Three New Steroidal Glycosides from the Roots of *Cynanchum auriculatum*

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Three new C_{21} steroidal glycosides with a cinnamoyl group at C(12) and a 2-methylbutanoyl group at C(20), and a straight sugar chain at C(3), namely cyanoauriculosides C–E (**1**–**3**, resp.), together with three known steroidal derivatives, were isolated from the roots of *Cynanchum auriculatum* (Asclepiadaceae). Their structures were determined by spectroscopic analyses and chemical methods. The known constituents were identified as wilfoside K1N (**4**), cynanauriculoside II (**5**), and auriculoside IV (**6**).

Introduction. – C_{21} Steroidal glycosides have been extensively studied as an important class of biological active compounds. They have been reported to possess cytotoxic [1], acetylchlolinesterase inhibitory [2], and immunosuppressive activities [3]. Several of these compounds have been isolated from plants, especially from those of the Asclepiadaceae family [4–8]. The roots of *Cynanchum auriculatum* ROYLE ex WIGHT (Asclepiadaceae), serve as a famous folk medicine in China, and has widely been used for the treatment of geriatric diseases, enhancing immunity, anti-aging effect, and prolonging life [9]. Previous phytochemical investigations on the genus *Cynanchum* revealed steroidal glycosides [10], acetophenones [11], and polysaccharides [12]. Steroidal glycosides were considered as the major biologically active constituents of the genus *Cynanchum* [10]. In our search for biologically active compounds from ethnomedicinal plants, three new C_{21} steroidal glycosides, **1**–**3**, together with three known compounds, **4**–**6**, were isolated from the roots of this plant (*Fig.*). Their structures were determined by detailed spectroscopic analyses, including 2D-NMR techniques, and acidic hydrolysis. Here, we describe the structure elucidation of these new compounds.

Results and Discussion. – Compound **1**, isolated as a colorless amorphous powder, showed positive reaction on *Libermann–Buchard* and *Keller–Kiliani* reactions, indicating the presence of a steroid skeleton with a 2-deoxysugar moiety. The molecular formula was established as $C_{62}H_{94}O_{22}$ according to HR-ESI-MS (*m/z* 1189.6166 ($[M-H]^-$)). The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) suggested that **1** was a C_{21} steroidal glycoside. The ^{1}H-NMR spectrum of **1** showed signals of three Me groups (δ(H) 1.56 (*s*), 1.18 (*s*), and 1.14 (*d*, $J = 6.3$)), three O-bearing CH groups (δ(H) 4.81–4.83 (*m*), 3.86–3.88 (*m*), and 3.52–3.54 (*m*)), one olefinic H-atom (δ(H) 5.36 (br. *s*)), one cinnamoyl group (δ(H) 6.38 (*d*, $J = 16.0$, 1 H), 7.64 (*d*, $J = 16.5$, 1 H), 7.65–7.67 (*m*, 2 H), and 7.42–7.44 (*m*, 3 H)), which was supported by the ion fragment

