A New 30-Noroleanane Saponin from *Wedelia chinensis*

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A novel 30-nortriterpenoid saponin, (3β)-3-hydroxy-30-noroleana-12,20(29)-dien-28-oic acid 3-(β-D-glucopyranosiduronic acid 6-methyl ester) (**1**), and a known compound, (3β)-oleanolic acid 3-(β-D-glucopyranosiduronic acid 6-methyl ester) (**2**), were isolated from the aerial parts of *Wedelia chinensis*. The structures were established by their spectral data including ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMBC, HSQC, NOESY, and HR-FAB-MS data.

Introduction. – *Wedelia chinensis* is a genus of the Asteraceae (alt. Compositae), mainly distributed in south China. It is traditionally used for the treatment of diphtheria, chincough, diarrhea, hemorrhoids, injuries due to falls, and faucitis. Now its clinic use is preferably for hepatitis and stomatitis. *W. chinensis* was reported to have extensive effects, *e.g.*, protective, antifebric, analgesic, and anti-inflammatory effects, in the form of its plant extract, but some of the specific pharmacologically active substance are unknown yet [1][2]. Chemical constituents isolated from *W. chinensis* include organic acids, sesquiterpenes, triterpenoids, flavonoids, and steroids [3].

In our phytochemical studies of the aerial parts of *W. chinensis*, we now isolated and characterized a new 30-nortriterpenoid saponin, (3β)-3-hydroxy-30-noroleana-12,20(29)-dien-28-oic acid 3-(β-D-glucopyranosiduronic acid 6-methyl ester) (**1**), and a known compound, (3β)-oleanolic acid 3-(β-D-glucopyranosiduronic acid 6-methyl ester) (**2**; *Fig. 1*). The 30-nortriterpenoids have been isolated in nature before, but this type of a saponin with a glucopyranosiduronic acid glycone at C(3) is described here for the first time.

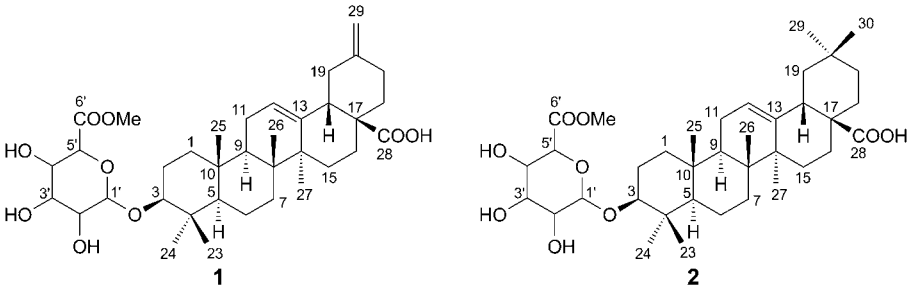

Fig. 1. *Compounds* **1** *and* **2**, *isolated from* Wedelia chinensis

Results and Discussion. – The crude AcOEt extract from the aerial parts of *W. chinensis* was separated by chromatographic procedures and led to the isolation of compounds **1** and **2**. Their structures were established by ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMBC, HSQC, NOESY, and HR-FAB-MS data.

