C_{21} Steroidal Glycosides from *Cynanchum wilfordii*

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Eight new C_{21} steroidal glycosides, named wilfosides A–H (**1**–**8**, resp.), along with one known compound wilfoside KIN (**9**), were isolated from the roots of *Cynanchum wilfordii*. The structures of the new glycosides were determined on the basis of spectroscopic analysis, including 1D- and 2D-NMR, and ESI-MS techniques, as well as by comparison of the spectral data with those of related compounds.

Introduction. – Steroidal glycosides are widely distributed in the *Cynanchum* species. Medicinal plants containing steroidal glycosides are commonly used for medicinal purposes in many Asian countries. Modern biological studies have shown that the extracts and fractions of *Cynanchum* species have varieties of biological activities, including antitumor, antiepileptic, immunoregulation, anti-oxidation, and antiviral effects [1]. *Cynanchum wilfordii* (MAXIM.) HEMSL. (former Asclepiadaceae, now Apocynaceae, subfamily Asclepiadoideae) is widely distributed in China and used as traditional Chinese medicine for the treatment of impotency, neurasthenia, lumbago, and abscesses [2]. C_{21} Steroidal glycosides have established themselves as an important class of biologically active compounds in the *Cynanchum* species. Previous investigation on the C_{21} steroidal glycosides had shown the presence of normal and aberrant pregnane skeletons [3–6]. The C_{21} steroidal glycosides with a normal pregnane skeleton are sarcostin derivatives with an oligosaccharide at C(3) and acetyl, ikemaoyl, cinnamoyl, isovaleroyl, *p*-hydrobenzoyl, and nicotinoyl esters at C(12) or C(20) [7]. Two sorts of aberrant pregnane glycosides with aglycone skeletons of the 13,14;14,15-disecopregnane type and the 14,15-secopregnane type are known from the *Cynanchum* species [8][9]. In these steroidal glycosides, the linkage sites of the sugar moieties are at C(3) of their aglycones and the sugar portion is generally composed of a linear rather than a branched oligosaccharide chain. The sugar residues include 2,6-dideoxysugar units, 6-deoxysugar units, and glucose units with the mode of $1 \rightarrow 4$ linkage [3–6].

As part of our phytochemical investigation of traditional Chinese medicinal plants, we describe here the isolation and structure elucidation of eight new C_{21} steroidal glycosides, wilfosides A–H (**1**–**8**, resp.), along with the known compound wilfoside KIN (**9**) from the roots of *C. wilfordii*. Like many wilfosides [10], wilfosides A–F (**1**–**6**, resp.) are sarcostin derivatives with an oligosaccharide at C(3) and esterified at C(20).

Wilfosides G and H (**7** and **8**, resp.) have an 8,14-secopregnane skeleton previously observed in *Cynanchum* species [8][9] and evidently arising from oxidative cleavage of the 8,14 glycol of sarcotins. All compounds possessed an oligosaccharide chain consisting of two, three, four, or five 2,6-dideoxysugar units at C(3) of the aglycone [7][11]. The structures of the new glycosides were determined on the basis of spectroscopic analysis, including 1D- and 2D-NMR and HR-ESI-MS techniques (*Fig. 1*).

Results and Discussion. – Compounds **1**–**9** all showed positive *Liebermann–Burchard* reactions, suggesting that they were steroidal glycosides, and positive *Keller–Killiani* reactions, suggesting the presence of 2-deoxysugar moieties. Compound **9** was elucidated to be wilfoside KIN by comparison of ^{1}H- and ^{13}C-NMR data with the literature [12].

The identity of the monosaccharides in the hydrolysates after acid hydrolysis of each compound was confirmed by co-TLC comparison with authentic sugars. For the oleandrose and digitoxose, to the best of our knowledge, β-linked digitoxopyranosyl and oleandropyranosyl units were so far only found in D-configuration in the Asclepiadaceae family [10][13]. Due to their similar ^{1}H- and ^{13}C-NMR chemical shifts with those reported in the literatures [9][10][14–16], all digitoxopyranosyl and oleandropyranosyl units in this study were determined to be of D-configuration.

Wilfoside A (**1**) was isolated as a colorless, amorphous powder. HR-ESI-MS indicated a molecular formula of $C_{50}H_{67}NO_{14}$. The ^{13}C-NMR and DEPT spectra (*Tables 1* and *2*) showed signals of two CO groups, two C=C bonds, and seven Me, nine CH_2, and 21 CH groups, as well as of seven quaternary C-atoms. The ^{1}H-NMR of the aglycone portion (*Table 3*) showed signals for three Me groups at δ(H) 1.09 (*s*, Me(19)), 1.34 (*d*, $J = 6.2$, Me(21)), and 1.60 (*s*, Me(18)), and three signals at 3.52–3.55 (*m*, H–C(3)), 4.79–4.81 (*m*, H–C(12)), 4.85–4.87 (*m*, H–C(20)) corresponding to secondary O-bearing C-atoms. The data were in good agreement with previously published data for similar compounds [3][17][18], which indicated that the structure of **1** was based on the skeleton of sarcostin. The extensive 1D- and 2D-NMR study revealed that the aglycone contained each a cinnamoyl and nicotinoyl moiety. Diagnostic long-range correlations (HMBC) were observed between H–C(12) (δ(H) 4.79–4.81) and C($1'_{cin}$) (δ(C) 166.2), and between H–C(20) (δ(H) 4.85–4.87) and C($1'_{nic}$) (δ(C) 163.7), revealing the connectivity between the cinnamoyl group and C(12), and between the nicotinoyl group and C(20). The aglycone of **1** was determined to be gagaminin by comparing with the NMR data of known C_{21} steroidal aglycones [19]. The presence of gagaminin in the acid hydrolysate of **1** was confirmed by TLC comparsion with an authentic sample.

The anomeric-C-atom resonances at δ(C) 96.0 and 101.4 correlating with the corresponding H-atoms at δ(H) 4.83 (*d*, $J = 8.1$) and 4.47 (*d*, $J = 7.8$) in the HSQC spectrum revealed the presence of two sugar residues. The coupling constants of the anomeric H-atom signals indicated that **1** had two sugar units with β-linkages. The ^{1}H- and ^{13}C-NMR spectra (*Tables 4* and *2*, resp.), and the TLC behavior of the two sugar units suggested that the sugar moieties were β-cymaropyranosyl and β-oleandropyranosyl, which was also supported by HSQC and HMBC data (*Fig. 2*). To determine the absolute configuration of cymarose, the hydrolysate of **1** was analyzed by HPLC with

