Four New C_{21} Steroidal Glycosides from the Roots of *Cynanchum auriculatum*

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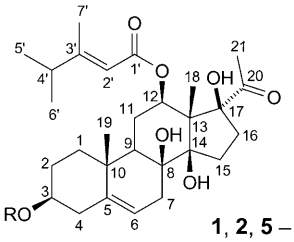
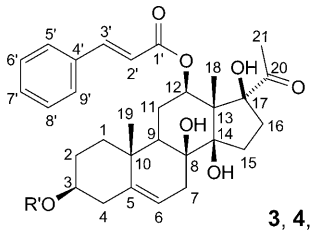
Four new C_{21} steroidal glycosides with an acyl group at C(12) and a straight sugar chain at C(3), namely auriculosides I–IV (**1**–**4**, resp.), along with seven known steroidal derivatives, were isolated from the roots of *Cynanchum auriculatum* ROYLE EX WIGHT. Their structures were established on the basis of spectroscopic evidences and chemical methods. The known constituents were identified as wilfoside C_1N (**5**), wilfoside C_3N (**6**), caudatin (**7**), cynanauriculoside I (**8**), cynanauriculoside II (**9**), wilfoside K_1N (**10**), and kidjoranin (**11**).

Introduction. – The root of *Cynanchum auriculatum* ROYLE EX WIGHT (Asclepiadaceae), commonly known as '*bai shou wu*', is a famous traditional Chinese medicine (TCM), and has widely been used for the treatment of geriatric diseases and prolonging life [1]. Steroidal glycosides are considered as the major bioactive constituents of this medicine. In recent years, they have attracted much attention for their bioactivities, such as antitumor, cytotoxicity, antifungal, acetylcholine esterase inhibition, and antiosteoporosis [2–6]. Currently, four C_{21} steroidal aglycones and 14 glycosides have been reported in the roots of *C. auriculatum* [7–13]. As part of our investigation on this plant, we herein report the isolation and elucidation of four new C_{21} steroidal glycosides, auriculosides I–IV (**1**–**4**, resp.), together with seven known steroidal derivatives, **5**–**11**, from the roots of this plant.

Results and Discussion. – Compounds **1**–**11** were isolated from the EtOH extract of the roots of *C. auriculatum* through repeated column chromatography. All of them showed positive *Libermann–Buchard* and *Keller–Kiliani* reactions, indicating the presence of a steroidal skeleton with a 2-deoxysugar moiety. Spectroscopic analysis demonstrated that all the glycosides had a pregnane skeleton with an acyl group at C(12) and a straight sugar chain consisting of three to seven sugar units at C(3) of the aglycone. Compounds **5**–**11** were identified as wilfoside C_1N (**5**) [14][15], wilfoside C_3N (**6**) [14], caudatin (**7**) [15], cynanauriculoside I (**8**) [11], cynanauriculoside II (**9**) [11], wilfoside K_1N (**10**) [15], and kidjoranin (**11**) [15], respectively, based on the comparison of their physico-chemical and spectral data with those reported in the literature.

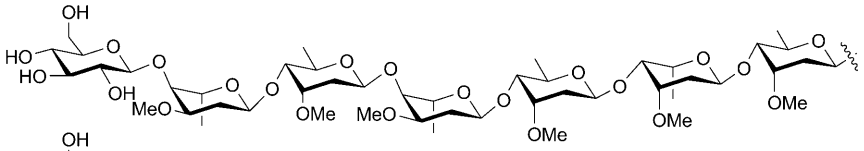


1, **2**, **5** – **7**



3, **4**, **8** – **11**

1 R =



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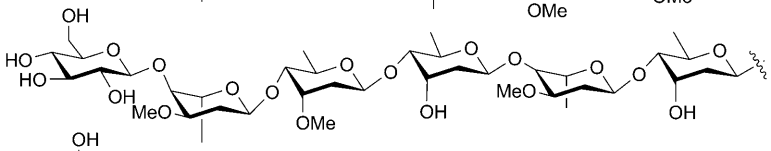

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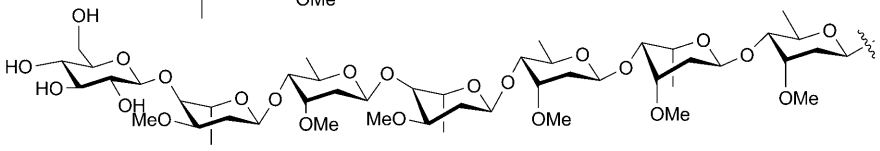

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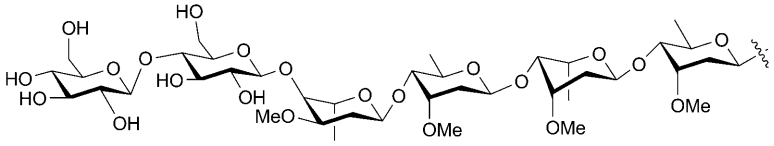

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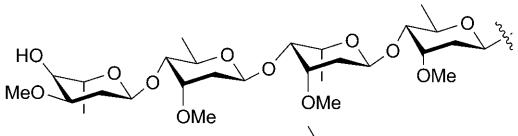

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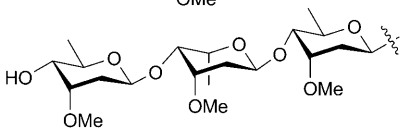
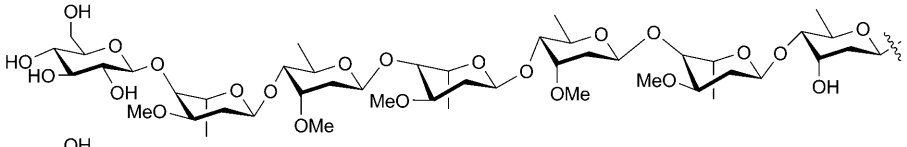


7 R = H

8 R' =



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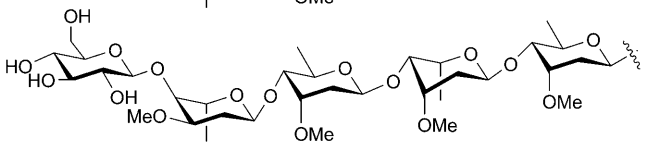
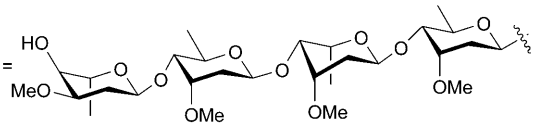


10 R' =



11 R' = H

Compound **1**, obtained as a white amorphous powder, had the molecular formula $C_{76}H_{124}O_{30}$ according to HR-ESI-MS (*m/z* 1539.6566 ($[M+Na]^+$)). The detailed analysis of the ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), HMBC, HSQC, ROESY, and TOCSY data, and comparison with literature data established the structure of **1** as caudatin 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranoside, named auriculoside I.