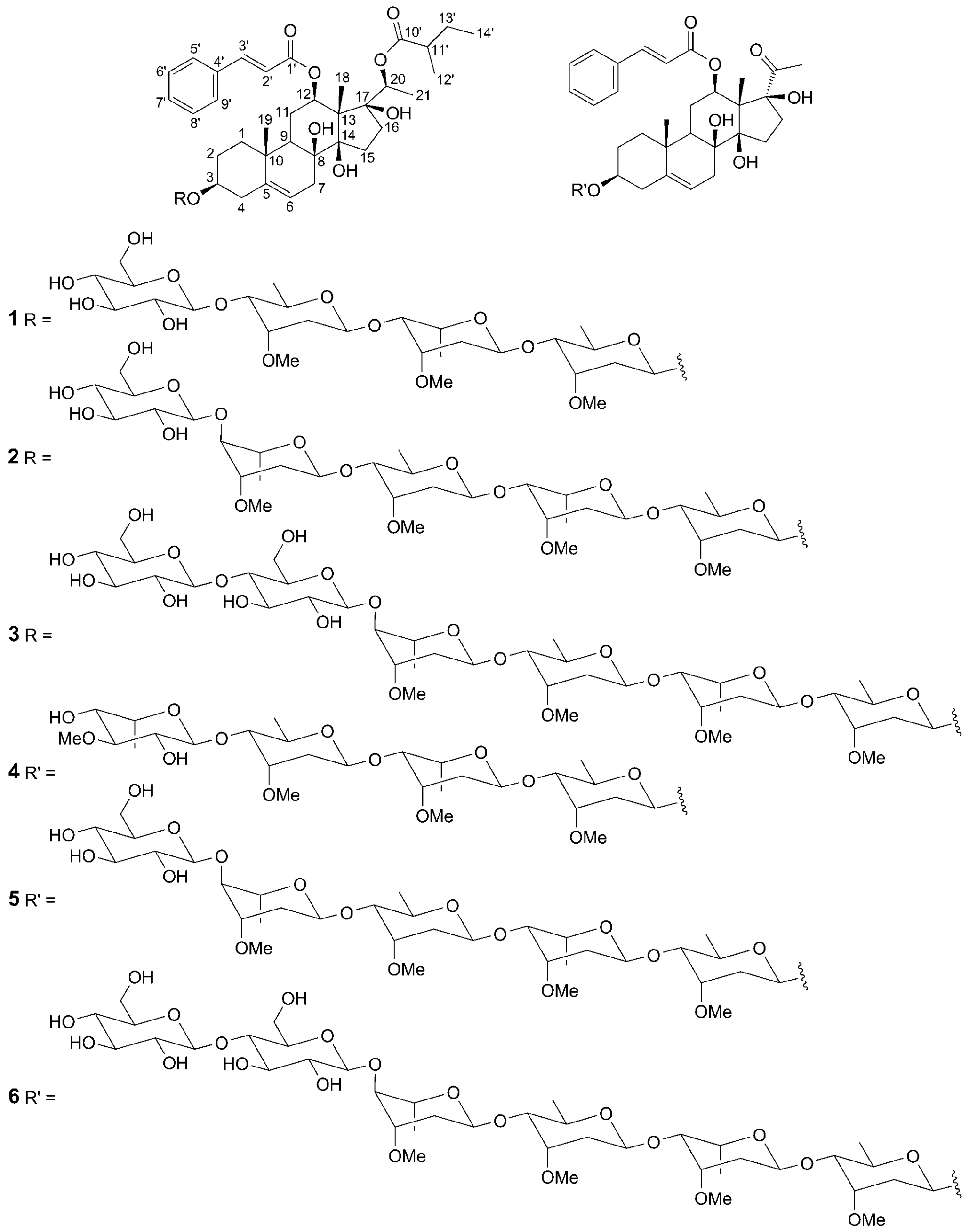

Figure. *Structures of compounds* **1**–**6** at *m*/*z* 147 ($[C_9H_7O_2]^+$) arising from the aglycone moiety. From ^{1}H,^{1}H-COSY and HSQC, one 2-methylbutyanoyl group (δ(H) 2.28–2.30 (*m*, 1 H), 1.17–1.19 (*m*, 3 H), 1.36–1.38, 1.68–1.70 (2*m*, 2 H), and 0.91 (*t*, *J* = 7.5, 3 H); δ(C) 42.6 (*d*), 16.7 (*q*), 27.2 (*t*), 12.1 (*q*), and 176.9 (*s*)) was also identified in aglycone moiety. When comparing the ^{13}C-NMR data of the aglycone portion of **1** with the data of penupogenin [13][14], it was found that the major difference was the presence of an additional 2-methylbutanoyl group in **1**, and the chemical shift of C(20) of **1** was deshielded by *ca.* 5 ppm. This suggested that a 2-methylbutanoyl group was located at C(20). Thus, the aglycone of compound **1** was proposed to be 20-*O*-(2-methylbutanoyl)penupogenin. The glycosidic portion contained four anomeric C-atoms with signals at δ(C) 96.9 (C(1^{I})), 101.7 (C(1^{II})), 100.3 (C(1^{III})), and 106.3 (C(1^{IV})), corresponding to anomeric H-atom signals at δ(H) 4.89 (*d*, *J* = 9.9), 4.99 (*d*, *J* = 3.6), 4.82 (*d*, *J* = 9.6), and 4.36 (*d*, *J* = 7.8), respectively, which indicated four sugar units in **1** with three β-linkages and one α-linkage. Upon acidic hydrolysis, this glycoside liberated cymarose, diginose, and glucose as sugar components, by TLC comparison with authentic samples. Compared with penupogenin [14], the glycosidation shifts were observed at