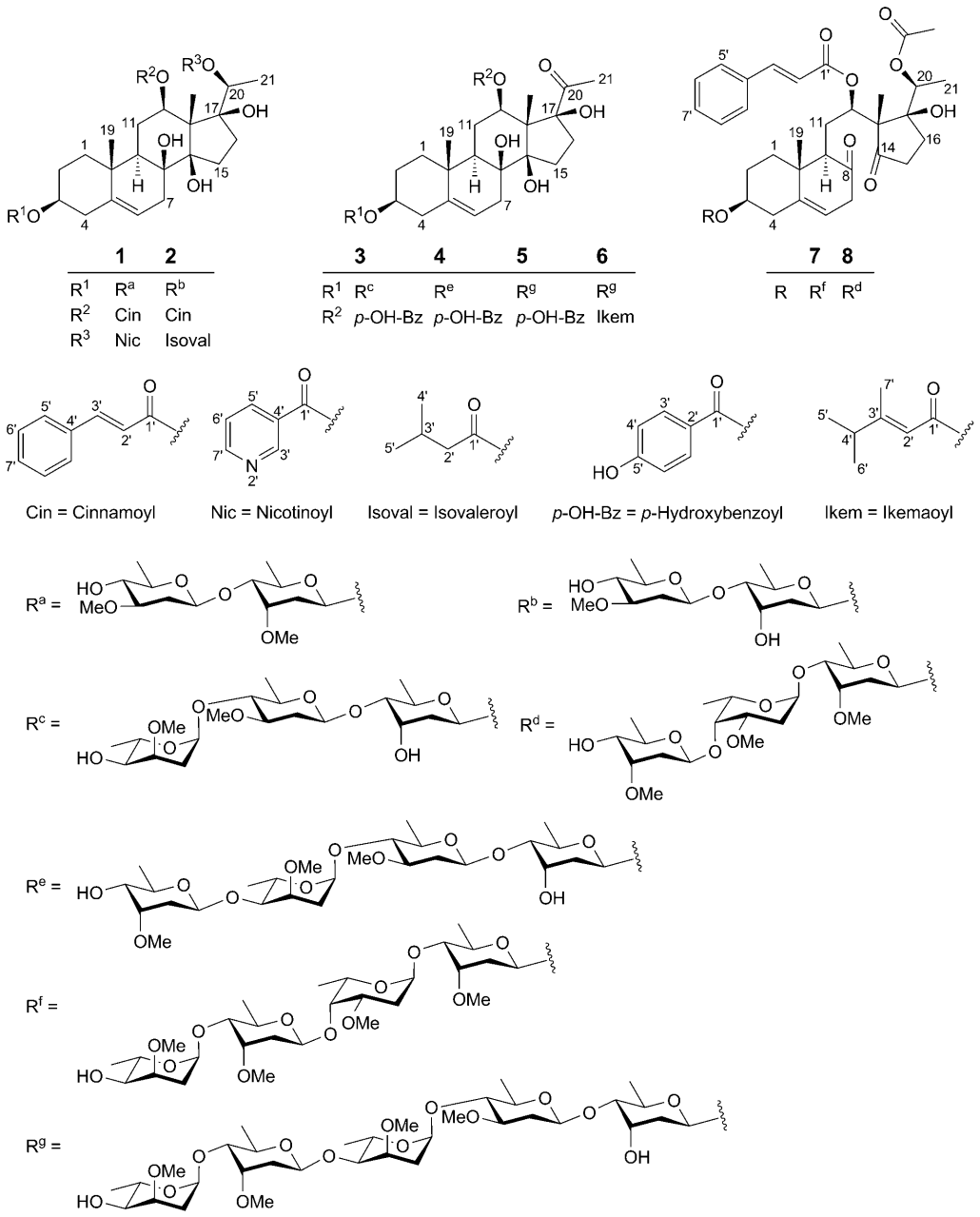


Fig. 1. *Structures of the new compounds* **1**–**8** *isolated from* Cynanchum wilfordii

Table 1. *^{13}C-NMR Data* (100 MHz) *of the Aglycone Moiety of Compounds* **1**–**8** *in $CDCl_3$* (δ in ppm)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
C(1)	38.7	38.8	38.9	38.8	38.8	38.8	36.9	36.9
C(2)	28.8	29.0	28.9	28.9	28.9	28.9	28.7	28.7
C(3)	77.8	78.0	78.0	78.0	77.9	77.9	76.6	76.6
C(4)	38.7	38.9	38.9	38.8	38.8	38.8	37.8	37.8
C(5)	140.2	140.1	140.3	140.5	140.4	140.4	141.2	141.2
C(6)	117.8	118.2	118.0	118.1	117.9	117.9	118.3	118.3
C(7)	34.1	34.4	34.3	34.3	34.3	34.3	40.8	40.7
C(8)	74.2	74.4	74.4	74.4	74.3	74.3	209.5	209.5
C(9)	43.2	43.5	43.7	43.7	43.7	43.7	55.2	55.1
C(10)	37.2	37.1	37.2	37.2	37.2	37.2	42.7	42.6
C(11)	24.8	25.2	24.4	24.4	24.4	24.4	25.4	25.3
C(12)	73.3	73.8	72.9	72.9	72.9	71.5	70.8	70.8
C(13)	56.4	56.3	58.5	58.6	58.5	58.5	61.6	61.5
C(14)	87.8	88.0	88.2	88.2	88.2	88.2	217.0	217.2
C(15)	32.9	33.1	33.4	33.4	33.4	33.4	33.4	33.3
C(16)	32.5	32.4	32.0	32.0	32.1	32.1	28.7	28.7
C(17)	87.2	87.3	91.6	91.7	91.6	91.6	82.4	82.3
C(18)	10.4	10.3	9.7	9.7	9.7	9.7	11.2	11.1
C(19)	18.3	18.4	18.6	18.6	18.6	18.4	18.6	18.6
C(20)	75.6	74.6	210.0	210.0	209.9	209.9	73.6	73.6
C(21)	14.9	15.2	27.6	27.6	27.6	27.6	14.1	14.0
Substituent at C(12)	Cin[a])	Cin	*p*-OH-Bz[b])	*p*-OH-Bz[b])	*p*-OH-Bz[b])	Ikem[c])	Cin	Cin
C(1′)	166.2	167.0	165.4	165.4	165.4	166.0	166.8	166.8
C(2′)	118.6	118.6	121.9	121.9	121.7	113.1	117.6	117.6
C(3′)	144.2	145.3	132.0	132.0	131.9	167.0	145.3	145.3
C(4′)	134.0	134.3	115.5	115.5	115.5	38.3	134.0	133.9
C(5′/9′)	127.9	128.5	161.0	161.1	161.2	21.1	127.9	127.9
C(6′/8′)	128.7	129.1	115.5	115.5	115.5	21.0	128.9	128.8
C(7′)	130.2	130.6	132.0	132.0	131.9	16.7	130.6	130.5
Substituent at C(20)	Nic[d])	Isoval[e])					Ac[f])	Ac
C(1′)	163.7	171.4					168.9	168.9
C(2′)		43.1					21.2	21.1
C(3′)	153.2	26.5						
C(4′)	126.1	22.8						
C(5′)	137.3	22.8						
C(6′)	123.3							
C(7′)	150.7							

[a]) Cin = cinnamoyl; [b]) *p*-OH-Bz = *p*-hydroxybenzoyl; [c]) Ikem = ikemaoyl; [d]) Nic = nicotinoyl; [e]) Isoval = isovaleryl; [f]) Ac = acetyl.

refractive index (*RI*) and optical rotation (*OR*) detectors [5]. The *OR* detection exhibited a positive signal for the cymarose, suggesting that the cymarose was of D-type. The presence of β-D-oleandropyranosyl was confirmed by comparison with the spectroscopic data in the literature [13]. The HMBC correlations from δ(H) 3.52–3.55 (H–C(3) of aglycone) to δ(C) 96.0 (C(1^{I})), and from δ(H) 3.20–3.22 (H–C(4^{I})) to δ(C) 101.4 (C(1^{II})) led us to establish the linkage positions and sequences of the two

Table 2. *^{13}C-NMR Data* (100 MHz) *of the Sugar Moieties of Compounds* **1**–**8** *in $CDCl_3$* (δ in ppm)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Moiety I	D-Cym[c])	D-Digit[b])	D-Digit	D-Digit	D-Digit	D-Digit	D-Cym	D-Cym
C(1^{I})	96.0	95.9	95.9	95.7	95.6	95.7	95.6	95.6
C(2^{I})	35.6	37.3	37.2	37.1	37.1	37.1	34.1	34.1
C(3^{I})	77.1	66.7	66.8	66.5	66.7	66.5	77.2	77.4
C(4^{I})	82.7	83.0	82.8	82.8	82.7	82.7	81.7	81.7
C(5^{I})	68.3	68.0	68.1	67.9	67.9	67.9	68.7	68.6
C(6^{I})	18.1	18.3	18.3	18.1	18.1	18.1	18.1	18.1
MeO–C(3^{I})	58.3						57.4	56.9
Moiety II	D-Ole[d])	D-Ole	D-Ole	D-Ole	D-Ole	D-Ole	L-Digin[a])	L-Digin
C(1^{II})	101.4	100.5	100.5	100.2	100.1	100.2	100.8	100.7
C(2^{II})	35.2	35.5	36.0	35.8	35.7	35.9	31.5	31.4
C(3^{II})	80.5	80.5	78.8	78.4	78.3	78.5	73.7	73.7
C(4^{II})	75.3	75.3	81.6	81.8	81.8	81.7	74.3	74.0
C(5^{II})	71.4	71.9	72.0	71.7	71.7	71.8	66.7	66.6
C(6^{II})	17.9	18.1	18.5	18.1	18.1	18.0	18.0	17.3
MeO–C(3^{II})	56.4	56.6	56.5	56.4	56.2	56.3	55.5	55.4
Moiety III			L-Cym	L-Cym	L-Cym	L-Cym	D-Cym	D-Cym
C(1^{III})			97.2	97.5	97.5	97.5	99.2	99.0
C(2^{III})			31.1	31.5	31.6	31.6	34.2	32.8
C(3^{III})			75.1	72.3	72.7	72.7	77.0	76.9
C(4^{III})			72.3	76.7	76.8	76.9	81.7	71.9
C(5^{III})			65.3	64.1	63.8	63.9	68.9	70.9
C(6^{III})			17.8	17.7	17.9	17.7	18.2	17.9
MeO–C(3^{III})			56.5	56.9	56.8	57.8	56.9	57.8
Moiety IV				D-Cym	D-Cym	D-Cym	L-Cym	
C(1^{IV})				94.4	94.5	94.6	98.5	
C(2^{IV})				33.4	35.0	35.2	30.9	
C(3^{IV})				77.2	77.2	77.1	74.7	
C(4^{IV})				72.3	81.9	81.7	71.9	
C(5^{IV})				70.8	68.7	68.6	65.5	
C(6^{IV})				18.2	18.1	18.2	17.4	
MeO–C(3^{IV})				57.2	57.6	56.8	56.3	
Moiety V					L-Cym	L-Cym		
C(1^{V})					98.3	98.2		
C(2^{V})					30.9	30.9		
C(3^{V})					74.7	74.7		
C(4^{V})					72.0	72.0		
C(5^{V})					65.5	65.6		
C(6^{V})					18.0	18.1		
MeO–C(3^{V})					56.4	56.2		