Acidic hydrolysis of **1** with 5% HCl afforded a sugar mixture of cymarose, diginose and glucose, and the aglycone, which was proved to be caudatin (**7**) by co-TLC comparison with authentic samples. Inspection of the NMR spectral data of **1** (*Table 1*) showed that besides the signals arising from the aglycone, it contained seven anomeric C-atoms with signals at δ(C) 96.2 (C(1^{I})), 100.9 (C(1^{II})), 99.4 (C(1^{III})), 98.9 (C(1^{IV})), 95.7 (C(1^{V})), 99.1 (C(1^{VI})), and 102.4 (C(1^{VII})), corresponding to seven anomeric H-atom signals at δ(H) 5.21 (*d*, *J* = 8.5), 5.13 (*d*, *J* = 3.4), 5.08 (*d*, *J* = 10.4), 4.92 (*d*, *J* = 3.1), 5.21 (*d*, *J* = 8.5), 4.92 (*d*, *J* = 3.1), and 4.98 (*d*, *J* = 7.7), respectively. Signals of each sugar unit (*Table 2*) were assigned by HSQC and TOCSY analyses, suggesting the existence of three β-D-cymaropyranosyl, one α-L-diginopyranosyl, two α-L-cymaropyranosyl, and one β-D-glucopyranosyl units. The absolute configuration of the sugar residues were tentatively assigned according to the configuration previously found for these monosaccharides in the family Asclepiadaceae and by comparison of their NMR spectral data [14][15]. Compared to the chemical shifts of **7**, glycosylation shift effects on the signals of C(2) (−2.2 ppm), C(3) (+6.2 ppm), and C(4) (−3.9 ppm) exhibited that the attachment of the sugar chain was at C(3) of the aglycone. The location and sequence of the sugar moieties was demonstrated by ROESY and HMBC data. A ROESY correlation was observed between H−C(3) at δ(H) 3.84–3.86 and H−C(1^{I}) at δ(H) 5.21, and HMBC correlations were observed between H−C(3) at δ(H) 3.84–3.86 and C(1^{I}) at δ(C) 96.2, as well as between H−C(1^{I}) at δ(H) 5.21 and C(3) at δ(C) 77.7, confirming that the β-D-Cym unit was attached at C(3)−O of the aglycone. HMBC Cross-peaks were detected from H−C(1^{II}) at δ(H) 5.13 to C(4^{I}) at δ(C) 82.4, from H−C(1^{III}) at δ(H) 5.08 to C(4^{II}) at δ(C) 74.7, from H−C(1^{IV}) at δ(H) 4.92 to C(4^{III}) at δ(C) 82.5, from H−C(1^{V}) at δ(H) 5.21 to C(4^{IV}) at δ(C) 77.5, from H−C(1^{VI}) at δ(H) 4.92 to C(4^{V}) at δ(C) 82.4, and from H−C(1^{VII}) at δ(H) 4.98 to C(4^{VI}) at δ(C) 79.0, revealing that the sugar chain was a β-D-Glc-(1 → 4)-α-L-Cym-(1 → 4)-β-D-Cym-(1 → 4)-α-L-Cym-(1 → 4)-β-D-Cym-(1 → 4)-α-L-Dig-(1 → 4)-β-D-Cym unit (*Fig. 1,a*).

Compound **2**, obtained as a white amorphous powder, was shown to have the molecular formula $C_{67}H_{108}O_{27}$ based on HR-ESI-MS (*m/z* 1367.4348 ($[M+Na]^+$)). After analysis of the ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), HMBC, HSQC, ROESY, and TOCSY data, the structure of **2** was established as caudatin 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside, named auriculoside II.

The acidic hydrolysis of **2** gave the same aglycone as in **1**. Its sugar components were identified by TLC comparison with standard samples. Six anomeric H-atom signals at δ(H) 5.18 (*d*, *J* = 9.3), 5.04 (*d*, *J* = 3.0), 5.44 (*d*, *J* = 9.2), 5.09 (*d*, *J* = 9.6), 4.92 (*d*, *J* = 3.4), and 4.98 (*d*, *J* = 7.7), and six anomeric C-atoms at δ(C) 95.6 (C(1^{I})), 98.9 (C(1^{II})), 96.4 (C(1^{III})), 99.5 (C(1^{IV})), 99.3 (C(1^{V})), and 102.4 (C(1^{VI})) were

Table 1. *^1H- (500 MHz) and ^{13}C-NMR (125 MHz) Data for the Aglycone Moieties of* **1**–**4** *in (D$_5$)Pyridine*[a])[b]). δ in ppm, J in Hz.

