molecular formula $C_{29}H_{40}O_5$, based on the HR-EIMS of the molecular ion peak at *m/z* 468.3125 (Calcd. 468.3130), indicating ten degrees of unsaturation. ^{1}H and ^{13}C NMR (**Tables I** and **II**) of **1** revealed signals due to five tertiary methyl groups , seven methylenes, seven methines and ten quaternary carbons, which suggest the presence of twenty nine carbons. Its IR spectrum exhibited absorption 3428 (OH), 2946 (C-H, aliphatic), 1624 (C=C), 1618 (carboxylic acid), 1612 (aromatic), 960-942 cm^{-1} (C=C-H). The ^{13}C NMR spectra of **1** shows a carboxylic function, a tri-substituted double bond, three carbons bearing OH groups, an aromatic group and five tertiary methyl groups and three olefinic methines. By analyzing the 2D NMR spectra, it revealed to have a 28-nor-ursame skeleton. Its ^{1}H NMR spectrum shows the presence of five tertiary methyl groups (δ 2.35, 2.34, 1.82, 1.25, 1.06), two oxymethine protons (δ 4.36, m; 4.73, d, *J* = 9.5 Hz), two *o*-positioned aromatic protons (d, δ 7.08, *J* = 7.5 Hz; d, 6.95, *J*=7.3 Hz), and one trisubstituted olefinic proton (brs, δ 5.67).

The structure of **1** was deduced on the basis of ^{1}H-^{1}H COSY, HMBC (**Table II**) data and compared with structurally related **4** (ref. 2), its spectral data (^{1}H, ^{13}C NMR, IR and MS) being very similar to those of **4** suggested that **1** and **4** have a similar skeleton. The difference of the ^{1}H NMR spectral data between **1** and **4** were that the former was lacking signal of methyl group (δ 0.92), but showed signals at δ 3.49 (d, *J* = 11.0 Hz, H-27a) and 3.17 (d, *J* = 11.0 Hz, H-27b). These observations favoured the hydroxyl group at C-27. The presence of the hydroxyl group at more labile position (C-27) was evident from MS spectrum fragment ion peak at *m/z* 437.2894 (M-CH_2OH^+), as well as from the ^{13}C NMR spectrum (**Table II**) in which C-13 suffered an upfield shift (δ 138.7) while C-12 shifted downfield (125.3, ref. 11). The NOESY plot showed interaction of oxymethylene protons (H-27a/b) with one another as well as with H-9, confirming its α-orientation.

HMBC correlations are observed from Me-29 to quaternary C-18, C-19 and C-20, as well as from Me-30 to quarternary C-19, C-20 and C-21. One of the two aromatic protons shows HMBC correlations with quarternary C-17, C-19 and Me-30. The other shows

Table I - ^{1}H NMR spectral data of compounds **1-4** (400 MHz, δ, C_5D_5N, TMS)*