[a]) Digin = diginopyranosyl; [b]) Digit = digitoxopyranosyl; [c]) Cym = cymaropyranosyl; [d]) Ole = oleandropyranosyl.

sugar units. Therefore, **1** was elucidated as 3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl gagaminin.

Wilfoside B (**2**) was isolated as a colorless, amorphous powder. The molecular formula was established as $C_{48}H_{70}O_{14}$ by HR-ESI-MS. The NMR data analysis of **2**

Table 3. *^{1}H-NMR Data* (400 MHz) *of the Aglycone Moiety of Compounds* **1**–**8** *in* $CDCl_3$ (δ in ppm, J in Hz)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
CH_2(1)	1.07–1.11 (*m*), 1.81–1.85 (*m*)	1.05–1.09 (*m*), 1.82–1.86 (*m*)	1.08–1.12 (*m*), 1.83–1.87 (*m*)	1.09–1.13 (*m*), 1.82–1.86 (*m*)	1.08–1.12 (*m*), 1.84–1.88 (*m*)	1.07–1.11 (*m*), 1.82–1.86 (*m*)	1.13–1.17 (*m*), 1.76–1.80 (*m*)	1.13–1.17 (*m*), 1.78–1.82 (*m*)
CH_2(2)	1.57–1.59 (*m*), 1.88–1.90 (*m*)	1.61–1.63 (*m*), 1.86–1.88 (*m*)	1.59–1.61 (*m*), 1.86–1.89 (*m*)	1.60–1.62 (*m*) 1.87–1.89 (*m*)	1.57–1.59 (*m*), 1.87–1.89 (*m*)	1.61–1.63 (*m*), 1.90–1.92 (*m*)	1.46–1.48 (*m*), 1.93–1.95 (*m*)	1.45–1.47 (*m*), 1.90–1.93 (*m*)
H–C(3)	3.52–3.55 (*m*)	3.52–3.55 (*m*)	3.54–3.57 (*m*)	3.56–3.59 (*m*)	3.56–3.59 (*m*)	3.56–3.59 (*m*)	3.52–3.55 (*m*)	3.52–3.55 (*m*)
CH_2(4)	2.26–2.28 (*m*), 2.37–2.39 (*m*)	2.24–2.26 (*m*), 2.36–2.38 (*m*)	2.23–2.25 (*m*), 2.36–2.38 (*m*)	2.24–2.26 (*m*), 2.36–2.38 (*m*)	2.24–2.26 (*m*), 2.37–2.39 (*m*)	2.27–2.29 (*m*), 2.37–2.39 (*m*)	2.07–2.09 (*m*), 2.39–2.41 (*m*)	2.05–2.07 (*m*), 2.39–2.41 (*m*)
H–C(6)	5.36 (br. *s*)	5.35 (br. *s*)	5.37 (br. *s*)	5.34 (br. *s*)	5.36 (br. *s*)	5.34 (br. *s*)	5.32 (br. *s*)	5.32 (br. *s*)
CH_2(7)	2.19–2.22 (*m*)	2.16–2.19 (*m*)	2.17–2.20 (*m*)	2.19–2.21 (*m*)	2.19–2.21 (*m*)	2.19–2.21 (*m*)	2.60–2.63 (*m*), 3.01–3.04 (*m*)	2.60–2.63 (*m*), 3.01–3.04 (*m*)
H–C(9)	1.49–1.53 (*m*)	1.48–1.52 (*m*)	1.54–1.58 (*m*)	1.54–1.58 (*m*)	1.55–1.59 (*m*)	1.50–1.54 (*m*)	2.26–2.30 (*m*)	2.24–2.28 (*m*)
CH_2(11)	1.67–1.70 (*m*), 1.92–1.94 (*m*)	1.67–1.69 (*m*), 1.94–1.96 (*m*)	1.87–1.89 (*m*)	1.89–1.91 (*m*)	1.89–1.91 (*m*)	1.82–1.84 (*m*)	1.43–1.45 (*m*), 1.85–1.87 (*m*)	1.40–1.43 (*m*), 1.83–1.85 (*m*)
H–C(12)	4.79–4.81 (*m*)	4.77–4.80 (*m*)	4.77–4.79 (*m*)	4.77–4.79 (*m*)	4.77–4.79 (*m*)	4.54–4.56 (*m*)	5.22–5.24 (*m*)	5.23–5.25 (*m*)
CH_2(15)	1.97–1.99 (*m*)	1.94–1.96 (*m*)	1.97–1.99 (*m*)	1.97–1.99 (*m*)	1.99–2.01 (*m*)	1.94–1.96 (*m*)	2.43–2.45 (*m*), 2.70–2.73 (*m*)	2.42–2.44 (*m*), 2.69–2.71 (*m*)
CH_2(16)	1.90–1.93 (*m*), 1.94–1.97 (*m*)	1.88–1.91 (*m*), 1.89–2.01 (*m*)	2.80–2.83 (*m*)	1.86–1.88 (*m*), 2.82–2.85 (*m*)	1.90–1.93 (*m*), 2.81–2.83 (*m*)	1.85–1.87 (*m*), 2.79–2.81 (*m*)	1.95–1.97 (*m*), 2.13–2.15 (*m*)	1.93–1.95 (*m*), 2.09–2.11 (*m*)
Me(18)	1.60 (*s*)	1.48 (*s*)	1.50 (*s*)	1.51 (*s*)	1.50 (*s*)	1.36 (*s*)	1.26 (*s*)	1.26 (*s*)
Me(19)	1.09 (*s*)	1.12 (*s*)	1.09 (*s*)	1.10 (*s*)	1.09 (*s*)	1.11 (*s*)	0.74 (*s*)	0.74 (*s*)
H–C(20)	4.85–4.87 (*m*)	4.68–4.71 (*m*)					4.82–4.85 (*m*)	4.82–4.85 (*m*)
Me(21)	1.34 (*d*, *J* = 6.2)	1.22 (*d*, *J* = 6.0)	2.06 (*s*)	2.06 (*s*)	2.06 (*s*)	2.11 (*s*)	1.23 (*d*, *J* = 6.2)	1.22 (*d*, *J* = 6.2)
Substituent at C(12)	Cin[a])	Cin	*p*-OH-Bz[b])	*p*-OH-Bz	*p*-OH-Bz	Ikem[c])	Cin	Cin
H–C(2′)	6.08 (*d*, *J* = 15.9)	6.34 (*d*, *J* = 16.2)				5.50 (*s*)	6.32 (*d*, *J* = 15.9)	6.30 (*d*, *J* = 15.9)
H–C(3′)	7.35 (*d*, *J* = 17.7)	7.64 (*d*, *J* = 16.2)	7.82 (*d*, *J* = 8.4)	7.82 (*d*, *J* = 8.7)	7.82 (*d*, *J* = 8.4)		7.60 (*d*, *J* = 15.9)	7.62 (*d*, *J* = 15.9)
H–C(4′)			6.84 (*d*, *J* = 8.4)	6.84 (*d*, *J* = 8.7)	6.84 (*d*, *J* = 8.4)	2.33–2.36 (*m*)		
H–C(5′/9′)	7.18–7.21 (*m*)	7.52–7.55 (*m*)				1.04 (*s*)	7.52–7.55 (*m*)	7.52–7.55 (*m*)
H–C(6′/8′)	7.28–7.31 (*m*)	7.36–7.39 (*m*)				1.06 (*s*)	7.38–7.41 (*m*)	7.39–7.42 (*m*)
H–C(7′)	7.30–7.33 (*m*)	7.34–7.37 (*m*)				2.06 (*s*)	7.40–7.43 (*m*)	7.40–7.43 (*m*)
Substituent at C(20)	Nic[d])	Isoval[e])					Ac[f])	Ac
H–C(2′)		2.36–2.39 (*m*), 2.44–2.47 (*m*)					1.86 (*s*)	1.85 (*s*)
H–C(3′)	8.7 (*s*)	2.00–2.02 (*m*)						
H–C(4′)		1.17 (*s*)						
H–C(5′)	8.09 (*d*, *J* = 8.1)	1.17 (*s*)						
H–C(6′)	7.17–7.19 (*m*)							
H–C(7′)	9.17 (*s*)							