	1		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.08–1.10 (*m*), 1.80–1.83 (*m*)	39.0 (*t*)	1.07–1.09 (*m*), 1.80–1.83 (*m*)	39.0 (*t*)	1.09–1.11 (*m*), 1.80–1.83 (*m*)	38.2 (*t*)	1.09–1.11 (*m*), 1.80–1.83 (*m*)	39.0 (*t*)
2	1.78–1.80 (*m*), 2.06–2.08 (*m*)	29.9 (*t*)	1.76–1.78 (*m*), 2.05–2.07 (*m*)	29.9 (*t*)	1.79–1.81 (*m*), 2.08–2.10 (*m*)	29.1 (*t*)	1.79–1.81 (*m*), 2.07–2.09 (*m*)	29.9 (*t*)
3	3.84–3.86 (*m*)	77.7 (*d*)	3.84–3.86 (*m*)	77.7 (*d*)	3.81–3.83 (*m*)	76.8 (*d*)	3.81–3.83 (*m*)	77.7 (*d*)
4	2.40–2.43 (*m*), 2.51–2.53 (*m*)	39.3 (*t*)	2.40–2.42 (*m*), 2.51–2.53 (*m*)	39.3 (*t*)	2.40–2.42 (*m*), 2.52–2.54 (*m*)	38.5 (*t*)	2.41–2.43 (*m*), 2.52–2.54 (*m*)	39.4 (*t*)
5		139.4 (*s*)		139.4 (*s*)		138.7 (*s*)		139.4 (*s*)
6	5.28 (br. *s*)	119.2 (*d*)	5.26 (br. *s*)	119.1 (*d*)	5.28 (br. *s*)	118.3 (*d*)	5.30 (br. *s*)	119.2 (*d*)
7	2.33–2.35 (*m*), 2.42–2.45 (*m*)	34.9 (*t*)	2.30–2.32 (*m*), 2.41–2.43 (*m*)	34.8 (*t*)	2.32–2.35 (*m*), 2.44–2.46 (*m*)	34.1 (*t*)	2.33–2.36 (*m*), 2.44–2.47 (*m*)	34.9 (*t*)
8	5.14 (br. *s*, OH)	74.3 (*s*)	5.15 (br. *s*, OH)	74.4 (*s*)	5.14 (br. *s*, OH)	73.6 (*s*)	5.15 (br. *s*, OH)	74.4 (*s*)
9	1.70–1.72 (*m*)	44.6 (*d*)	1.69–1.71 (*m*)	44.7 (*d*)	1.72–1.74 (*m*)	43.8 (*d*)	1.73–1.75 (*m*)	44.6 (*d*)
10		37.4 (*s*)		37.4 (*s*)		36.7 (*s*)		37.5 (*s*)
11	2.13–2.15 (*m*), 2.22–2.24 (*m*)	25.1 (*t*)	2.11–2.13 (*m*), 2.20–2.22 (*m*)	25.1 (*t*)	2.17–2.19 (*m*), 2.29–2.31 (*m*)	24.3 (*t*)	2.18–2.20 (*m*), 2.30–2.33 (*m*)	25.1 (*t*)
12	5.01–5.03 (*m*)	72.6 (*d*)	5.00–5.02 (*m*)	72.7 (*d*)	5.17–5.19 (*m*)	72.9 (*d*)	5.17–5.19 (*m*)	73.7 (*d*)
13		58.0 (*s*)		58.0 (*s*)		57.4 (*s*)		58.2 (*s*)
14	6.17 (br. *s*, OH)	89.5 (*s*)	6.20 (br. *s*, OH)	89.5 (*s*)	6.19 (br. *s*, OH)	88.7 (*s*)	6.16 (br. *s*, OH)	89.5 (*s*)
15	2.09–2.11 (*m*), 2.09–2.11 (*m*)	33.9 (*t*)	2.06–2.08 (*m*), 2.06–2.08 (*m*)	33.8 (*t*)	2.09–2.11 (*m*), 2.09–2.11 (*m*)	33.1 (*t*)	2.10–2.13 (*m*), 2.10–2.13 (*m*)	33.9 (*t*)
16	2.01–2.03 (*m*), 3.25–3.27 (*m*)	33.0 (*t*)	2.00–2.02 (*m*), 3.25–3.27 (*m*)	33.0 (*t*)	2.01–2.03 (*m*), 3.25–3.27 (*m*)	32.3 (*t*)	2.01–2.03 (*m*), 3.25–3.27 (*m*)	33.1 (*t*)
17	6.48 (br. *s*, OH)	92.4 (*s*)	6.47 (br. *s*, OH)	92.4 (*s*)	6.51 (br. *s*, OH)	91.6 (*s*)	6.47 (br. *s*, OH)	92.5 (*s*)
18	1.97 (*s*)	10.7 (*q*)	1.96 (*s*)	10.7 (*q*)	2.01 (*s*)	9.9 (*q*)	2.02 (*s*)	10.7 (*q*)
19	1.31 (*s*)	18.2 (*q*)	1.30 (*s*)	18.2 (*q*)	1.32 (*s*)	17.8 (*q*)	1.32 (*s*)	18.3 (*q*)
20		209.3 (*s*)		209.3 (*s*)		208.9 (*s*)		209.7 (*s*)
21	2.48 (*s*)	27.5 (*q*)	2.49 (*s*)	27.5 (*q*)	2.48 (*s*)	26.9 (*q*)	2.48 (*s*)	27.6 (*q*)
1′		166.0 (*s*)		166.0 (*s*)		165.0 (*s*)		165.8 (*s*)
2′	5.85 (*s*)	114.2 (*d*)	5.85 (*s*)	114.2 (*d*)	6.80 (*d*, J = 16.1)	118.5 (*d*)	6.80 (*d*, J = 16.0)	119.3 (*d*)
3′		165.3 (*s*)		165.3 (*s*)	7.98 (*d*, J = 16.2)	144.1 (*d*)	7.99 (*d*, J = 16.0)	144.9 (*d*)
4′	2.25–2.27 (*m*)	38.2 (*d*)	2.25–2.27 (*m*)	38.2 (*d*)		134.3 (*s*)		135.1 (*s*)
5′	0.93 (*d*, J = 7.4)	21.0 (*q*)	0.93 (*d*, J = 7.3)	21.0 (*q*)	7.60–7.62 (*m*)	127.8 (*d*)	7.61–7.63 (*m*)	128.6 (*d*)
6′	0.95 (*d*, J = 7.4)	20.9 (*q*)	0.95 (*d*, J = 7.1)	20.9 (*q*)	7.31–7.33 (*m*)	128.5 (*d*)	7.32–7.34 (*m*)	129.3 (*d*)
7′	2.26 (*s*)	16.5 (*q*)	2.26 (*s*)	16.5 (*q*)	7.31–7.33 (*m*)	129.8 (*d*)	7.32–7.34 (*m*)	130.6 (*d*)
8′					7.31–7.33 (*m*)	128.5 (*d*)	7.32–7.34 (*m*)	129.3 (*d*)
9′					7.60–7.62 (*m*)	127.8 (*d*)	7.61–7.63 (*m*)	128.6 (*d*)

[a]) Me_4Si Was used as an internal standard in all experiments. [b]) Assignments were based on HMBC, HSQC, ROESY, and TOCSY experiments; due to severe overlapping, only detectable J values are reported.