C(2) (−1.9 ppm), C(3) (+7.7 ppm), and C(4) (−3.6 ppm) in aglycone moiety, indicating the attachment of the sugar chain at C(3)–*O* of the aglycone. This was corroborated by the HMBC between H–C(1^{I}) at δ(H) 4.89 and C(3) at δ(C) 79.2, and ROESY correlation was observed between H–C(1^{I}) at δ(H) 4.89 and H–C(3) at δ(H) 3.52–3.54. On the basis of the ^{1}H,^{1}H-COSY, HSQC, HMBC, and ROESY spectra, the data of these four sugars were assigned as two β-cymaropyranosyls, one α-diginopyranosyl and one β-glucopyranosyl. By comparing the ^{1}H- and ^{13}C-NMR data of the deoxysugar moieties of **1** with those of the corresponding deoxysugar moieties of cynanoside K [15] and cynanauriculoside II [16], as well as with those which have been reported in contrast for D-diginopyranosyl [17] and L-cymaropyranosyl [13][18] residues, the configuration of the diginopyranose and cymaropyranose units in **1** were assigned to L and D, respectively. The sugar sequence and the interglycosidic linkages were established from the HMBC spectrum, in which distinct correlations between H–C(1^{I}) at δ(H) 4.89 and C(3) at δ(C) 79.2, between H–C(1^{II}) at δ(H) 4.99 and C(4^{I}) at δ(C) 82.1, between H–C(1^{III}) at δ(H) 4.82 and C(4^{II}) at δ(C) 75.3, and between H–C(1^{IV}) at δ(H) 4.36 and C(4^{III}) at δ(C) 83.7 were detected. Thus, compound **1** was determined to be 20-*O*-(2-methylbutanoyl)penupogenin-3-*O*-β-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranoside, named cyanoauriculoside C.