[a]) Cin = cinnamoyl; [b]) *p*-OH-Bz = *p*-hydroxybenzoyl; [c]) Ikem = ikemaoyl; [d]) Nic = nicotinoyl; [e]) Isoval = isovaleryl; [f]) Ac = acetyl.

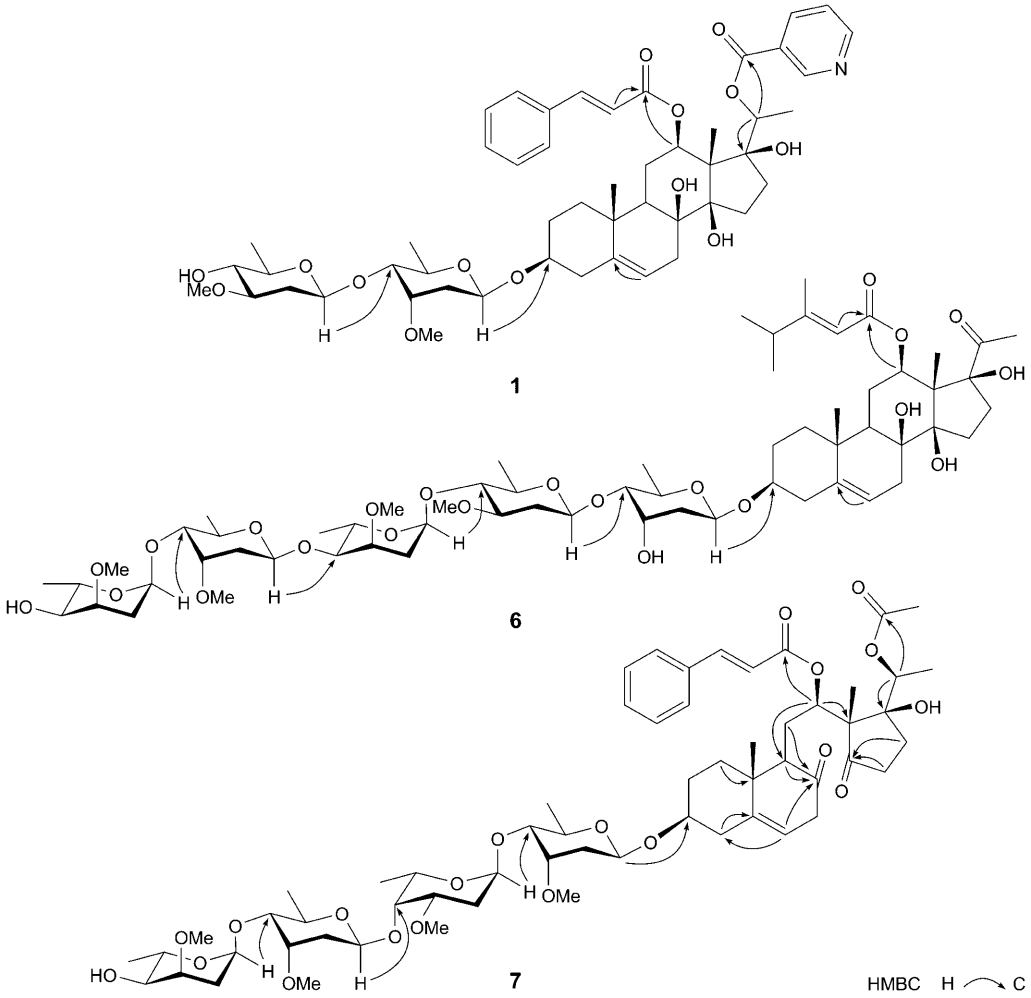


Fig. 2. *Key HMBCs of compounds* **1**, **6**, *and* **7**

indicated that it possessed the same steroid part as **1** (*Tables 1* and *3*). The difference was that there was an isovaleroyl group instead of a nicotinoyl group linked to C(20) [5]. The linkage positions of the isovaleryl and cinnamoyl groups were ascertained by the HMBC spectrum. The aglycone obtained upon acid hydrolysis of **2** was identified as 12-*O*-cinnamoyl-20-*O*-isovaleroylsarcostin by comparsion with an authentic sample [20].

The ^{1}H-NMR spectrum of **2** showed two anomeric H-atom signals (δ(H) 4.83, *d*, $J = 8.7$, and 4.52, *d*, $J = 9.9$) for two β-linked sugar units (*Table 4*). On the basis of the HSQC, HMBC, and acid hydrolysis experiments, as well as by comparison with the literature [14], the two sugar moieties were identified as β-D-digitoxopyranosyl and β-D-oleandropyranosyl. The sugar sequence was confirmed by the HMBC correlations

Table 4. *^{1}H-NMR Data* (400 MHz) *of the Sugar Moieties of Compounds* **1**–**8** *in $CDCl_3$* (δ in ppm, J in Hz)

	1	**2**	**3**	**4**
Moiety I	D-Cym[a])	D-Digit[b])	D-Digit	D-Digit
H–C(1^{I})	4.83 (d, J = 8.1)	4.83 (d, J = 8.7)	4.92 (d, J = 8.7)	4.92 (d, J = 8.7)
H–C(2^{I})	1.55–1.57 (m), 2.07–2.08 (m)	1.66–1.69 (m), 2.05–2.07 (m)	1.66–1.69 (m), 2.07–2.08 (m)	1.66–1.69 (m), 2.07–2.08 (m)
H–C(3^{I})	3.77–3.80 (m)	4.22–4.25 (m)	4.21–4.23 (m)	4.19–4.21 (m)
H–C(4^{I})	3.20–3.22 (m)	3.19–3.22 (m)	3.18–3.21 (m)	3.19–3.22 (m)
H–C(5^{I})	3.82–3.86 (m)	3.78–3.81 (m)	3.78–3.81 (m)	3.78–3.81 (m)
Me(6^{I})	1.20 (d, J = 6.3)	1.24 (d, J = 6.0)	1.23 (d, J = 6.0)	1.23 (d, J = 6.0)
MeO–C(3^{I})	3.43 (s)			
Moiety II	D-Ole[c])	D-Ole	D-Ole	D-Ole
H–C(1^{II})	4.47 (d, J = 7.8)	4.52 (d, J = 9.9)	4.49 (d, J = 8.7)	4.48 (d, J = 8.7)
H–C(2^{II})	1.47–1.49 (m), 2.17–2.19 (m)	1.43–1.46 (m), 2.14–2.17 (m)	1.47–1.50 (m), 2.30–2.33 (m)	1.44–1.47 (m), 2.29–2.32 (m)
H–C(3^{II})	3.14–3.16 (m)	3.17–3.19 (m)	3.23–3.25 (m)	3.23–3.25 (m)
H–C(4^{II})	3.09–2.12 (m)	3.10–3.12 (m)	3.07–3.09 (m)	3.07–3.09 (m)
H–C(5^{II})	3.26–3.28 (m)	3.29–3.31 (m)	3.27–3.29 (m)	3.30–3.32 (m)
Me(6^{II})	1.30 (d, J = 6.2)	1.31 (d, J = 6.0)	1.25 (d, J = 6.0)	1.25 (d, J = 6.0)
MeO–C(3^{II})	3.36 (s)	3.38 (s)	3.35 (s)	3.34 (s)
Moiety III			L-Cym	L-Cym
H–C(1^{III})			4.84 (d, J = 3.0)	4.84 (d, J = 3.0)
H–C(2^{III})			1.70–1.73 (m), 2.20–2.23 (m)	1.72–1.76 (m), 2.20–2.23 (m)
H–C(3^{III})			3.55–2.58 (m)	3.67–3.70 (m)
H–C(4^{III})			3.27–3.29 (m)	3.57–3.59 (m)
H–C(5^{III})			4.05–4.07 (m)	4.25–4.28 (m)
Me(6^{III})			1.22 (d, J = 6.5)	1.22 (d, J = 6.4)
MeO–C(3^{III})			3.36 (s)	3.38 (s)
Moiety IV				D-Cym
H–C(1^{IV})				4.76 (d, J = 8.7)
H–C(2^{IV})				1.57–1.59 (m), 2.27–2.29 (m)
H–C(3^{IV})				3.60–3.64 (m)
H–C(4^{IV})				3.20–3.23 (m)
H–C(5^{IV})				3.53–3.55 (m)
Me(6^{IV})				1.25 (d, J = 6.4)
MeO–C(3^{IV})				3.40 (s)
Moiety V				
H–C(1^{V})				
H–C(2^{V})				
H–C(3^{V})				
H–C(4^{V})				
H–C(5^{V})				
Me(6^{V})				
MeO–C(3^{V})				