observed in the ^{1}H- and ^{13}C-NMR spectra, respectively (*Table 2*). Based on the HSQC and TOCSY experiments, the data of these six sugars were assigned to be two β-D-digitoxopyranosyl, two α-L-cymaropyranosyl, one β-D-cymaropyranosyl, and one β-D-

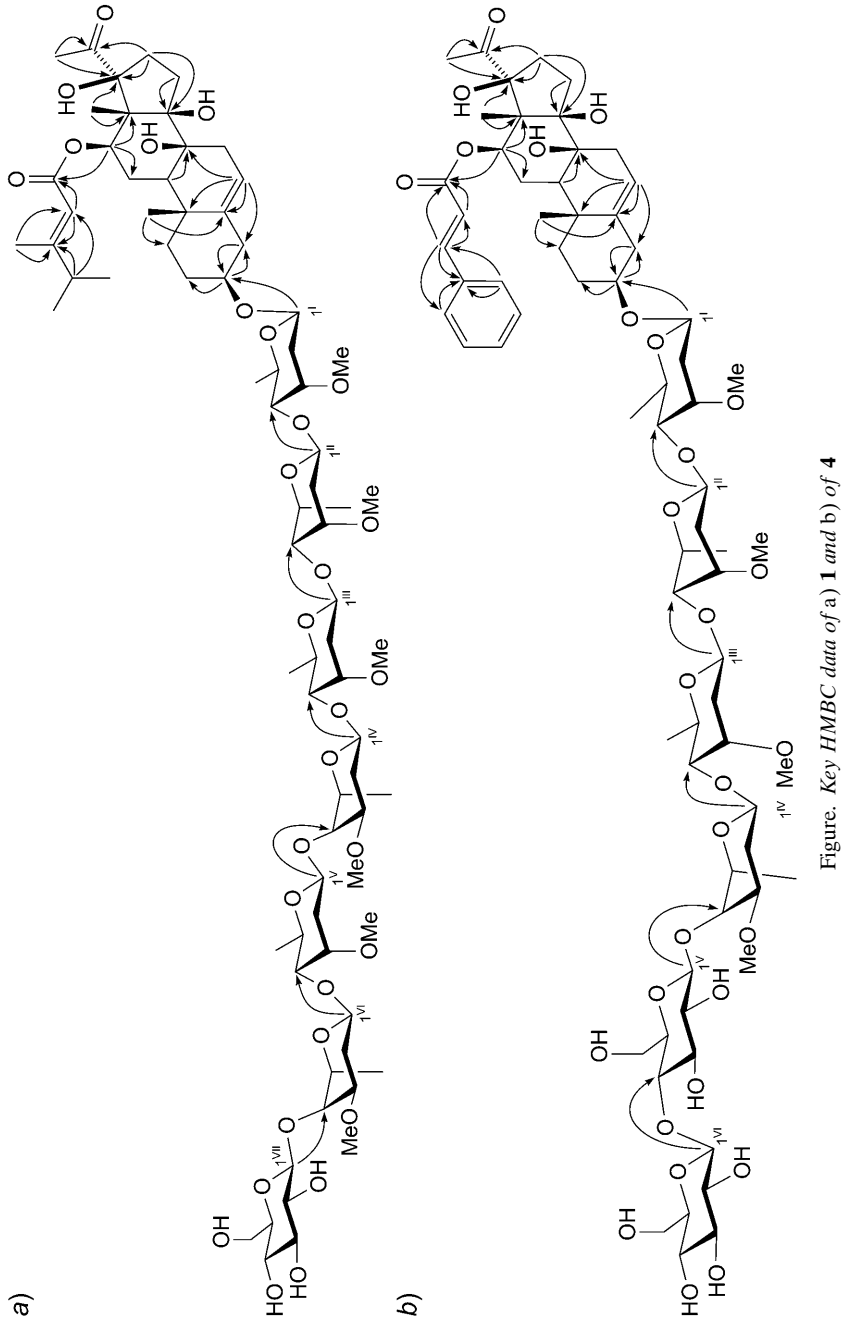


Figure. *Key HMBC data of* a) **1** *and* b) *of* **4**

Table 2. *^1H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data for the Sugar Moieties of* **1**–**4** *in (D$_5$)Pyridine*[a])[b]). δ in ppm, J in Hz.