No	**1** δ_H coupling (Hz)	**2** δ_H coupling (Hz)	**3** δ_H coupling (Hz)	**4** δ_H coupling (Hz)
1α	1.64 (1H)	1.65 (1H)	1.64 (1H)	1.67 (1H)
1β	2.53 (1H)	2.52 (1H)	2.55 (1H)	2.54 (1H)
2α				
2β	4.36 (m, 1H)	4.38 (m, 1H)	4.39 (m, 1H)	4.40 (m, 1H)
3	4.73 (1H, d, *J* = 9.5Hz)	4.76 (1H, d, *J* = 9.5Hz)	4.74 (1H, d, *J* = 9.5Hz)	4.75 (1H, d, *J* = 9.5Hz)
5	2.31 (1H)	2.34 (1H)	2.32 (1H)	2.23 (1H)
6α	1.90 (1H)	1.90 (1H)	1.93 (1H)	1.92 (1H)
6β	1.77 (1H)	1.76 (1H)	1.76 (1H	1.77 (1H)
7α	1.61 (1H)	1.61 (1H)	1.63 (1H)	1.62 (1H)
7β	1.74 (1H)	1.75 (1H)	1.76 (1H)	1.76 (1H)
9	2.02 (1H)	2.03 (1H)	2.03 (1H)	2.04 (1H)
11	1.18~2.23 (2H)	1.19~2.30 (2H)	1.18~2.33 (2H)	2.19~2.33 (2H)
12	5.67 (brs, 1H)	5.66 (brs, 1H)	5.65 (brs, 1H)	5.65 (brs, 1H)
15α	2.21 (1H)	2.22 (1H)	1.76 (1H)	2.20 (1H)
15β	0.90 (1H)	0.91 (1H)	0.94 (1H)	0.92 (1H)
16	2.48 (2H)	2.47 (2H)	2.49 (2H)	2.48 (2H)
21	7.08 (1H, d, *J* = 7.5 Hz)	7.09 (1H, d, *J* = 7.5 Hz)	7.10 (1H, d, *J* = 7.5 Hz)	7.10 (1H, d, *J* = 7.5 Hz)
22	6.95 (1H, d, *J* = 7.3 Hz)			6.98 (1H, d, *J*=7.3 Hz)
24	1.82 (s, 3H)	1.85 (s, 3H)	1.82 (s, 3H)	1.84(s, 3H)
25	1.25 (s, 3H)	1.25 (s, 3H)	1.26 (s, 3H)	1.26 (s, 3H)
26	1.06 (s, 3H)	1.07 (s, 3H)	1.08 (s, 3H)	1.08 (s, 3H)
27 *a*	3.49 (d, *J* = 11.0 Hz)	3.47 (d, *J* = 11.0 Hz)	3.82 (s, 3H)	0.91 (s, 3H)
27β	3.17 (d, *J* = 11.0 Hz)	3.18 (d, *J* = 11.0 Hz)		
28				
29	2.35 (s, 3H)	2.35 (s, 3H)	2.36 (s, 3H)	2.37 (s, 3H)
30	2.34 (s, 3H)	2.38 (s, 3H)	2.33 (s, 3H)	2.33 (s, 3H)
COOMe		2.37 (s, 3H)	-	-

*Assignments from ^{1}H-^{1}H COSY, HMBC (from proton to carbon) and HMQC

HMBC correlations with quarternary C-18, C-20 and methylene C-16. Therefore, the two aromatic protons are then assigned to be H-21 and H-22 of ring E.

Using ^{1}H-^{1}H COSY, HMQC and HMBC spectrum a detailed partial structure was build up. The two hydroxyl groups are at C-2 and C-3 positions, respectively, this were deduced from the signals at δ 4.36 (dd, *J* = 6.8, 8.8 Hz) and 4.37 (d, *J* = 9.5 Hz). A strong NOESY correlation between H-2 and Me-25 indicated an α-orientation of the C-2 hydroxyl group. Consequently, the C-3 hydroxyl group must take β-orientation due to the multiplet and coupling constants of H-3 (d, *J* = 9.5 Hz). Carboxyl group shows a HMBC correlation with H-3 indicating that it is substituted at C-4. The Me-24 (δ 1.84, s, 3H) showed a cross-peak with the Me-25 (1.25, s, 3H), which suggested that the carboxyl group takes α-orientation. Based on detailed consideration of molecular models, structure **1** was established for the new natural product named as Changyediyuine **I** (**Figure 1**).

Compound 2 was obtained as a white amorphous powder. The IR spectrum of showed absorption bonds for hydroxyl (3436), methyl group (2953), ester carbonyl (1748), double bonds (1626, 952), aromatic group (1614) cm^{-1}. In the molecule of **2**, its ^{13}C NMR and DEPT spectrum (**Table II**) clearly exhibited 30 carbon signals (6 × CH_3, 7 × CH_2, 7 × CH, 10 × C). It was shown by HR-EIMS to have the molecular formula $C_{30}H_{42}O_5$, based on the HR-EIMS of the

Table II - ^{13}C NMR spectral data of compounds **1-4** (100 MHz, δ , C_5D_5N, TMS)*