[a]) Cym: cymaropyranosyl; [b]) Digit: digitoxopyranosyl; [c]) Ole: oleandropyranosyl; [d]) Digin: diginopyranosyl.

Table 4 (cont.)

5	**6**	**7**	**8**
D-Digit	D-Digit	D-Cym	D-Cym
4.93 (*d*, *J* = 9.9)	4.92 (*d*, *J* = 9.9)	4.77 (*d*, *J* = 8.1)	4.75 (*d*, *J* = 8.1)
1.67 – 1.70 (*m*),	1.66 – 1.69 (*m*),	1.52 – 1.55 (*m*),	1.52 – 1.55 (*m*),
2.09 – 2.11 (*m*)	2.05 – 2.07 (*m*)	2.13 – 2.16 (*m*)	2.13 – 2.16 (*m*)
4.19 – 4.21 (*m*)	4.20 – 4.23 (*m*)	3.62 – 3.65 (*m*)	3.64 – 3.66 (*m*)
3.19 – 3.22 (*m*)	3.20 – 3.23 (*m*)	3.17 – 3.19 (*m*)	3.17 – 3.19 (*m*)
3.78 – 3.81 (*m*)	3.77 – 3.79 (*m*)	3.70 – 3.74 (*m*)	3.70 – 3.74 (*m*)
1.24 (*d*, *J* = 6.0)	1.26 (*d*, *J* = 6.0)	1.20 (*d*, *J* = 6.3)	1.12 (*d*, *J* = 6.3)
		3.44 (*s*)	3.38 (*s*)
D-Ole	D-Ole	L-Digin[d])	L-Digin
4.48 (*d*, *J* = 9.9)	4.50 (*d*, *J* = 9.9)	4.93 (*d*, *J* = 3.0)	4.93 (*d*, *J* = 3.0)
1.44 – 1.47 (*m*),	1.48 – 1.51 (*m*),	1.79 – 1.81 (*m*),	1.79 – 1.81 (*m*),
2.30 – 2.33 (*m*)	2.30 – 2.33 (*m*)	1.99 – 2.01 (*m*)	1.99 – 2.01 (*m*)
3.27 – 3.29 (*m*)	3.25 – 3.27 (*m*)	3.55 – 3.57 (*m*)	3.56 – 3.58 (*m*)
3.07 – 3.09 (*m*)	3.07 – 3.10 (*m*)	3.81 – 3.84 (*m*)	3.81 – 3.84 (*m*)
3.27 – 3.29 (*m*)	3.30 – 3.33 (*m*)	3.89 – 3.91 (*m*)	3.90 – 3.92 (*m*)
1.22 (*d*, *J* = 6.0)	1.24 (*d*, *J* = 6.0)	1.21 (*d*, *J* = 6.0)	1.19 (*d*, *J* = 6.0)
3.32 (*s*)	3.38 (*s*)	3.44 (*s*)	3.40 (*s*)
L-Cym	L-Cym	D-Cym	D-Cym
4.82 (*d*, *J* = 3.0)	4.82 (*d*, *J* = 3.0)	4.75 (*d*, *J* = 8.7)	4.64 (*d*, *J* = 8.1)
1.72 – 1.75 (*m*),	1.70 – 1.74 (*m*),	1.72 – 1.76 (*m*),	1.60 – 1.63 (*m*),
2.20 – 2.22 (*m*)	2.21 – 2.24 (*m*)	2.27 – 2.29 (*m*)	2.35 – 2.37 (*m*)
3.72 – 3.75 (*m*)	3.52 – 3.55 (*m*)	3.74 – 3.76 (*m*)	3.64 – 3.67 (*m*)
3.55 – 3.57 (*m*)	3.70 – 3.74 (*m*)	3.25 – 3.27 (*m*)	3.25 – 3.27 (*m*)
4.26 – 4.29 (*m*)	4.24 – 4.28 (*m*)	3.82 – 3.85 (*m*)	3.52 – 3.56 (*m*)
1.18 (*d*, *J* = 6.5)	1.20 (*d*, *J* = 6.5)	1.14 (*d*, *J* = 6.5)	1.26 (*d*, *J* = 6.5)
3.46 (*s*)	3.46 (*s*)	3.39 (*s*)	3.38 (*s*)
D-Cym	D-Cym	L-Cym	
4.86, (*d*, *J* = 8.7)	4.85, (*d*, *J* = 8.7)	4.76 (*d*, *J* = 3.0)	
1.60 – 1.63 (*m*),	1.60 – 1.63 (*m*),	1.70 – 1.72 (*m*),	
2.14 – 2.17 (*m*)	2.14 – 2.16 (*m*)	2.23 – 2.25 (*m*)	
3.70 – 3.71 (*m*)	3.67 – 3.69 (*m*)	3.55 – 3.57 (*m*)	
3.23 – 3.25 (*m*)	3.23 – 3.25 (*m*)	3.23 – 3.25 (*m*)	
3.83 – 3.85 (*m*)	3.82 – 3.84 (*m*)	4.00 – 4.02 (*m*)	
1.22 (*d*, *J* = 6.4)	1.24 (*d*, *J* = 6.0)	1.20 (*d*, *J* = 6.4)	
3.35 (*s*)	3.35 (*s*)	3.40 (*s*)	
L-Cym	L-Cym		
4.80 (*d*, *J* = 3.0)	4.79 (*d*, *J* = 3.0)		
1.73 – 1.76 (*m*),	1.70 – 1.73 (*m*),		
2.27 – 2.30 (*m*)	2.27 – 2.30 (*m*)		
3.57 – 3.60 (*m*)	3.57 – 3.60 (*m*)		
3.27 – 3.29 (*m*)	3.27 – 3.29 (*m*)		
4.03 – 4.05 (*m*)	4.03 – 4.05 (*m*)		
1.19 (*d*, *J* = 6.4)	1.21 (*d*, *J* = 6.4)		
3.38 (*s*)	3.43 (*s*)		

between *i*) H–C(3) (δ(H) 3.52–3.55, *m*) of the aglycone and C(1^{I}) (δ(C) 95.9) of the β-D-digitoxopyranosyl moiety; *ii*) H–C(4^{I}) (δ(H) 3.19–3.22, *m*) of the β-D-digitoxopyranosyl moiety and C(1^{II}) (δ(H) 100.5) of the β-D-oleandropyranosyl moiety [21]. Therefore, **2** was elucidated as 3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl 12-*O*-cinnamoyl-20-*O*-isovaleroylsarcostin.

Wilfoside C (**3**) was isolated as a colorless, amorphous powder. The molecular formula was determined as $C_{48}H_{70}O_{17}$ by HR-ESI-MS. The aglycone of **3** was identified as qingyangshengenin by comparing its spectroscopic data with those of known C_{21} sterodial aglycones [10] as well as upon acid hydrolysis.