	1		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
	β-D-Cym		β-D-Dit		β-D-Cym		β-D-Cym	
1^{I}	5.21 (*d*, $J = 8.5$)	96.2 (*d*)	5.18 (*d*, $J = 9.3$)	95.6 (*d*)	5.22 (*d*, $J = 9.1$)	95.4 (*d*)	5.21 (*d*, $J = 9.2$)	96.2 (*d*)
2^{I}	1.79–1.81 (*m*), 2.22–2.24 (*m*)	35.4 (*t*)	1.78–1.80 (*m*), 2.22–2.24 (*m*)	39.1 (*t*)	1.75–1.77 (*m*), 2.27–2.29 (*m*)	35.9 (*t*)	1.82–1.84 (*m*), 2.40–2.42 (*m*)	35.5 (*t*)
3^{I}	3.84–3.86 (*m*)	77.8 (*d*)	4.44–4.46 (*m*)	65.2 (*d*)	3.87–3.88 (*m*)	76.9 (*d*)	3.88–3.90 (*m*)	77.5 (*d*)
4^{I}	3.41–3.43 (*m*)	82.4 (*d*)	3.47–3.49 (*m*)	83.5 (*d*)	3.41–3.43 (*m*)	81.7 (*d*)	3.45–3.47 (*m*)	82.4 (*d*)
5^{I}	4.14–4.16 (*m*)	69.3 (*d*)	4.22–4.24 (*m*)	68.5 (*d*)	4.14–4.16 (*m*)	68.6 (*d*)	4.15–4.17 (*m*)	69.3 (*d*)
6^{I}	1.45 (*d*, $J = 6.4$)	18.8 (*q*)	1.38 (*d*, $J = 6.2$)	18.4 (*q*)	1.45 (*d*, $J = 6.4$)	18.0 (*q*)	1.36 (*d*, $J = 6.1$)	18.8 (*q*)
MeO^{I}	3.50 (*s*)	57.3 (*q*)			3.52 (*s*)	56.5 (*q*)	3.51 (*s*)	57.3 (*q*)
	α-L-Dig		α-L-Cym		α-L-Dig		α-L-Dig	
1^{II}	5.13 (*d*, $J = 3.4$)	100.9 (*d*)	5.04 (*d*, $J = 3.0$)	98.9 (*d*)	5.14 (*d*, $J = 3.3$)	100.1 (*d*)	5.14 (*d*, $J = 3.3$)	100.9 (*d*)
2^{II}	1.98–2.01 (*m*), 2.30–2.33 (*m*)	32.5 (*t*)	1.76–1.78 (*m*), 2.26–2.28 (*m*)	32.3 (*t*)	1.79–1.81 (*m*), 2.32–2.34 (*m*)	31.7 (*t*)	1.77–1.79 (*m*), 2.30–2.33 (*m*)	32.5 (*t*)
3^{II}	4.00–4.02 (*m*)	74.0 (*d*)	3.70–3.72 (*m*)	73.4 (*d*)	4.00–4.02 (*m*)	73.2 (*d*)	4.00–4.02 (*m*)	74.0 (*d*)
4^{II}	3.78–3.40 (*m*)	74.7 (*d*)	3.83–3.85 (*m*)	77.3 (*d*)	3.78–3.40 (*m*)	73.9 (*d*)	3.80–3.82 (*m*)	74.7 (*d*)
5^{II}	4.20–4.22 (*m*)	67.6 (*d*)	4.64–4.66 (*m*)	65.3 (*d*)	4.20–4.22 (*m*)	66.8 (*d*)	4.21–4.23 (*m*)	67.6 (*d*)
6^{II}	1.39 (*d*, $J = 6.4$)	17.9 (*q*)	1.30 (*d*, $J = 6.1$)	18.2 (*q*)	1.40 (*d*, $J = 6.4$)	17.1 (*q*)	1.42 (*d*, $J = 6.1$)	17.9 (*q*)
MeO^{II}	3.40 (*s*)	55.4 (*q*)	3.40 (*s*)	56.8 (*q*)	3.40 (*s*)	54.6 (*q*)	3.41 (*s*)	55.4 (*q*)
	β-D-Cym		β-D-Dit		β-D-Cym		β-D-Cym	
1^{III}	5.08 (*d*, $J = 10.4$)	99.4 (*d*)	5.44 (*d*, $J = 9.2$)	96.4 (*d*)	5.09 (*d*, $J = 9.0$)	98.6 (*d*)	5.10 (*d*, $J = 9.8$)	99.5 (*d*)
2^{III}	1.78–1.80 (*m*), 2.28–2.30 (*m*)	36.3 (*t*)	1.75–1.77 (*m*), 2.22–2.24 (*m*)	36.7 (*t*)	1.80–1.82 (*m*), 2.27–2.29 (*m*)	35.5 (*t*)	1.80–1.82 (*m*), 2.40–2.42 (*m*)	36.4 (*t*)
3^{III}	3.84–3.86 (*m*)	77.8 (*d*)	4.59–4.61 (*m*)	67.5 (*d*)	3.86–3.88 (*m*)	77.0 (*d*)	3.85–3.87 (*m*)	77.8 (*d*)
4^{III}	3.41–3.43 (*m*)	82.5 (*d*)	3.47–3.49 (*m*)	83.5 (*d*)	3.41–3.43 (*m*)	81.6 (*d*)	3.41–3.43 (*m*)	82.5 (*d*)
5^{III}	4.15–4.17 (*m*)	69.4 (*d*)	4.14–4.16 (*m*)	69.4 (*d*)	4.19–4.21 (*m*)	68.5 (*d*)	4.20–4.22 (*m*)	69.5 (*d*)
6^{III}	1.47 (*d*, $J = 6.4$)	18.5 (*q*)	1.35 (*d*, $J = 6.2$)	18.4 (*q*)	1.44–1.46 (*m*)	17.7 (*q*)	1.36 (*d*, $J = 6.1$)	18.6 (*q*)
MeO^{III}	3.52 (*s*)	58.3 (*q*)			3.51 (*s*)	57.5 (*q*)	3.53 (*s*)	58.4 (*q*)
	α-L-Cym		β-D-Cym		α-L-Cym		α-L-Cym	
1^{IV}	4.92 (*d*, $J = 3.1$)	98.9 (*d*)	5.09 (*d*, $J = 9.6$)	99.5 (*d*)	4.93 (*d*, $J = 3.0$)	98.2 (*d*)	4.92 (*d*, $J = 3.2$)	99.0 (*d*)
2^{IV}	1.77–1.79 (*m*), 2.29–2.31 (*m*)	32.4 (*t*)	1.84–1.86 (*m*), 2.32–2.34 (*m*)	36.2 (*t*)	1.78–1.80 (*m*), 2.30–2.32 (*m*)	31.6 (*t*)	1.77–1.79 (*m*), 2.31–2.33 (*m*)	32.5 (*t*)
3^{IV}	3.70–3.72 (*m*)	73.4 (*d*)	3.82–3.84 (*m*)	77.8 (*d*)	3.71–3.73 (*m*)	72.6 (*d*)	3.87–3.89 (*m*)	73.5 (*d*)
4^{IV}	3.81–3.83 (*m*)	77.5 (*d*)	3.39–3.41 (*m*)	82.4 (*d*)	3.92–3.94 (*m*)	76.8 (*d*)	3.84–3.86 (*m*)	79.3 (*d*)
5^{IV}	4.65–4.68 (*m*)	65.3 (*d*)	4.14–4.16 (*m*)	69.3 (*d*)	4.60–4.63 (*m*)	64.8 (*d*)	4.65–4.67 (*m*)	65.2 (*d*)
6^{IV}	1.35 (*d*, $J = 6.4$)	18.4 (*q*)	1.50 (*d*, $J = 6.3$)	18.6 (*q*)	1.37–1.39 (*m*)	17.4 (*q*)	1.41 (*d*, $J = 6.1$)	18.5 (*q*)
MeO^{IV}	3.35 (*s*)	56.9 (*q*)	3.50 (*s*)	58.3 (*q*)	3.35 (*s*)	56.1 (*q*)	3.42 (*s*)	57.0 (*q*)
	β-D-Cym		α-L-Cym		β-D-Cym		β-D-Glc	
1^{V}	5.21 (*d*, $J = 8.5$)	95.7 (*d*)	4.92 (*d*, $J = 3.4$)	99.3 (*d*)	5.22 (*d*, $J = 9.1$)	94.9 (*d*)	4.91 (*d*, $J = 7.6$)	102.3 (*d*)
2^{V}	1.75–1.77 (*m*), 2.26–2.28 (*m*)	36.7 (*t*)	1.76–1.78 (*m*), 2.26–2.28 (*m*)	32.4 (*t*)	1.79–1.81 (*m*), 2.38–2.408 (*m*)	34.6 (*t*)	3.93–3.95 (*m*)	74.7 (*d*)

Table 2 (cont.)