Compound **1** showed an ion peak at *m*/*z* 669.3401 ($[M+K]^+$) in the HR-FAB-MS, from which the molecular formula $C_{36}H_{54}O_9$ was deduced. The ^{1}H-NMR spectrum (*Table*) exhibited six Me *s*. Two of them (δ(H) 0.84 and 1.04) were assigned to be in geminal position, as confirmed by the common ^2J- and ^3J-coupling partners in the HMBC spectrum (*Table*, *Fig. 2*). C(5) (δ(C) 57.0) showed correlations with both Me(24) and Me(25) (δ(H) 0.94); C(9) (δ(C) 48.60) displayed correlations with both Me(25) and Me(26) (δ(H) 0.82). Both C(8) (δ(C) 40.6) and C(14) (δ(C) 42.9) were involved in the correlation with Me(26) and Me(27) (δ(H) 1.18). A C=C bond could be placed between C(12) and C(13) due to the correlation between Me(27) and C(13) (δ(C) 144.7) in the HMBC spectrum, and an olefinic H-atom (δ(H) 5.27–5.31) was locateded at C(12) (δ(C) 124.0). This allowed to assign 1 H–C(11) (δ(H) 1.87–1.93) which correlated with the olefinic H–C(12) at δ(H) 5.27–5.31 in the COSY plot, and was further confirmed by an NOE correlation with Me(25). The broad signals at δ(H) 4.57 and 4.61 were from two protons attached to the C-atom resonating at δ(C) 107.1, the latter chemical shift indicating that this C-atom was part of an exocyclic methylene group (CH_2(29)). In the COSY plot, these olefinic CH_2 group correlated (allyl coupling) with a br. *dd* at δ(H) 2.54 arising from the CH_2(19) group (δ(C) 42.8; δ(H) 2.54 and 2.04). Both of the two geminal H–C(19) correlated with H–C(18) at δ(H) 2.72 (corresponding C-atom in HSQC spectrum, C(18) at δ(C) 48.6) establishing a link between these C-atoms. Correlations between H_{eq}–C(19) (δ(H) 2.04) and C(13), C(17), and C(21) were in good agreement with the proposed structure fragment (*Fig. 2*). The anomeric proton signal of **1** at δ(H) 4.38 ($^3J(1',2')=7.9$ Hz, H–C(1′)) indicated the presence of a monosaccharide unit. The sugar moiety was identified as β-glucuropyranosiduronic acid 6-methyl ester by the HMBCs between C(6′) (δ(C) 171.4) and H–C(5′) (δ(H) 3.82), H–C(4′) (δ(H) 3.50), and Me (δ(H) 3.76) [4]. The HMBC cross-peaks Me(24)/C(3) and H–C(1′)/C(3) demonstrated that the glycosidation by the glucopyranosuronic acid 6-methyl ester was positioned at C(3) of the triterpene core. Some H-atoms overlapped but often one of these H-atoms could be identified by its obvious NOE signals (*Fig. 3*, *Table*). It is surprising to observe that Me(27) has a NOE correlation with H–C(9) (δ(H) 1.56–1.63), H_{ax}–C(22) (δ(H) 1.51–1.59) and H_{ax}–C(19) suggesting that rings *C* and *D* a probably adopted a half-chair conformation due to the C=C bond between C(12) and C(13) which made the two rings more flat than normal. Combining all the information above, the structure of compound **1** was

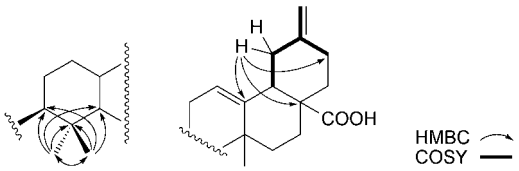

Fig. 2. *Correlation of structure fragments of* **1**

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; CD_3OD) *of* **1**. δ in ppm, J in Hz.

	δ(H)	δ(C)	HMBC	NOESY
H–C(1′)	4.38 (*d*, *J* = 7.9)	107.0	C(3)	
H–C(2′)	3.22 (*dd*, *J* = 9.2, 8.0)	75.3		
H–C(3′)	3.35 (*dd*, *J* = 9.2, 9.3)	77.5		
H–C(4′)	3.50 (*dd*, *J* = 9.4, 9.3)	73.2		
H–C(5′)	3.82 (*d*, *J* = 9.8)	76.6		
C(6′)		171.4	C(4′), C(5′)	
MeO	3.76 (*s*)	52.8	C(6′)	
CH_2(1)	1.57 – 1.63 (*m*, H_{eq})	39.7		
	0.97 (*ddd*, *J* = 13.6, 3.2, 3.2, H_{ax})			
CH_2(2)	1.75 – 1.82 (*m*, H_{eq})	27.0	C(1), C(3), C(10)	
	1.62 – 1.71 (*m*, H_{ax})			
H–C(3)	3.15 (*dd*, *J* = 11.7, 4.5)	91.1	C(1′), C(1), C(2), Me(23), Me(24)	H_{ax}–C(1), Me(24)
C(4)		40.2		
H_{ax}–C(5)	0.79 (*d*, *J* = 11.3)	57.0		H_{ax}–C(1), H_{ax}–C(7), H–C(9), Me(24)
CH_2(6)	1.52 – 1.59 (*m*, H_{eq})	19.3		
	1.37 – 1.45 (*m*, H_{ax})			
CH_2(7)	1.28 – 1.34 (*m*, H_{eq})	34.0		
	1.48 – 1.55 (*m*, H_{ax})			
C(8)		40.6		
H–C(9)	1.56 – 1.63 (*m*)	48.60 (o)		
C(10)		37.9		
CH_2(11)	1.87 – 1.93,	24.5	C(8), C(10), C(13)	
	1.68 – 1.72 (2*m*)			
H–C(12)	5.27 – 5.31 (*m*)	124.0	C(9), C(14), C(18)	H–C(18), H_{eq}–C(19), CH_2(11)
C(13)		144.7		
C(14)		42.9		
CH_2(15)	1.12 (br. *d*, *J* = 13.6, H_{eq})	28.9		
	1.78 – 1.85 (*m*, H_{ax})			
CH_2(16)	2.09 – 2.16,	24.3 (br.)		
	1.69 – 1.75 (2*m*)			
C(17)		49		
H–C(18)	2.72 (br. *d*, *J* = 11.5)	48.6 (o)	C(14)	
CH_2(19)	2.04 (*dd*, *J* = 13.0, 2.6, H_{eq})	42.8	C(13), C(17), C(21)	
	2.54 (br. *dd*, *J* = 13.5, 13.5, H_{ax})			
C(20)		148	C(19), C(21)	
CH_2(21)	2.09 – 2.14 (*m*)	31.0		
	2.18 – 2.25 (br. *dd*, *J* ≈ 12.4, 12.4, H_{ax})			
CH_2(22)	1.81 – 1.87 (*m*, H_{eq})	39.2 (br.)		
	1.51 – 1.59 (*m*, H_{ax})			
Me(23)	1.04 (*s*)	28.5	C(3), C(4), C(5), Me(24)	
Me(24)	0.84 (*s*)	17.0	C(3), C(4), C(5), Me(23)	
Me(25)	0.94 (*s*)	15.9	C(1), C(5), C(9), C(10)	Me(23), H_{ax}–C(2), H_{ax}–C(6)
Me(26)	0.82 (br.)	17.8	C(7), C(8), C(9), C(14)	Me(25), H_{eq}–C(7), CH_2(11)
Me(27)	1.18 (*s*)	26.4	C(8), C(13), C(14), C(15)	H–C(9), H_{ax}–C(22), H_{ax}–C(19)
C(28)		180.0		
CH_2(29)	4.61, 4.57 (2br. *s*)	107.1 (br.)		H_{eq}–C(19), H_{eq}–C(21)