Comparing the structure of the sugar moiety of **3** with that of **2**, there was one more sugar unit linked with the β-D-oleandropyranosyl. The coupling constant of the anomeric H-atom signal of the third sugar (δ(H) 4.84, *d*, $J = 3.0$) indicated an α-configuration. On acid hydrolysis, **3** afforded cymarose, digitoxose, and oleandrose as component sugars. According to the literatures [4–22], the chemical shift of C(2) is < 33.0 ppm in an L-cymaropyranosyl unit and > 35.0 ppm in a D-cymaropyranosyl unit [10]. The ^{13}C-NMR signals of the third sugar unit assigned by HSQC and HMBC analyses, and in particular the resonance of C(2^{III}) at δ(C) 31.1, indicated the presence of one α-L-cymaropyranosyl moiety. The *OR* detection exhibited a negative signal for the cymarose, which further confirmed the L-configuration of cymarose. Following the methodology described above, the sequence of the sugar moieties was assigned from HSQC and HMBC analysis using the well-defined anomeric H-atoms as starting signals. Therefore, **3** was elucidated as 3-*O*-α-L-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl qingyangshengenin.

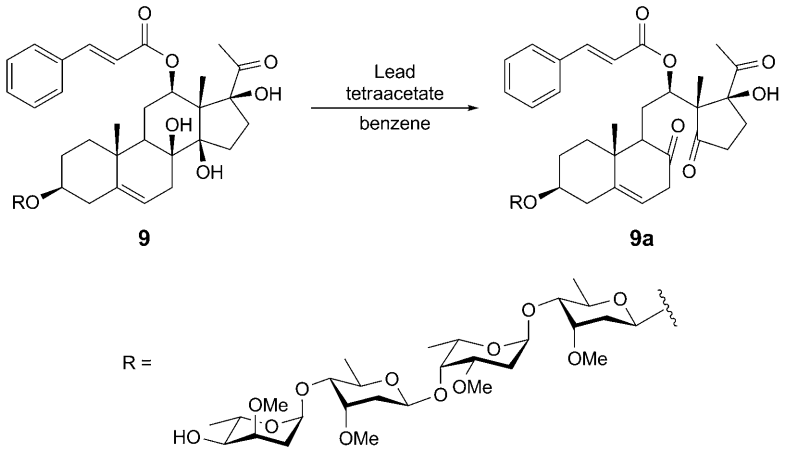
Wilfoside D (**4**) was isolated as a colorless, amorphous powder. The positive HR-ESI-MS gave the molecular formula $C_{55}H_{82}O_{20}$. Compound **4** was determined to possess the same aglycone as **3** from the NMR data and the acid hydrolysis experiment, and the structure of the sugar moiety was corresponding to that of **3** except that one more sugar unit was linked to the α-L-cymaropyranosyl moiety. The coupling constant of the anomeric H-atom signal in the fourth sugar (δ(H) 4.76, *d*, $J = 8.7$) indicated a β-configuration. The chemical shifts and TLC behavior suggested that the sugar was a β-D-cymaropyranosyl moiety. Similarly as what was carried out on **3**, HPLC analysis revealed that both D- and L-cymaroses occurred in **4** since *OR* of the cymarose was detected to be zero. Following the same methodology described above, the sequence of the sugar moieties of **4** was assigned. Therefore, **4** was elucidated as 3-*O*-β-D-cymaropyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl qingyangshengenin.

Wilfoside E (**5**) was isolated as a colorless, amorphous powder. Based on the HR-ESI-MS data, the molecular formula was established as $C_{62}H_{94}O_{23}$. The NMR of **5** were similar to **4**, indicating the same aglycone, and the sugar moiety in **5** was corresponding to that of **4** except that one more sugar unit was linked to the β-D-cymaropyranosyl moiety. The sugar unit was identified as α-L-cymaropyranosyl by the ^{1}H-, ^{13}C-NMR, TLC, and HPLC data. An extensive study of the HMBC experiment enabled to establish the linkage positions and sequence of the sugar moieties of **5**. Therefore, **5** was elucidated as 3-*O*-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl qingyangshengenin.

Wilfoside F (**6**) was isolated as a colorless, amorphous powder. The molecular formula was obtained as $C_{62}H_{100}O_{22}$ by HR-ESI-MS. The NMR data analysis of **6** indicated that it possessed the same steroid part and sugar chain as those of **5**. The difference was that there was an ikemaoyl group instead of a *p*-hydroxybenzoyl substituent at C(12). The aglycone of **6** was determined to be caudatin (*Table 1*) by comparing the NMR data with those of known C_{21} steroidal aglycones [18]. The structure of **6** was further confirmed by acid hydrolysis, HSQC, HMBC (*Fig. 2*), and ^{1}H,^{1}H-COSY experiments to be 3-*O*-*α*-L-cymaropyranosyl-(1 → 4)-*β*-D-cymaropyranosyl-(1 → 4)-*α*-L-cymaropyranosyl-(1 → 4)-*β*-D-oleandropyranosyl-(1 → 4)-*β*-D-digitoxopyranosyl caudatin.

Wilfoside G (**7**) was isolated as a colorless, amorphous powder. The molecular formula was established as $C_{60}H_{88}O_{20}$ by HR-ESI-MS. When comparing the NMR data of **7** with those of wilfoside KIN (**9**) [12], the two O-bearing quaternary C-atom signals C(8) and C(14) (δ(C) 74.2 and 88.1) of **9** replaced by two CO group (δ(C) 209.5 and 217.0) signals in **7**, suggesting the oxidation of the two OH groups. Oxidative cleavage of the 8*β*,14*β*-diol of **9** (30 mg) with lead tetraacetate yielded 24.5 mg of diketone product (**9a**) [17] (*Scheme*). Lead tetraacetate is widely used as an oxidizing agent in organic chemistry for 1,2-diol cleavage to obtain ketone products. The reaction should occur in organic solvent such as benzene. The ^{1}H-NMR spectra of **9** and **9a** (*Table 5*) showed different chemical shifts of H–C(12) (δ(H) 5.20 – 5.24, *m*, in **9a** *vs.* δ(H) 4.71 – 4.73, *m*, in **9**) and the HO–C(8/14) (δ(H) 4.36, 4.19 in **9**) were disappeared in **9a**. **9a** was elucidated as 3-*O*-*α*-L-cymaropyranosyl-(1 → 4)-*β*-D-cymaropyranosyl-(1 → 4)-*α*-L-diginopyranosyl-(1 → 4)-*β*-D-cymaropyranosyl kidjoranin-8,14-dione.

Scheme. *Lead Tetraacetate Oxidation of* **9**



The close resemblance of the NMR data of **7** and **9a** indicated that they possessed the same steroid part and sugar chain, but that an acetyl substituent was linked to C(20) in **7**, which was confirmed from the HMBC correlation between C(1′$_{Ac}$) (δ(C) 168.9) and H–C(20) (δ(H) 4.82 – 4.85). A Me group (δ(C) 14.1) was assigned to be linked to

Table 5. *^{1}H-NMR* (400 MHz) *and ^{13}C-NMR* (100 MHz) *Data of Compounds* **9** *and* **9a** *in $CDCl_3$* (δ in ppm, J in Hz)