	1		**2**		**3**		**4**	
No	δc	DEPT	δc	DEPT	δc	DEPT	δc	DEPT
1	48.2	CH_2	48.4	CH_2	48.5	CH_2	48.7	CH_2
2	68.9	CH	68.5	CH	68.4	CH	68.8	CH
3	82.0	CH	81.7	CH	81.5	CH	81.1	CH
4	54.7	C	54.7	C	54.8	C	55.0	C
5	52.3	CH	52.4	CH	52.3	CH	52.6	CH
6	21.5	CH_2	21.4	CH_2	21.6	CH_2	21.3	CH_2
7	33.9	CH_2	34.0	CH_2	34.2	CH_2	34.1	CH_2
8	39.9	C	39.9	C	40.1	C	40.4	C
9	48.3	CH	48.5	CH	48.4	CH	48.6	CH
10	38.2	C	38.2	C	38.7	C	38.6	C
11	23.9	CH_2	23.7	CH_2	23.8	CH_2	23.9	CH_2
12	125.3	CH	125.4	CH	125.6	CH	125.5	CH
13	138.7	C	139.0	C	138.9	C	139.1	C
14	44.0	C	44.2	C	75.7	C	44.4	C
15	33.4	CH_2	33.2	CH_2	33.6	CH_2	32.6	CH_2
16	32.0	CH_2	32.0	CH_2	31.8	CH_2	31.4	CH_2
17	138.4	C	138.5	C	138.4	C	138.8	C
18	139.0	C	133.6	C	139.2	C	139.4	C
19	133.5	C	133.6	C	133.7	C	133.9	C
20	135.4	C	135.4	C	135.6	C	135.4	C
21	128.2	CH	128.0	CH	128.2	CH	127.9	CH
22	123.5	CH	123.4	CH	123.6	CH	123.6	CH
23	138.1	C	169.1	C	138.4	C	138.2	C
24	13.9	CH_3	13.8	CH_3	13.9	CH_3	13.7	CH_3
25	18.2	CH_3	18.2	CH_3	18.3	CH_3	18.1	CH_3
26	17.2	CH_3	17.0	CH_3	17.3	CH_3	17.1	CH_3
27	29.4	CH_2	29.2	CH_2	59.5	OCH_3	27.5	C
28	-	-	-	-	-	-	-	-
29	19.3	CH_3	19.2	CH_3	19.4	CH_3	19.3	CH_3
30	21.3	CH_3	21.2	CH_3	21.3	CH_3	21.0	CH_3
$COOCH_3$	-	-	20.8	CH_3	-	-	-	-

molecular ion peak at *m/z* 482.3293 (Calcd. 482.3288), indicating ten degrees of unsaturation.

The ^1H, ^{13}C NMR and DEPT (**Tables I** and **II**), IR, MS and NOESY spectral data of **2** were very similar to those of **1**. Comparing the ^1H NMR and ^{13}C NMR spectra of **2** with those of **1** led to the conclusion that the main difference was the **2** showed the presence of one $–COOCH_3$ (δ_H 2.37, δ_C 169.1, 20.8) at C-4. Furthermore, in the HMBC spectrum the correlation between H-3 and CO of $COOCH_3$ was clearly observed. So this group must be attached to C-4. A strong NOESY correlation between Me-24 and Me-25 suggests that the formate group takes α-orientation. From the above information, Changyediyuine **II** was determined to have the structure as shown in **2**.

Compound 3 was obtained as a white amorphous powder. The HR-EIMS, ^1H NMR, ^{13}C NMR (**Table II**) and DEPT data indicate a molecular formula of $C_{29}H_{40}O_5$ indicating ten degrees of unsaturation. Its spectra indicated that **3** has an almost similar set of signals as that of **1**, but it showed slight difference in the ^1H NMR spectra owing to the presence of one methoxy group at δ 3.82 (s, 3H). It was further supported by the highfield shifting of C-14 signal by δ 75.7 in ^{13}C NMR spectra of **3** (**Table II**). Hence it was placed on C-14 position. In HMBC spectrum, the

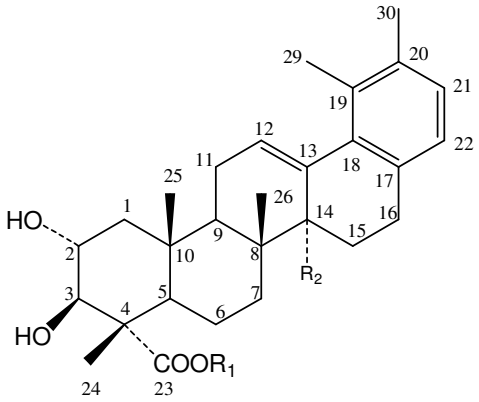

Figure 1 — The structure of compounds **1-4**

cross peak between Me-27/C-14, C-15, C-8, concluded that **3** was 2α,3β-dihydroxy-14-methoxy-28-norurs-12,17(18),19(20),21-tetraen-23-oic acid named Changyediyuine **III**.