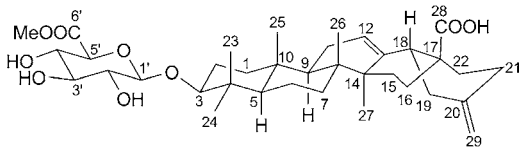

Fig. 3. *Conformation of compound* **1**

confirmed as (3β)-3-hydroxy-30-noroleana-12,20(29)-dien-28-oic acid 3-(β-D-glucopyranosiduronic acid 6-methyl ester) (**1**).

Compound **2** was closly related to compound **1**. High-resolution fast-atom-bombardment (HR-FAB) MS data (*m*/*z* 685.3715 ($[M+K]^+$)) suggested that the molecular formula of **2** was $C_{37}H_{58}O_9$. It had the same sugar moiety and a similar type of complexity for the triterpene core. The difference was the absence of the exocyclic methylene group; instead there was an extra geminal-dimethyl moiety in **2** (δ(H) 0.89 and 0.93). The HMBC spectrum revealed good correlations for the Me groups. Thus, Me(24) (δ(H) 0.84) exhibted correlations with C(3) (δ(C) 91.1), C(5) (δ(C) 56.6), as well as C(23) (δ(C) 15.4). Me(27) (δ(H) 1.15) was easy to identify as it correlated with the olefinic C(13) (δ(C) 145.4). Based on the above-mentioned evidence and comparison of the spectral data with those reported, the structure of this compound was assigned as (3β)-oleanolic acid 3-(β-D-glucopyranosiduronic acid 6-methyl ester) (**2**) [4].

The triterpenoids are a large and structurally diverse group of natural products derived from squalene or related acyclic C_{30} precursors with more than 100 distinct skeletons [5–8]. Noroleanane-type triterpenes have been reported historically but mainly from callus tissue [9–11]. In 1998, three 30-nortriterpenoid saponins were isolated from *Zygophyllum decumbens* DEL. [12]. Recently, rare 24,30-dinortriterpenoids had been identified [13][14]. To the best of our knowledge, a 30-noroleanane saponin with a glucopyranosiduronic acid 6-methyl ester moiety at C(3) is reported for the first time. It is interesting that 30-nortriterpenoids are always accompanied by corresponding normal oleanane triterpenoids. Biosynthesis of the nortriterpenoids is not clear yet, and it is generally presumed that nortriterpenoids stem from corresponding normal triterpenes as suggested by the co-occurrence of different degrees of oxidation at C(29). It is reported that more often in nature observed nortriterpenoids were 24-noroleanane derivatives [15][16].