	9		**9a**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.08–1.11 (*m*), 1.80–1.83 (*m*)	38.8	1.14–1.16 (*m*), 1.77–1.79 (*m*)	37.1
CH_2(2)	1.58–1.62 (*m*), 1.89–1.91 (*m*)	28.9	1.46–1.48 (*m*), 1.93–1.95 (*m*)	29.0
H–C(3)	3.60–3.63 (*m*)	72.7	3.50–3.53 (*m*)	76.9
CH_2(4)	2.23–2.27 (*m*), 2.35–2.37 (*m*)	38.8	2.07–2.09 (*m*), 2.39–2.41 (*m*)	38.1
C(5)		140.5		141.5
H–C(6)	5.38 (br. *s*)	117.8	5.32 (br. *s*)	118.7
CH_2(7)	2.13–2.17 (*m*)	34.3	2.60–2.64 (*m*), 3.01–3.03 (*m*)	41.0
C(8)		74.2		209.7
H–C(9)	1.47–1.51 (*m*)	43.6	2.27–2.29 (*m*)	55.3
C(10)		37.2		42.9
CH_2(11)	1.77–1.79 (*m*), 2.04–2.06 (*m*)	24.2	1.42–1.45 (*m*), 1.85–1.87 (*m*)	23.4
H–C(12)	4.71–4.73 (*m*)	72.1	5.20–5.24 (*m*)	71.8
C(13)		58.0		61.4
C(14)		88.1		214.0
CH_2(15)	2.00–2.01 (*m*)	33.2	2.43–2.45 (*m*), 2.70–2.74 (*m*)	34.1
CH_2(16)	1.90–1.91 (*m*), 2.83–2.86 (*m*)	32.0	1.95–1.97 (*m*), 2.13–2.15 (*m*)	29.5
C(17)		91.5		85.7
Me(18)	1.55 (*s*)	9.5	1.36 (*s*)	14.1
Me(19)	1.09 (*s*)	18.5	1.07 (*s*)	18.9
C(20)		209.5		209.5
Me(21)	2.08 (*s*)	27.5	2.01 (*s*)	27.1
Cin at C(12)				
C(1′)		165.8		166.4
H–C(2′)	6.08 (*d*, J = 15.9)	117.6	6.08 (*d*, J = 15.9)	116.8
H–C(3′)	7.35 (*d*, J = 17.7)	145.5	7.35 (*d*, J = 17.7)	146.3
C(4′)		134.2		134.1
H–C(5′/9′)	7.18–7.21 (*m*)	128.2	7.18–7.21 (*m*)	128.3
H–C(6′/8′)	7.28–7.31 (*m*)	128.9	7.28–7.31 (*m*)	129.1
H–C(7′)	7.30–7.33 (*m*)	130.5	7.30–7.33 (*m*)	130.8
Moiety I: D-Cym				
H–C(1^I)	4.81 (*d*, J = 8.7)	95.7	4.83 (*d*, J = 8.7)	95.9
H–C(2^I)	1.55–1.58 (*m*), 2.10–2.13 (*m*)	34.3	1.57–1.59 (*m*), 2.10–2.13 (*m*)	34.5
H–C(3^I)	3.65–3.68 (*m*)	77.2	3.67–3.69 (*m*)	77.2
H–C(4^I)	3.20–3.22 (*m*)	81.9	3.20–3.23 (*m*)	81.9
H–C(5^I)	3.90–3.93 (*m*)	69.1	3.87–3.89 (*m*)	68.9
Me(6^I)	1.20 (overlap)	18.3	1.22 (overlap)	18.3
MeO–C(3^I)	3.38 (*s*)	57.1	3.42 (*s*)	57.1
Moiety II: L-Digin				
H–C(1^{II})	4.96 (*d*, J = 3.0)	100.9	4.98 (*d*, J = 3.0)	100.9
H–C(2^{II})	1.70–1.73 (*m*), 1.95–1.97 (*m*)	31.1	1.73–1.76 (*m*), 1.93–1.96 (*m*)	31.2
H–C(3^{II})	3.73–3.75 (*m*)	73.9	3.72–3.75 (*m*)	74.0
H–C(4^{II})	3.85–3.88 (*m*)	74.5	3.87–3.89 (*m*)	74.5
H–C(5^{II})	3.94–3.97 (*m*)	66.8	3.96–3.99 (*m*)	66.9
Me(6^{II})	1.20 (overlap)	18.2	1.22 (overlap)	18.2
MeO–C(3^{II})	3.40 (*s*)	55.6	3.41 (*s*)	55.7
Moiety III: D-Cym				
H–C(1^{III})	4.76 (*d*, J = 9.0)	99.4	4.75 (*d*, J = 9.0)	99.4

Table 5 (cont.)

	9		**9a**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(2^{III})	1.70–1.73 (*m*), 2.21–2.23 (*m*)	34.3	1.70–1.71 (*m*), 2.23–2.25 (*m*)	34.5
H–C(3^{III})	3.82–3.85 (*m*)	77.2	3.82–3.85 (*m*)	77.3
H–C(4^{III})	3.26–2.29 (*m*)	81.9	3.26–2.29 (*m*)	81.9
H–C(5^{III})	3.86–3.88 (*m*)	69.1	3.86–3.89 (*m*)	69.1
Me(6^{III})	1.20 (overlap)	18.2	1.22 (overlap)	18.2
MeO–C(3^{III})	3.45 (*s*)	57.5	3.46 (*s*)	57.6
Moiety IV: L-Cym				
H–C(1^{IV})	4.84 (*d*, *J* = 3.0)	98.6	4.83 (*d*, *J* = 3.0)	98.6
H–C(2^{IV})	1.64–1.66 (*m*), 2.17–2.19 (*m*)	31.7	1.64–1.67 (*m*), 2.17–2.19 (*m*)	31.7
H–C(3^{IV})	3.61–3.62 (*m*)	74.9	3.61–3.63 (*m*)	74.9
H–C(4^{IV})	3.23–3.25 (*m*)	72.1	3.23–2.26 (*m*)	72.2
H–C(5^{IV})	4.01–4.03 (*m*)	65.7	4.01–4.04 (*m*)	65.8
Me(6^{IV})	1.20 (overlap)	17.6	1.22 (overlap)	17.6
MeO–C(3^{IV})	3.37 (*s*)	56.4	3.39 (*s*)	56.5

Cin = cinnamoyl; Digin = diginopyranosyl; Cym = cymaropyranosyl; Ole = oleandropyranosyl

C(20) from the cross-peaks of δ(H) 4.82–4.85 (H–C(20)) to δ(C) 14.1 in the HMBC spectrum. Two CO C-atoms were identified as C(8) and C(14) based on the HMBC correlations between C(8) (δ(C) 209.5) and H–C(6) (δ(H) 5.32), CH_2(7) (δ(H) 2.60–2.63, 3.01–3.04), H–C(9) (δ(H) 2.26–2.30), and CH_2(11) (δ(H) 1.43–1.45, 1.85–1.87), and between C(14) (δ(C) 217.0) and CH_2(15) (δ(H) 2.43–2.45, 2.70–2.73) and CH_2(16) (δ(H) 1.95–1.97, 2.13–2.15) [4][23][24]. There were key HMBCs observed between C(5) (δ(C) 141.2) and CH_2(7), between C(2) (δ(C) 28.7) and CH_2(4) (δ(H) 2.07–2.09, 2.39–2.41), between C(9) (δ(C) 55.2) and CH_2(1) (δ(H) 1.13–1.17, 1.76–1.80), and between C(10) (δ(C) 42.7) and CH_2(4) (*Fig. 2*). Therefore, the aglycone of **7** was determined to be 12-*O*-cinnamoyl-20-*O*-acetyl-8,14-secosarcostin-8,14-dione. Compared with NMR data in the literature [23–25], **7** was elucidated as 3-*O*-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranosyl 12-*O*-cinnamoyl-20-*O*-acetyl-8,14-secosarcostin-8,14-dione.

Wilfoside H (**8**) was isolated as a colorless, amorphous powder. The molecular formula was established as $C_{53}H_{77}O_{17}$ by HR-ESI-MS. The similar NMR data of **7** and **8** indicated that they possessed the same aglycone. The chemical shifts of C(4^{III}) (δ(C) 71.9 in **8** *vs.* δ(C) 81.7 in **7**) of the third sugar unit indicated one less β-D-cymaropyranosyl unit than in **7**. On acid hydrolysis, **8** afforded cymarose, diginose, and oleandrose as sugar components. Therefore, **8** was elucidated as 3-*O*-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranosyl 12-*O*-cinnamoyl-20-*O*-acetyl-8,14-secosarcostin-8,14-dione.

Experimental Part

General. All solvents used were of anal. grade (*Shanghai Chemical Plant*). TLC: Silica gel *GF254* (*Yantai Huiyou Inc.*). Column chromatography (CC): *silica gel H* (SiO_2; 200–300 mesh; *Qingdao*

Marine Chemical Ltd.), *Sephadex LH-20* (25–100 μm; *Pharmacia Fine Chemicals*), *MCI* gel *CHP 20P* (high porous polymer, 75–150 μm; *Mitsubishi Chemical Ind.*), and *RP-18* (20–45 μm; *Fuji Silysia Chemical Ltd.*). HPLC Separations: *JASCO PU-2080* HPLC system, equipped with *RI* (*G1632A 1100 RID*) and *OR* (*Shodex OR-2*) detectors. Optical rotations: in MeOH soln.; *Perkin-Elmer PE-241* polarimeter; *JASCO DIP-370* digital polarimeter; in a 0.5 dm length cell. UV Spectra: *Varian CARY 300 Bio* spectrometer; λ_{max} in nm (log ε). IR Spectra: *Perkin-Elmer 16-PC* FT-IR spectrophotometer; in cm^{-1}. ^1H- (400 MHz) and ^{13}C-NMR (100 MHz) Spectra: *Bruker AMX-400* spectrometer; δ in ppm, *J* in Hz, with Me_4Si as internal standard. HR-ESI-MS: *Bruker Daltonics FTMS APEX III* mass spectrometer in *m*/*z*.