	1		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
3^{V}	3.84–3.86 (*m*)	77.8 (*d*)	3.70–3.72 (*m*)	73.1 (*d*)	3.86–3.88 (*m*)	76.9 (*d*)	4.25–4.27 (*m*)	76.7 (*d*)
4^{V}	3.41–3.43 (*m*)	82.4 (*d*)	3.93–3.95 (*m*)	79.0 (*d*)	3.41–3.43 (*m*)	81.6 (*d*)	4.27–4.29 (*m*)	81.5 (*d*)
5^{V}	4.15–4.17 (*m*)	69.4 (*d*)	4.19–4.21 (*m*)	65.0 (*d*)	4.14–4.16 (*m*)	68.6 (*d*)	3.95–3.97 (*m*)	76.7 (*d*)
6^{V}	1.47 (*d*, *J* = 6.4)	18.6 (*q*)	1.43 (*d*, *J* = 6.4)	18.5 (*q*)	1.44–1.46 (*m*)	17.8 (*q*)	4.27–4.30 (*m*), 4.50 (*d*, *J* = 10.1)	62.5 (*t*)
MeO^{V}	3.52 (*s*) α-L-Cym	58.3 (*q*)	3.34 (*s*) β-D-Glc	57.0 (*q*)	3.49 (*s*) α-L-Cym	56.3 (*q*)	β-D-Glc	
1^{VI}	4.92 (*d*, *J* = 3.1)	99.1 (*d*)	4.98 (*d*, *J* = 7.7)	102.4 (*d*)	4.93 (*d*, *J* = 3.1)	98.3 (*d*)	5.17 (*d*, *J* = 7.9)	105.1 (*d*)
2^{VI}	1.77–1.79 (*m*), 2.29–2.31 (*m*)	32.3 (*t*)	3.94–3.96 (*m*)	75.3 (*d*)	1.78–1.80 (*m*), 2.30–2.32 (*m*)	31.5 (*t*)	3.93–3.95 (*m*)	74.8 (*d*)
3^{VI}	3.70–3.72 (*m*)	73.4 (*d*)	4.20–4.22 (*m*)	78.5 (*d*)	3.79–3.81 (*m*)	72.6 (*d*)	4.17–4.19 (*m*)	78.4 (*d*)
4^{VI}	3.90–3.93 (*m*)	79.0 (*d*)	4.18–4.20 (*m*)	71.9 (*d*)	3.91–3.93 (*m*)	78.2 (*d*)	4.16–4.18 (*m*)	71.6 (*d*)
5^{VI}	4.60–4.62 (*m*)	65.5 (*d*)	3.95–3.97 (*m*)	78.6 (*d*)	4.66–4.68 (*m*)	64.5 (*d*)	3.98–4.00(*m*)	78.5 (*d*)
6^{VI}	1.32 (*d*, *J* = 6.1)	18.4 (*q*)	4.33–4.35 (*m*), 4.53 (*d*, *J* = 11.5)	63.1 (*t*)	1.34–1.36 (*m*)	17.6 (*q*)	4.27–4.30 (*m*), 4.50 (*d*, *J* = 10.1)	62.5 (*t*)
MeO^{VI}	3.42 (*s*) β-D-Glc	56.8 (*q*)			3.35 (*s*) β-D-Glc	56.0 (*q*)		
1^{VII}	4.98 (*d*, *J* = 7.7)	102.4 (*d*)			4.99 (*d*, *J* = 7.7)	101.6 (*d*)		
2^{VII}	3.93–3.95 (*m*)	75.3 (*d*)			3.95–3.97 (*m*)	74.5 (*d*)		
3^{VII}	4.19–4.21 (*m*)	78.5 (*d*)			4.20–4.22 (*m*)	77.7 (*d*)		
4^{VII}	4.16–4.18 (*m*)	71.9 (*d*)			4.18–4.20 (*m*)	71.1 (*d*)		
5^{VII}	3.92–3.94 (*m*)	78.6 (*d*)			3.93–3.95 (*m*)	77.8 (*d*)		
6^{VII}	4.34–4.36 (*m*), 4.54 (*d*, *J* = 10.5)	63.1 (*t*)			4.34–4.36 (*m*), 4.52–4.54 (*m*)	62.3 (*t*)		

[a]) Me_4Si Was used as an internal standard in spectra experiments. [b]) Assignments were based on HMBC, HSQC, ROESY, and TOCSY experiments; due to severe overlapping, only detectable *J* values are reported.

glucopyranosyl residues when compared with reference data [14][15]. The absolute configuration of the β-digitoxopyranosyl unit was tentatively assigned as D according to the configuration found so far for this residue in the family Asclepiadaceae. According to the glycosylation shifts of the signals of C(2) (−2.2 ppm), C(3) (+6.2 ppm), and C(4) (−3.9 ppm), the sugar chain was determined to be attached at C(3)−O of the aglycone. In the HMBC spectrum of **2**, long-range correlations of δ(H) 5.18 (H−C(1^{I})) with δ(C) 77.7 (C(3)), of δ(H) 5.04 (H−C(1^{II})) with δ(C) 83.5 (C(4^{I})), of δ(H) 5.44 (H−C(1^{III})) with δ(C) 77.3 (C(4^{II})), of δ(H) 5.09 (H−C(1^{IV})) with δ(C) 83.5 (C(4^{III})), of δ(H) 4.92 (H−C(1^{V})) with δ(C) 82.4 (C(4^{IV})), and of δ(H) 4.98 (H−C(1^{VI})) with δ(C) 79.0 (C(4^{V})) were observed. From the above evidence, the sugar chain was characterized as a β-D-Glc-(1→4)-α-L-Cym-(1→4)-β-D-Cym-(1→4)-β-D-Dit-(1→4)-α-L-Cym-(1→4)-β-D-Dit unit.