Experimental Section

Melting Points were determined using a kofler melting point apparatus and optical rotations were made on a DIP-181 instrument. Spectra were recorded with the following instrument. IR and UV spectra were taken in Perkin-Elmer 599B and Shimadzu UV-300 spectrometer. ^{1}H NMR, ^{13}C NMR and 2D NMR spectra were recorded on a Bruker AM-400 FT NMR with TMS as internal standard and HR-EIMS and EIMS data were obtained on a MAT-12 (70 eV). Silica gel (100-200, 200-300 mesh) was used for column chromatography and Silica gel GF_{254} for TLC.

Collection of plant material

The plant material was collected from Neimenggu of P. R. China, in August 2005, and identified by Prof Yong-shan Lian of Northwest Normal University, A voucher specimen (No.35492) is deposited in the herbarium of the College of Life Science, Northwest Normal University, Lanzhou 730070, P.R. China.

Extraction and isolation

Powdered dried roots (5.6 kg) were extracted three times with 95% EtOH at room temperature. After removing solvents under reduced pressure, about 560 g residue was obtained. The residue was defatted with cyclohexane, and partitioned sequentially with $CHCl_3$ and *n*-BuOH layer was dried in *vacuo*, respectively. The $CHCl_3$ extract (85 g) was subjected to silica gel (750 g, 100-200 mesh) column chromatography and used *n*-hexane-EtOAC gradient, three major fractions (A 13 g, B 16 g, C 18 g) being obtained. Rechromatography on silica gel (200-300 mesh), with *n*-hexane-$CHCl_3$ (1:10, v/v) and $CHCl_3$-Me_2CO (8:1, V/V) gradient fractions A and B were obtained.and purified by preparative TLC, yielded **6** (8 mg), **7** (13 mg), **8** (21 mg), **9** (17 mg). Fraction C (18 g) was purified by rechromatography on silica gel column (200-300 mesh) with $CHCl_3$-EtOAc (3:7, v/v) as eluent and preparative TLC, **10** (20 mg), **11** (28 mg), **12** (14 mg) and **13** (11 mg) were obtained. The *n*-BuOH layer was dried *in vacuo* to yield a triterpene-enriched fraction. Which was then separated by silica gel column chromatography (200-300 mesh) using $CHCl_3$-CH_3OH as solvent to yield three major fraction (D 30 g, E 38 g, F 34 g) being obtained. The extract of fraction D was purified by preparative TLC [$CHCl_3$:EtOAc:CH_3OH (5:4:1, v/v)] to afford **1** (18 mg) and **4** (22 mg). The extract of Fraction E was purified by rechromatography on silica gel column (200-300 mesh, 300 g), with Me_2CO:CH_3OH (4:7, v/v) as eluent and preparative TLC gave compounds **2** (22 mg), **3** (14 mg) and **5** (18 mg).

Compound 1: $C_{29}H_{40}O_5$, white amorphous powder, $[\alpha]_D^{20}$ +19.6° (MeOH, *c*, 0.22). IR (KBr): 3428, 2946, 1624, 1618, 1612, 960-942 cm^{-1}; HR-EIMS *m/z*: Found 468.3125, (Calcd 468.3130); UV λ_{max}^{MeOH} (nm): 214, 244.

Compound 2: $C_{30}H_{42}O_5$, white amorphous powder, $[\alpha]_D^{20}$ +19.9° (MeOH, *c*, 0.21). IR (KBr): 3436, 2953, 1748, 1626, 1614, 952 cm^{-1}; HR-EIMS *m/z*: Found 482.3293, (Calcd 482.3288); UV λ_{max}^{MeOH} (nm): 215, 244.

Compound 3: $C_{29}H_{40}O_5$, white amorphous powder, $[\alpha]_D^{20}$ +20.1° (MeOH, *c*, 0.23). IR (KBr): 3430, 2941, 1622, 1616, 956 cm^{-1}; UV λ_{max}^{MeOH} (nm): 214, 242; HR-EIMS *m/z*: Found 468.3132, (Calcd 468.3130).

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