Compound **2** was obtained as a colorless amorphous powder. The molecular formula was established as $C_{69}H_{106}O_{25}$ according to HR-ESI-MS (*m*/*z* 1369.6713 ($[M + Cl]^-$)). Acidic hydrolysis of **2** afforded a mixture of sugars, which were identified as cymarose, diginose, and glucose by TLC comparison with authentic samples. The ^{1}H- and ^{13}C-NMR data indicated that **2** possessed the same aglycone as **1**. Comparison of the spectroscopic data of the sugar moieties with those of **1** indicated the presence of one additional cymaropyranosyl moiety in **2**. The sugar sequence and the interglycosidic linkages were determined by HMBC spectrum, in which correlations between H–C(1^{II}) at δ(H) 4.96 and C(4^{I}) at δ(C) 82.7, between H–C(1^{III}) at δ(H) 4.79 and C(4^{II}) at δ(C) 75.3, between H–C(1^{IV}) at δ(H) 4.83 and C(4^{III}) at δ(C) 83.1, and between H–C(1^{V}) at δ(H) 4.40 and C(4^{IV}) at δ(C) 78.6 were observed. Thus,

Table 1. *NMR Data* (CD_3OD) *of the Aglycone Moiety of Compounds* **1**–**3**. δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.10–1.12 (*m*), 1.79–1.82 (*m*)	39.7	1.10–1.12 (*m*), 1.78–1.80 (*m*)	39.7	1.14–1.16 (*m*), 1.78–1.81 (*m*)	39.8
2	1.56–1.58 (*m*), 1.85–1.87 (*m*)	30.1	1.57–1.59 (*m*), 1.84–1.86 (*m*)	30.1	1.57–1.59 (*m*), 1.85–1.87 (*m*)	30.1
3	3.52–3.54 (*m*)	79.2	3.51–3.53 (*m*)	79.1	3.53–3.55 (*m*)	79.2
4	2.20–2.22 (*m*), 2.34–2.36 (*m*)	39.7	2.20–2.22 (*m*), 2.34–2.36 (*m*)	39.7	2.20–2.22 (*m*), 2.35–2.37 (*m*)	39.8
5		140.0		140.0		140.1
6	5.36 (br. *s*)	119.7	5.34 (br. *s*)	119.9	5.33 (br. *s*)	119.8
7	2.14–2.16 (*m*), 2.22–2.24 (*m*)	35.1	2.15–2.17 (*m*), 2.23–2.25 (*m*)	35.1	2.15–2.17 (*m*), 2.21–2.23 (*m*)	35.2
8		75.0		75.0		74.9
9	1.52–1.54 (*m*)	44.7	1.52–1.54 (*m*)	44.7	1.52–1.54 (*m*)	44.7
10		38.0		38.0		38.0
11	1.62–1.64 (*m*), 2.03–2.05 (*m*)	26.2	1.62–1.64 (*m*), 2.01–2.03 (*m*)	26.2	1.64–1.66 (*m*), 2.02–2.04 (*m*)	26.2
12	4.81–4.83 (*m*)	75.4	4.82–4.84 (*m*)	75.0	4.82–4.84 (*m*)	75.4
13		57.4		57.5		57.6
14		88.3		88.4		88.4
15	1.98–2.00 (*m*)	34.0	1.94–1.96 (*m*)	34.0	1.96–1.98 (*m*)	34.1
16	1.87–1.89 (*m*)	34.1	1.87–1.89 (*m*)	34.0	1.88–1.90 (*m*)	34.1
17		89.5		89.4		89.6
18	1.56 (*s*)	11.0	1.54 (*s*)	11.0	1.56 (*s*)	11.0
19	1.18 (*s*)	18.5	1.15 (*s*)	18.3	1.15 (*s*)	18.6
20	3.86–3.88 (*m*)	75.3	3.82–3.84 (*m*)	75.2	3.80–3.82 (*m*)	74.1
21	1.14 (*d*, $J = 6.3$)	15.2	1.14 (*d*, $J = 6.4$)	15.2	1.14 (*d*, $J = 6.3$)	15.2
1′		168.4		168.4		168.4
2′	6.38 (*d*, $J = 16.0$)	120.3	6.35 (*d*, $J = 16.0$)	120.3	6.35 (*d*, $J = 15.8$)	120.3
3′	7.64 (*d*, $J = 16.5$)	145.5	7.60 (*d*, $J = 16.0$)	145.5	7.60 (*d*, $J = 15.5$)	145.5
4′		135.9		135.8		135.8
5′	7.65–7.67 (*m*)	129.2	7.56–7.58 (*m*)	129.2	7.56–7.58 (*m*)	129.2
6′	7.42–7.44 (*m*)	130.0	7.39–7.41 (*m*)	130.0	7.39–7.41 (*m*)	130.0
7′	7.42–7.44 (*m*)	131.5	7.39–7.41 (*m*)	131.5	7.39–7.41 (*m*)	131.5
8′	7.42–7.44 (*m*)	130.0	7.39–7.41 (*m*)	130.0	7.39–7.41 (*m*)	130.0
9′	7.65–7.67 (*m*)	129.2	7.56–7.58 (*m*)	129.2	7.56–7.58 (*m*)	129.2
10′		176.9		176.9		177.0
11′	2.28–2.30 (*m*)	42.6	2.29–2.31 (*m*)	42.6	2.29–2.31 (*m*)	42.6
12′	1.17–1.19 (*m*)	16.7	1.15–1.17 (*m*)	16.8	1.13–1.15 (*m*)	16.7
13′	1.36–1.38 (*m*), 1.68–1.70 (*m*)	27.2	1.36–1.38 (*m*), 1.67–1.69 (*m*)	27.2	1.36–1.38 (*m*), 1.67–1.69 (*m*)	27.2
14′	0.91 (*t*, $J = 7.5$)	12.1	0.89 (*t*, $J = 7.5$)	12.2	0.89 (*t*, $J = 7.5$)	12.2

compound **2** was determined to be 20-*O*-(2-methylbutanoyl)penupogenin-3-*O*-β-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranoside, named cyanoauriculoside D.

Compound **3** was obtained as a colorless amorphous powder. The molecular formula was established as $C_{75}H_{116}O_{30}$ according to HR-ESI-MS (*m*/*z* 1531.7179

Table 2. *NMR Data* (CD_3OD) *of the Sugar Moieties of Compounds* **1**–**3**. δ in ppm, J in Hz[a])