Compound **3**, obtained as a white amorphous powder, was assigned the molecular formula $C_{78}H_{120}O_{30}$ by HR-ESI-MS (*m/z* 1559.6584 ($[M+Na]^+$)). The 1D- and 2D-NMR data (*Tables 1* and *2*), as well as comparison with those of **1** and **11** determined the structure of **3** to be kidjoranin 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranoside, named auriculoside III.

Acid hydrolysis of **3** gave kidjoranin (**11**) as aglycone, and cymarose, diginose, as well as glucose as sugar residues. The NMR spectroscopic data for the sugar part of **3** showed a close resemblance to those of **1**, revealing that **3** had the same sugar substitution pattern as **1**. The glycosylation shift effects on the signals of C(2) (−2.9 ppm), C(3) (+5.2 ppm), and C(4) (−4.8 ppm) showed the linkage position of the sugar chain was at C(3)−O of the aglycone.

Compound **4**, obtained as a white amorphous powder, was suggested to have the molecular formula as $C_{70}H_{106}O_{29}$ based on HR-ESI-MS (*m/z* 1433.6759 ($[M+Na]^+$)). Its structure was established as kidjoranin 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranoside, and named auriculoside IV.

In the acid hydrolysis experiment, kidjoranin (**11**) as the aglycone and cymarose, diginose and glucose as sugar residues were obtained and identified by TLC comparison with standard samples. Six anomeric H-atom signals at δ(H) 5.21 (*d*, *J* = 9.2), 5.14 (*d*, *J* = 3.3), 5.10 (*d*, *J* = 9.8), 4.92 (*d*, *J* = 3.2), 4.91 (*d*, *J* = 7.6), and 5.17 (*d*, *J* = 7.9), and six anomeric C-atom signals at δ(C) 96.2 (C(1^{I})), 100.9 (C(1^{II})), 99.5 (C(1^{III})), 99.0 (C(1^{IV})), 102.3 (C(1^{V})), and 105.0 (C(1^{VI})) were observed in the NMR spectra, respectively. Based on the HMBC, HSQC, ROESY, and TOCSY spectra, the data of these six sugars were assigned to be two β-D-cymaropyranosyl, one α-L-diginopyranosyl, one α-L-cymaropyranosyl, and two β-D-glucopyranosyl units comparing with the spectroscopic data in the literature [8]. The comparison of the ^{13}C-NMR spectral data of **4** with those of **11** showed glycosylation shifts of the signals of C(2) (−2.1 ppm), C(3) (+6.1 ppm), and C(4) (−3.9 ppm). Thus, **4** was a kidjoranin 3-*O*-hexosaccharide. According to the same methodology described above, the sequence of the sugar chain was established as β-D-Glc-(1 → 4)-β-D-Glc-(1 → 4)-α-L-Cym-(1 → 4)-β-D-Cym-(1 → 4)-α-L-Dig-(1 → 4)-β-D-Cym unit (*Fig. 1*, *b*).

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Factory*); *RP-C_{18}* gel (250 mesh; *Merck*). Semi-prep. HPLC: *ODS* column (250 × 9.4 mm, 5 μm; *Zorbax*, *Agilent*), with *Waters 2487* dual-wavelength UV detector (220 or 280 nm) and *Waters 600* pump; flow rate, 2.5 ml/min. Optical rotations: *Jasco-P-1020* spectropolarimeter. UV Spectra: *UV-240* spectrometer (*Shimadzu*); λ_{max} (log ε) in nm. IR Spectra: *FT-IR-8900* spectrophotometer (*Shimadzu*); KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AV-500* spectrometer; (D_5)pyridine soln.; δ in ppm rel. to Me_4Si, *J* in Hz. HR-TOF-MS: ESI mode; *Wiff Agilent TOF* mass spectrometer (HR); in *m/z*.

Plant Material. The roots of *C. auriculatum* were collected in Binhai County, Jiangsu Province, P. R. China, in November 2004, and were identified by Prof. *Ping Li* (China Pharmaceutical University). A

voucher specimen (No. WFC-2004525-3) was deposited with the Laboratory of Phytochemistry, Jiangsu Provincial Institute of Traditional Chinese Medicine.

Extraction and Isolation. The dried roots of *C. auriculatum* (10 kg) were cut into small pieces and extracted three times with 95% EtOH (3 × 100 l) under reflux for 2 h each time. The extract was evaporated under reduced pressure. The residue (1.1 kg) was suspended in H_2O (3 l), and then partitioned sequentially with same volume petroleum ether (PE), $CHCl_3$, and BuOH. The $CHCl_3$-soluble part (320 g) was separated by CC (SiO_2, $CHCl_3$/MeOH 50 : 1 → 2 : 1) to give five fractions, *Fr. A – E*. *Fr. A* (66.7 g) was subjected to CC (1. SiO_2, $CHCl_3$/MeOH 98 : 2 → 94 : 6; 2. C_{18}, MeOH/H_2O 80 – 90%) to yield **5** (68.7 mg), **6** (128.2 mg), and **10** (110.5 mg). *Fr. B* (48.3 g) was purified repeatedly by CC (SiO_2, $CHCl_3$/MeOH 98 : 5 → 92 : 8) to afford **7** (96.3 mg) and **11** (83.5 mg). *Fr. C* (46.5 g) was chromatographed on CC (1. SiO_2, $CHCl_3$/MeOH 95 : 5 → 85 : 15) to give five subfractions (*C1 – C5*). *Fr. C1* (5.1 g) was subjected to semi-prep. HPLC (MeOH/H_2O 81 : 19, 280 nm) to provide **3** (13.3 mg, t_R 34.8 min), **9** (14.8 mg, t_R 36.3 min), and **8** (10.2 mg, t_R 40.9 min). *Fr. C2* (1.5 g) was subjected to semi-prep. HPLC (MeOH/H_2O 82 : 18, 220 nm) to provide **1** (9.2 mg, t_R 34.4 min) and **2** (11.8 mg, t_R 24.9 min). *Fr. D* (77.0 g) was separated over CC (1. SiO_2, $CHCl_3$/MeOH 85 : 15 → 75 : 25; 2. C_{18}, MeOH/H_2O 65 – 80%) to yield **4** (88.2 mg).