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Experimental Part

General. Na_2SO_4 was the drying agent used in all workup procedures. Flash chromatography (FC): silica gel *60* (SiO_2, 230–400 mesh; *EM Science*). TLC: SiO_2 *60* F_{254} pre-coated TLC plates (0.25 mm or 0.5 mm; *EM Science*); visualization by spraying with 10% H_2SO_4 EtOH followed by heating on a hot plate. Anal. HPLC: *Waters-600-FHU* delivery system coupled to a *PDA-996* detector; *Whatman-Partisil-*

10-ODS-2 anal. columns (4.6 × 250 mm) in series. Prep. and semi-prep. HPLC: *Waters-Delta-Prep-3000* instrument coupled to a *UV-486* tunable absorbance detector set (*Waters*, Montreal, Quebec, Canada); *Whatman-Partisil-10-ODS-2 Mag-9* semi-prep. columns (9.4 × 250 mm) in series; elution with a 50 min linear gradient of MeCN (25 → 100%) in H_2O, flow rate 3 ml/min (prep. HPLC). Optical rotations: *Jasco DIP-370* digital polarimeter. NMR Spectra: *Bruker-Avance-500* spectrometer; at 500.13 (^{1}H) and 125.77 MHz (^{13}C), at r.t.; internal reference, solvent CD_3OD (δ(H) 3.34 and δ(C) 49.86); δ in ppm, *J* in Hz; recording of the various 2D spectra by standard procedures; phase-sensitive 2D experiments (NOESY and HSQC), data acquisition by the TPPI phase mode; NOESY experiment by using a mixing time of 0.3 s and a relaxation delay of 1.5 s. FAB-MS (pos.): *Vacuum-Generators-ZAB-HS* double-focussing instrument; xenon beam of 8 kV energy at 1 mA equivalent neutral current; low-resolution MS in glycerol; samples were dissolved in 0.2 µl of DMSO before addition of 0.5 µl of glycerol; in *m*/*z*.

Plant Material. Aerial parts of the plant (3.0 kg) were collected at Hebei, China, in 2006 and were identified as *Wedelia chinensis* by Prof. *F. Z. Nie*, Laboratory of Pharmacognosy, Hebei Medical University. The specimen is deposited with the Laboratory of Natural Product Chemistry, Hebei Medical University, under accession voucher number Qw-2006-01.

Extraction and Isolation. The dry and well-crashed plant (3.0 kg) was extracted with cold EtOH for 3 d and extracted for 3 times. The extracts were combined and concentrated to yield an extract which was partitioned between CH_2Cl_2 and H_2O yielded the org. solvent-soluble fraction. The residual aq. phase was further partitioned with AcOEt. The dried AcOEt extract was subjected to CC (SiO_2, CH_2Cl_2 containing increasing proportions of MeOH) affording the crude saponin mixture. The mixture was purified by repeated prep. TLC and prep. HPLC: **1** and **2**.

*(3β)-3-Hydroxy-30-noroleana-12,20(29)-dien-28-oic Acid 3-(β-*D*-Glucopyranosiduronic Acid 6-Methyl Ester)* (*=(3β)-17-Carboxy-28,30-dinoroleana-12,20(29)-dien-3-yl β-*D*-Glucopyranosiduronic Acid 6-Methyl Ester*; **1**): Gum. $[\alpha]_D^{22} = +84$ (*c* = 0.05, MeOH). ^{1}H- and ^{13}C-NMR: *Table.* HR-FAB-MS: 669.3401 ($[M+K]^+$, $C_{36}H_{54}KO_3^+$; calc. 669.3405).

*(3β)-Oleanolic Acid 3-(β-*D*-glucopyranosiduronic Acid 6-Methyl Ester)* (*=(3b)-17-Carboxy-28-norolean-12-en-3-yl β-*D*-Glucopyranosiduronic Acid 6-Methyl Ester*; **2**): White needles. $[\alpha]_D^{22} = +16$ (*c* = 0.05, MeOH). ^{1}H-NMR (CD_3OD, 500 MHz): 3.14 (*dd*, *J* = 11.7, 4.5, H–C(3)); 5.23 (*s*, H–C(12)); 0.84 (*s*, Me(23)); 1.04 (*s*, Me(24)); 0.94 (*s*, Me(25)); 0.81 (br., Me(26)); 1.15 (*s*, Me(27)); 0.89 (*s*, Me(29)); 0.93 (*s*, Me(30)); 4.37 (*d*, *J* = 7.9, H–C(1′)); 3.22 (*dd*, *J* = 9.2, 8.0, H–C(2′)); 3.35 (*dd*, *J* = 9.2, 8.0, H–C(3′)); 3.50 (*dd*, *J* = 9.2, 8.0, H–C(4′)); 3.82 (*d*, *J* = 9.8, H–C(5′)); 3.76 (*s*, MeO). ^{13}C-NMR (CD_3OD, 125 MHz): 91.1 (C(3)); 123.3 (C(12)); 145.4 (C(13)); 180.0 (C(28)); 106.8 (C(1′)); 75.2 (C(2′)); 77.4 (C(3′)); 72.1 (C(4′)); 76.5 (C(5′)); 171.1 (C(6′)); 52.5 (MeO). HR-FAB-MS: 685.3715 ($[M+K]^+$, $C_{37}H_{58}KO_3^+$; calc. 685.3719).

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