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
	β-D-cym		β-D-cym		β-D-cym	
1^{I}	4.89 (d, $J = 9.9$)	96.9	4.84 (d, $J = 10.0$)	96.9	4.86 (overlap)	97.0
2^{I}	1.50–1.52 (*m*), 2.15–2.17 (*m*)	35.3	1.52–1.54 (*m*), 2.14–2.16 (*m*)	35.3	1.53–1.55 (*m*), 2.15–2.17 (*m*)	35.3
3^{I}	3.65–3.67 (*m*)	78.2	3.67–3.69 (*m*)	78.2	3.63–3.65 (*m*)	78.2
4^{I}	3.23–3.25 (*m*)	82.1	3.22–3.24 (*m*)	82.7	3.22–3.24 (*m*)	82.7
5^{I}	3.86–3.88 (*m*)	70.3	3.83–3.85 (*m*)	70.2	3.84–3.86 (*m*)	70.2
6^{I}	1.22 (d, $J = 6.0$)	18.5	1.19 (d, $J = 6.0$)	18.5	1.20 (d, $J = 6.5$)	18.5
MeO	3.44 (*s*)	57.4	3.47 (*s*)	57.4	3.42 (*s*)	57.5
	α-L-dig		α-L-dig		α-L-dig	
1^{II}	4.99 (d, $J = 3.6$)	101.7	4.96 (d, $J = 3.1$)	101.7	4.96 (br. *s*)	101.7
2^{II}	1.73–1.75 (*m*), 2.19–2.21 (*m*)	32.6	1.74–1.76 (*m*), 2.24–2.26 (*m*)	32.6	1.78–1.80 (*m*), 2.26–2.28 (*m*)	32.6
3^{II}	3.92–3.94 (*m*)	75.1	3.92–3.94 (*m*)	75.2	3.92–3.94 (*m*)	74.9
4^{II}	3.59–3.61 (*m*)	75.3	3.59–3.61 (*m*)	75.3	3.60–3.62 (*m*)	75.0
5^{II}	3.96–3.98 (*m*)	68.2	3.95–3.97 (*m*)	68.2	3.95–3.97 (*m*)	68.3
6^{II}	1.26 (d, $J = 6.4$)	17.8	1.24 (d, $J = 6.5$)	17.8	1.23 (d, $J = 6.5$)	17.8
MeO	3.43 (*s*)	56.1	3.39 (*s*)	56.1	3.39 (*s*)	56.1
	β-D-cym		β-D-cym		β-D-cym	
1^{III}	4.82 (d, $J = 9.6$)	100.3	4.79 (d, $J = 9.5$)	100.3	4.79 (d, $J = 9.5$)	100.3
2^{III}	1.62–1.64 (*m*), 2.23–2.25 (*m*)	35.6	1.61–1.63 (*m*), 2.24–2.26 (*m*)	35.7	1.61–1.63 (*m*), 2.26–2.28 (*m*)	35.7
3^{III}	3.70–3.72 (*m*)	78.6	3.68–3.70 (*m*)	78.7	3.63–3.65 (*m*)	78.7
4^{III}	3.24–3.26 (*m*)	83.7	3.22–3.24 (*m*)	83.1	3.22–3.24 (*m*)	83.2
5^{III}	3.86–3.88 (*m*)	70.2	3.83–3.85 (*m*)	70.1	3.84–3.86 (*m*)	70.2
6^{III}	1.23 (d, $J = 6.0$)	18.7	1.22 (d, $J = 6.0$)	18.7	1.22 (d, $J = 6.0$)	18.7
MeO	3.48 (*s*)	58.4	3.48 (*s*)	58.3	3.48 (*s*)	58.3
	β-glu		α-L-cym		α-L-cym	
1^{IV}	4.36 (d, $J = 7.8$)	106.3	4.83 (d, $J = 3.0$)	99.9	4.83 (d, $J = 3.0$)	100.0
2^{IV}	3.18–3.20 (*m*)	75.0	1.78–1.80 (*m*), 2.25–2.27 (*m*)	32.2	1.78–1.80 (*m*), 2.26–2.28 (*m*)	32.3
3^{IV}	3.26–3.28 (*m*)	78.0	3.78–3.80 (*m*)	74.0	3.81–3.83 (*m*)	74.1
4^{IV}	3.32–3.34 (*m*)	71.7	3.56–3.58 (*m*)	78.6	3.53–3.55 (*m*)	79.1
5^{IV}	3.24–3.26 (*m*)	77.9	4.28–4.30 (*m*)	65.9	4.26–4.28 (*m*)	66.0
6^{IV}	3.64–3.66 (*m*), 3.87–3.89 (*m*)	62.8	1.25 (d, $J = 6.5$)	18.4	1.25 (d, $J = 6.5$)	18.4
MeO			3.42 (*s*)	56.8	3.39 (*s*)	56.8
			β-glu		β-glu	
1^{V}			4.40 (d, $J = 7.7$)	101.7	4.41 (d, $J = 8.0$)	101.8
2^{V}			3.19–3.21 (*m*)	75.0	3.20–3.22 (*m*)	75.4
3^{V}			3.26–3.28 (*m*)	78.1	3.26–3.28 (*m*)	78.5
4^{V}			3.35–3.37 (*m*)	71.6	3.37–3.39 (*m*)	71.0
5^{V}			3.25–3.27 (*m*)	77.8	3.23–3.25 (*m*)	77.1
6^{V}			3.63–3.65 (*m*), 3.83–3.85 (*m*)	62.8	3.65–3.67 (*m*), 3.85–3.87 (*m*)	62.1

Table 2 (cont.)