Auriculoside I (= *Caudatin 3*-O-*β*-D-*Glucopyranosyl-(1 → 4)-α*-L-*cymaropyranosyl-(1 → 4)-β*-D-*cymaropyranosyl-(1 → 4)-α*-L-*cymaropyranosyl-(1 → 4)-β*-D-*cymaropyranosyl-(1 → 4)-α*-L-*diginopyranosyl-(1 → 4)-β*-D-*cymaropyranoside* = *(3α,9ξ,12β,14β,17α)-3-{[β*-D-*Glucopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-α*-L-ribo-*hexopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-β*-D-ribo-*hexopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-α*-L-ribo-*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-20-oxopregn-5-en-12-yl (2*E*)-3,4-Dimethylpent-2-enoate*; **1**). White amorphous powder. M.p. 149 – 151°. $[\alpha]_D^{25} = -20.1$ ($c = 0.20$, MeOH). UV (MeOH): 221 (3.85). IR (KBr): 3445, 1713, 1645, 1160. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ^{1}H,^{13}C-HMBC: *Fig. 1,a*. HR-ESI-MS: 1539.6566 ($[M + Na]^+$, $C_{76}H_{124}NaO_{30}^+$; calc. 1539.6547).

Auriculoside II (= *Caudatin 3*-O-*β*-D-*Glucopyranosyl-(1 → 4)-α*-L-*cymaropyranosyl-(1 → 4)-β*-D-*cymaropyranosyl-(1 → 4)-β*-D-*digitoxopyranosyl-(1 → 4)-α*-L-*cymaropyranosyl-(1 → 4)-β*-D-*digitoxopyranoside* = *(3α,9ξ,12β,14β,17α)-3-{[β*-D-*Glucopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-α*-L-ribo-*hexopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-β*-D-ribo-*hexopyranosyl-(1 → 5)-1,3,7-trideoxy-β*-D-ribo-*hept-2-ulopyranosyl-(2 → 4)-2,6-dideoxy-3*-O-*methyl-α*-L-ribo-*hexopyranosyl-(1 → 4)-(3ξ)-2,6-dideoxy-β*-D-erythro-*hexopyranosyl]oxy}-8,14,17-trihydroxy-20-oxopregn-5-en-12-yl (2*E*)-3,4-Dimethylpent-2-enoate*; **2**). White amorphous powder. M.p. 152 – 154°. $[\alpha]_D^{25} = -31.8$ ($c = 0.20$, MeOH). IR (KBr): 3443, 1715, 1643, 1160. ^{1}H- and ^{13}C-NMR (DEPT): *Tables 1* and *2*. HR-ESI-MS: 1367.4348 ($[M + Na]^+$, $C_{67}H_{108}NaO_{27}^+$; calc. 1367.4324).

Auriculoside III (= *Kidjoranin 3*-O-*β*-D-*Glucopyranosyl-(1 → 4)-α*-L-*cymaropyranosyl-(1 → 4)-β*-D-*cymaropyranosyl-(1 → 4)-α*-L-*cymaropyranosyl-(1 → 4)-β*-D-*cymaropyranosyl-(1 → 4)-α*-L-*diginopyranosyl-(1 → 4)-β*-D-*cymaropyranoside* = *(3α,9ξ,12β,14β,17α)-3-{[β*-D-*Glucopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-α*-L-ribo-*hexopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-β*-D-ribo-*hexopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-α*-L-ribo-*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-20-oxopregn-5-en-12-yl (2*E*)-3-Phenylprop-2-enoate*; **3**). White amorphous powder. M.p. 177 – 179°. $[\alpha]_D^{25} = +12.0$ ($c = 0.20$, MeOH). UV (MeOH): 280 (4.39), 221 (4.20), 217 (4.16). IR (KBr): 3438, 1710, 1635, 1600, 1580, 1495, 1465, 1160. ^{1}H- and ^{13}C-NMR (DEPT): *Tables 1* and *2*. HR-ESI-MS: 1559.6584 ($[M + Na]^+$, $C_{78}H_{120}NaO_{30}^+$; calc. 1559.6552).

Auriculoside IV (= *Kidjoranin 3-O-β*-D-*Glucopyranosyl-(1 → 4)-β*-D-*glucopyranosyl-(1 → 4)-α*-L-*cymaropyranosyl-(1 → 4)-β*-D-*cymaropyranosyl-(1 → 4)-α*-L-*diginopyranosyl-(1 → 4)-β*-D-*cymaropyranoside* = *(3α,9ξ,12β,14β,17α)-3-{[β*-D-*Glucopyranosyl-(1 → 4)-β*-D-*glucopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-α*-L-ribo-*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-20-oxopregn-5-en-12-yl (2*E*)-3-Phenylprop-2-enoate*; **4**). White amorphous powder. M.p. 181 – 182°. $[\alpha]_D^{25} = +8.1$ ($c = 0.20$, MeOH). IR (KBr): 3450, 1710, 1640, 1600, 1580, 1500,

1450, 1160. ^{1}H- and ^{13}C-NMR (DEPT): *Tables 1* and *2*. ^{1}H,^{13}C-HMBC: *Fig. 1*, *b*. HR-ESI-MS: 1433.6759 ($[M+Na]^+$, $C_{70}H_{106}NaO_{29}^+$; calc.1433.6712).

Acid Hydrolysis. A soln. of **1**–**4** (4 mg) in 5% HCl/1,4-dioxane 1:1 (4 ml) was heated at 95° for 1.5 h. The hydrolyzed mixture was neutralized with NaOH (4 mol/l) and evaporated to dryness under reduced pressure. The residue was dissolved in MeOH, and compared by TLC analysis with authentic samples of caudatin (**7**) and kidjoranin (**11**) which were assigned as the aglycones of glycosides **1** and **2**, and **3** and **4**, resp. The identity of the monosaccharides in the hydrolysates of each compound was confirmed by TLC comparison with authentic sugars, digitoxose was detected from **2**; diginose was detected from **1**, **3**, and **4**; cymarose and glucose were detected from **1**–**4**. The R_f values of caudatin, kidjoranin, digitoxose, diginose, and cymarose were 0.50, 0.36, 0.19, 0.13, and 0.10 with solvent $CHCl_3$/MeOH (9:1); 0.41, 0.14, 0.12, 0.08, and 0.04 with solvent PE/Me_2CO (3:2); 0.57, 0.26, 0.21, 0.15, and 0.08 with solvent hexane/Me_2CO (1:1), resp. The R_f values of glucose was 0.32 with solvent $CHCl_3$/MeOH/H_2O (7:3:0.5).

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