Position	1		2		3	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
					β-glu	
1^{VI}					4.37 (*d*, $J = 7.8$)	104.6
2^{VI}					3.21–3.23 (*m*)	74.9
3^{VI}					3.36–3.38 (*m*)	78.0
4^{VI}					3.44–3.46 (*m*)	71.3
5^{VI}					3.30–3.32 (*m*)	77.8
6^{VI}					3.65–3.67 (*m*), 3.88–3.90 (*m*)	62.4

[a]) cym = Cymaropyranosyl; dig = diginopyranosyl; glu = glucopyranosyl.

($[M+\mathrm{Cl}]^-$)). Acidic hydrolysis of **3** afforded a mixture of sugars, which were identified as cymarose, diginose, and glucose by TLC comparison with authentic samples. The ^{1}H- and ^{13}C-NMR data indicated that **3** possessed the same aglycone as **2**. Comparison of the spectroscopic data of the sugar moieties with those of **2** revealed the presence of one additional glucopyranosyl moiety in **3**. The sugar sequence and the interglycosidic linkages were determined by HMBC spectrum, in which correlations between H–C(1^{II}) at δ(H) 4.96 and C(4^{I}) at δ(C) 82.7, between H–C(1^{III}) at δ(H) 4.79 and C(4^{II}) at δ(C) 74.9, between H–C(1^{IV}) at δ(H) 4.83 and C(4^{III}) at δ(C) 83.2, between H–C(1^{V}) at δ(H) 4.41 and C(4^{IV}) at δ(C) 79.1, and between H–C(1^{VI}) at δ(H) 4.37 and C(4^{V}) at δ(C) 81.0 were observed. Thus, compound **3** was determined to be 20-*O*-(2-methylbutanoyl)penupogenin-3-*O*-β-glucopyranosyl-(1 → 4)-β-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranoside, named cyanoauriculoside E.

The three known compounds were identified as wilfoside K1N (**4**) [16], cynanauriculoside II (**5**) [17], and auriculoside IV (**6**) [10] by comparison of their spectroscopic data with those reported in the literature. They have been already reported from *Cynanchum auriculatum*.

Experimental Part

General. HPLC: *Ultimate 3000* HPLC system; *Ultimate 3000 Variable Wavelength*; *Ultimate 3000* pump; column, *Waters 5C$_{18}$-MS-II* (10 × 250 mm, 5 µm). Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Co., Ltd.*, P. R. China) and *C$_{18}$* reversed-phase (RP) silica gel (*YMC Co., Ltd.*, Japan). TLC: Precoated silica gel *GF$_{254}$* plates (*Qingdao Haiyang Chemical Co., Ltd.*, P. R. China). ^{1}H- and ^{13}C-NMR spectra: *Bruker DRX-500* instrument; δ in ppm rel. to Me_4Si as internal standard (= 0 ppm), *J* in Hz. HR-ESI-MS: *Finnigan LCQ-Deca* and *Waters/Micromass Q-Tof-Ultima* mass spectrometer in *m/z* (rel. int.).

Plant Material. The roots of the *C. auriculatum* were collected from Jishou, Hunan Province, P. R. China, in September 2007, and were identified by Prof. *Ding-Rong Wan* (College of Pharmacy, South Central University for Nationalities). The voucher specimen was deposited with the Herbarium of College of Pharmacy, South Central University for Nationalities.

Extraction and Isolation. The air-dried roots of *C. auriculatum* (4 kg) were powdered and extracted with 95% EtOH at r.t. for three times (3 × 6 l; 48, 48, and 24 h). The EtOH extract (0.6 kg) was suspended in H_2O (1.6 l) and then successively partitioned with petroleum ether (PE; 1.5 l × 3), $CHCl_3$

(1.5 l × 3), AcOEt (1.5 l × 3), and BuOH (1.5 l × 3). The $CHCl_3$ extract (195 g) was chromatographed on SiO_2 column using gradient solvents of cyclohexane/AcOEt (100 : 0 → 0 : 100) and AcOEt/MeOH (100 : 0 → 0 : 100) to yield seven fractions, *Frs. 1–7*. *Fr. 5* (7.9 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 9 : 1 → 0 : 100) to afford three subfractions, *Frs. 5.1–5.3*. *Fr. 5.3* (2.5 g) was subjected to CC (*ODS*; H_2O/MeOH 9 : 1 → 1 : 9) to afford five subfractions, *Frs. 5.3.1–5.3.5*. *Fr. 5.3.1* was purified by semi-prep. HPLC (MeOH/H_2O 82 : 18; 3 ml/min) to yield **1** (25 mg, t_R 24.6 min). *Fr. 5.3.4* was purified by semi-prep. HPLC (MeOH/H_2O 85 : 15; 3 ml/min) to yield **2** (37 mg, t_R 16.5 min). *Fr. 5.3.5* was purified by semi-prep. HPLC (MeOH/H_2O 85 : 15; 3 ml/min) to yield **3** (27 mg, t_R 14.9 min). *Fr. 2* (4.3 g) was repeatedly chromatographed on SiO_2, then purified on a *RP-C_{18}* SiO_2 column to afford **4** (28 mg) and **6** (32 mg). *Fr. 7* (18.0 g) was subjected to CC (SiO_2; AcOEt/MeOH 100 : 0 → 0 : 100) to afford five subfractions, *Frs. 7.1–7.5*. *Fr. 7.1* (2.1 g) was subjected to CC (*ODS*; H_2O/MeOH 9 : 1 → 1 : 9) to afford five subfractions, *Frs. 7.1.1–7.1.5*. *Fr. 7.1.1* was purified by semi-prep. HPLC (MeOH/H_2O 80 : 20; 3 ml/min) to yield **5** (38 mg, t_R 16.9 min).

Acid Hydrolysis. A soln. of **1**, **2**, and **3** (each 5 mg) in MeOH was treated with 0.05M HCl/4-dioxane 1 : 1 (1 ml) at 60° for 1.5 h, resp. [13][19]. After removing dioxane, the soln. was extracted with AcOEt (3 × 2 ml). The aq. layer was neutralized by NaOH and concentrated under reduced pressure to give the sugar fraction. The presence of the monosaccharides in the hydrolysates of each compound was confirmed by TLC comparison with authentic sugars. Diginose, cymarose, and glucose were detected from the hydrolysates of compounds **1**–**3**. The R_f values of diginose and cymarose were 0.66 and 0.76 with $CHCl_3$/MeOH 95 : 5, 0.18 and 0.23 with PE/Me_2CO 3 : 1, resp.

Cyanoauriculoside C (*=20-*O*-(2-Methylbutanoyl)penupogenin-3-*O*-β-glucopyranosyl-(1 → 4)-β-*D*-cymaropyranosyl-(1 → 4)-α-*L*-diginopyranosyl-(1 → 4)-β-*D*-cymaropyranoside = (3β,12β,14β,17α,20S)-3-{[β-Glucopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-*ribo*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-α-*L*-*lyxo*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-*ribo*-hexopyranosyl]oxy}-8,14,17-trihydroxy-12-{[(2*E*)-3-phenylprop-2-enoyl]oxy}pregn-5-en-20-yl 2-Methylbutanoate*; **1**). Colorless amorphous powder. $[\alpha]_D^{20} = +11.4$ ($c = 0.30$, MeOH). UV (MeOH): 278 (4.40), 216 (4.26), 203 (4.33). ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 1189.6166 ($[M - H]^-$, $C_{62}H_{93}O_{22}^-$; calc. 1189.6158).

Cyanoauriculoside D (*=20-*O*-(2-Methylbutanoyl)penupogenin-3-*O*-β-glucopyranosyl-(1 → 4)-α-*L*-cymaropyranosyl-(1 → 4)-β-*D*-cymaropyranosyl-(1 → 4)-α-*L*-diginopyranosyl-(1 → 4)-β-*D*-cymaropyranoside = (3β,12β,14β,17α,20*S*)-3-{[β-glucopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-α-*L*-*arabino*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-*ribo*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-α-*L*-*lyxo*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-*ribo*-hexopyranosyl]oxy}-8,14,17-trihydroxy-12-{[(2*E*)-3-phenylprop-2-enoyl]oxy}pregn-5-en-20-yl 2-Methylbutanoate*; **2**). Colorless amorphous powder. $[\alpha]_D^{20} = -18.3$ ($c = 0.14$, MeOH). UV (MeOH): 278 (4.38), 218 (4.22), 203 (4.30). ^1H- and ^{13}C- NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 1369.6713 ($[M + Cl]^-$, $C_{69}H_{106}ClO_{25}^-$; calc. 1369.6711).

Cyanoauriculoside E (*=20-*O*-(2-Methylbutanoyl)penupogenin-3-*O*-β-glucopyranosyl-(1 → 4)-β-glucopyranosyl-(1 → 4)-α-*L*-cymaropyranosyl-(1 → 4)-β-*D*-cymaropyranosyl-(1 → 4)-α-*L*-diginopyranosyl-(1 → 4)-β-*D*-cymaropyranoside = (3β,12β,14β,17α,20*S*)-3-{[β-Glucopyranosyl-(1 → 4)-β-glucopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-α-*L*-*arabino*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-*ribo*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-α-*L*-*lyxo*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-*ribo*-hexopyranosyl]oxy}-8,14,17-trihydroxy-12-{[(2*E*)-3-phenylprop-2-enoyl]oxy}pregn-5-en-20-yl 2-Methylbutanoate*; **3**). Colorless amorphous powder. $[\alpha]_D^{20} = +7.8$ ($c = 0.20$, MeOH). UV (MeOH): 278 (4.37), 216 (4.24), 203 (4.30). ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 1531.7179 ($[M + Cl]^-$, $C_{75}H_{116}ClO_{30}^-$; calc. 1531.7239).

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REFERENCES

[1] R.-S. Zhang, Y.-P. Ye, Y.-M. Shen, H.-L. Liang, *Tetrahedron* **2000**, *56*, 3875.
[2] K. Y. Lee, S. H. Sung, Y. C. Kim, *Helv. Chim. Acta* **2003**, *86*, 474.
[3] X. Li, H. Sun, Y. Ye, F. Chen, Y. Pan, *Steroids* **2006**, *71*, 61.
[4] S.-Q. Luo, L.-Z. Lin, G. A. Cordell, X. Liang, M. E. Johnson, *Phytochemistry* **1993**, *34*, 1615.
[5] K. Hayashi, K. Wada, H. Mitsuhashi, H. Bando, M. Takase, S. Terada, Y. Koide, T. Aiba, T. Narita, D. Mizuno, *Chem. Pharm. Bull.* **1980**, *28*, 1954.
[6] Q. Z. Mu, J. L. Lu, Q. L. Zhou, *Sci. China, Ser. B* **1985**, *8*, 724.
[7] Y. E. Zhang, J. L. Yuan, W. P. Ding, *Acta Pharm. Sin.* **1994**, *29*, 281.
[8] S.-L. Li, H. Tan, Y. M. Shen, K. Kawazoe, X.-J. Hao, *J. Nat. Prod.* **2004**, *67*, 82.
[9] Jiangsu College of New Medicine, 'A Dictionary of the Traditional Chinese Medicines', People's Hygiene Publisher, Shanghai, 2004, p. 732.
[10] X.-J. Gu, N. Yao, S.-H. Qian, Y.-B. Li, P. Li, *Helv. Chim. Acta* **2009**, *92*, 88.
[11] J. Li, S. Kadota, Y. Kawata, M. Hattori, G.-J. Xu, T. Namba, *Chem. Pharm. Bull.* **1992**, *40*, 3133.
[12] Y. M. Xu, R. A. Ren, *China J. Chin. Mat. Med.* **1991**, *16*, 164.
[13] H. Gan, W.-J. Xiang, L. Ma, L.-H. Hu, *Helv. Chim. Acta* **2008**, *91*, 2222.
[14] F. Abe, Y. Mori, H. Okabe, T. Yamauchi, *Chem. Pharm. Bull.* **1994**, *42*, 1777.
[15] H. Bai, W. Li, K. Koike, *Steroids* **2008**, *73*, 96.
[16] B. Y. Hwang, Y. H. Kim, J. S. Ro, K. S. Lee, J. J. Lee, *Arch. Pharmacal Res.* **1999**, *22*, 72.
[17] Y.-B. Zhao, H.-P. He, C.-H. Lu, Q.-Z. Mu, Y.-M. Shen, X.-J. Hao, *Steroids* **2006**, *71*, 935.
[18] S. Tsukamoto, K. Hayashi, H. Mitsuhashi, *Tetrahedron* **1985**, *41*, 927.
[19] X. S. Yao, 'Natural Medicinal Chemistry', People's Health Press, Beijing, 2006, p. 323.

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