Plant Material. The roots of *C. wilfordii* were collected in March 2007 in Hebei Province, P. R. China. A voucher specimen (No. 200706) was deposited with the Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Shanghai.

Extraction and Isolation. The dried roots of *C. wilfordii* (5 kg) were extracted three times with 95% EtOH (40 l) under reflux for 2 h, which afforded a dark residue (600 g) after evaporation. The residue was partitioned between AcOEt (3 l) and H_2O (3 l). The org. layer (200 g after evaporation) was subjected to CC (SiO_2, petroleum ether (PE)/acetone 10:1–1:1, and 100% acetone) to give five fractions (*Frs. A–E*). *Fr. B* was subjected to CC (*MCI* gel; gradient MeOH/H_2O 40:60 → 95:5), then to CC (SiO_2; $CHCl_3$/acetone 4:1), to CC (*RP-18*; MeCN/H_2O 50:50), and to CC (*RP-18*; MeOH/H_2O 70:30) to yield **1** (28 mg), **7** (25 mg), **8** (26 mg), and **9** (70 mg). *Fr. C* was subjected to CC (*MCI* gel, gradient MeOH/H_2O 40:60 → 95:5), then to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1), and to CC (*RP-18*; MeCN/H_2O 45:55) to yield **2** (25 mg) and **6** (16 mg). *Fr. D* (5.0 g) was subjected to CC (*RP-18*; MeCN/H_2O 30:70) to yield **3** (11 mg), **4** (14 mg), and **5** (30 mg).

Acid Hydrolysis. A soln. of **1**–**8** (each 6 mg) in MeOH (5 ml) was treated separately with 0.05M HCl (dioxane/H_2O 1:1, 1 ml) at 60° for 1.5 h. After dioxane was removed, the soln. was extracted with 2 ml of AcOEt for three times. The aq. layer was neutralized by NaOH (1M) and concentrated under reduced pressure to give the sugar fraction. The identification of the monosaccharides in the hydrolysates of each compound was confirmed by TLC comparison with authentic sugars: digitoxose was detected from **2**, **3**, **4**, and **5**; diginose was detected from **7** and **8**; cymarose was detected from **1**, **3**, **4**, **5**, **7**, and **8**; oleandrose was detected from **1** and **2**. The R_f values of digitoxose, diginose, cymarose, and oleandrose were 0.21, 0.37, 0.48, and 0.42 ($CHCl_3$/MeOH 9:1), 0.28, 0.39, 0.51, and 0.48 (CH_2Cl_2/EtOH 9:1), and 0.09, 0.19, 0.23, and 0.20 (PE/Me_2CO 3:2), resp. [5]. The AcOEt-soluble portion was concentrated to dryness to give the crude aglycone. The crude aglycones were identified by TLC comparison with authentic samples (the aglycone of **6** was caudatin, the aglycone of **1** was gagaminin, the aglycone of **2** was 12-*O*-cinnamoyl-20-*O*-isovaleroylsarcostin and the aglycone of **3**, **4**, and **5** was qingyangshengenin).

Determination of the Absolute Configuration of the Monosaccharides. The sugar fractions were obtained from the aq. layer by acid hydrolysis as described above. The absolute configurations were analyzed by HPLC [8][26][27]. Identification of D-cymarose and L-diginose in each sugar fraction was performed by comparing their retention time and optical rotation polarity with those of authentic samples: D-cymarose (t_R 14.687 min, pos. polarity) and L-diginose (t_R 15.794 min, neg. polarity; HPLC conditions: column, *Shodex Asahipak GS-220 HQ*, 300 × 7.5 mm i.d.; flow rate, 0.5 ml/min; column temp., 40°; solvent H_2O; detection with *RI* (Refractive Index Detector *G1632A 1100 RID*) and *OR* (Optical Rotation Detector *Shodex OR-2*) detectors. Cymarose (a mixture of D- and L-form in the ratio 1:1) was detected from **4** and **6**. D-Cymarose was detected from **1**, **7**, and **8**, and L-cymarose was detected from **3**, **5**, and **6**.

$Pb(OAc)_4$ Oxidation of **9**. To a soln. of **9** (30 mg) in 4 ml of benzene, 80 mg of lead tetraacetate was added, and the mixture was stirred for 30 min at r.t. H_2O was added, and the mixture was then extracted with Et_2O. The Et_2O soln. was washed successively with 5% $NaHCO_3$ soln. and H_2O, and dried over $MgSO_4$. After removal of the solvent and purification of the residue, 24.5 mg of diketone **9a** was obtained [17]. *(1*R*)-1-[(1*R,2S*)-2-Acetyl-2-hydroxy-1-methyl-5-oxocyclopentyl]-2-[(6*S,8*a*R*)-6-{[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}-8a-methyl-2-oxo-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-yl]ethyl (2*E*)-3-phenylprop-2-enoate* (**9a**). Colorless, amorphous powder. $[\alpha]_D^{20} = -53$ ($c = 0.3$, MeOH). UV (MeOH): 218 (4.20), 223 (4.16), 282

(4.40). IR (KBr): 3440, 2935, 1733, 1715, 1635, 1450, 1734, 1252, 1163, 1100, 1057. ^{1}H-NMR: *Table 5*. ^{13}C-NMR: *Table 5*. HR-ESI-MS: 1107.5468 ($[M+Na]^+$, $C_{53}H_{77}NaO_{17}^+$; calc. 1107.5454).

Wilfoside A (=*(3β,12β,14β,17α)-3-{[2,6-Dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-β-*D*-arabino-hexopyranosyl)-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 Pyridine-3-carboxylate*; **1**). Colorless, amorphous powder. $[\alpha]_D^{20} = +118$ ($c =$ 0.3, MeOH). UV (MeOH): 217 (4.44), 280 (4.37). IR (KBr): 3438, 2933, 1716, 1637, 1450. ^{1}H-NMR: *Tables 3* and *4*. ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 906.4648 ($[M+H]^+$, $C_{50}H_{68}NO_{14}^+$; calc. 906.4640).

Wilfoside B (=*(3β,12β,14β,17α)-3-{[2,6-Dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-β-*D*-arabino-hexopyranosyl)-β-*D*-ribo-hexopyranosyl]oxy}-8,14,17-trihydroxy-12-{[(2*E*)-3-phenylprop-2-enoyl]oxy}pregn-5-en-20-yl 3-Methylbutanoate*; **2**). Colorless, amorphous powder. $[\alpha]_D^{20} = +41$ ($c = 0.3$, MeOH). UV (MeOH): 205 (4.09), 217 (3.73), 278 (4.69). IR (KBr): 3446, 2933, 1710, 1637, 1450. ^{1}H-NMR: *Tables 3* and *4*. ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 893.4667 ($[M + Na]^+$, $C_{48}H_{70}NaO_{14}^+$; calc. 893.4663).

Wilfoside C (=*(3β,12β,14β,17α)-3-{[2,6-Dideoxy-3-*O*-methyl-α-*L*-ribo-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-arabino-hexopyranosyl-(1 → 4)-2,6-dideoxy-β-*D*-ribo-hexopyranosyl]oxy}-8,14,17-trihydroxy-20-oxopregn-5-en-12-yl 4-Hydroxybenzoate*; **3**). Colorless, amorphous powder. $[\alpha]_D^{20} = -27$ ($c = 0.3$, MeOH). UV (MeOH): 212 (3.40), 255 (4.14). IR (KBr): 3446, 2933, 1710, 1610, 1450. ^{1}H-NMR: *Tables 3* and *4*. ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 941.4524 ($[M+Na]^+$, $C_{48}H_{70}NaO_{17}^+$; calc. 941.4511).

Wilfoside D (=*(3α,12β,14β,17α)-3-{[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*-arabino-hexopyranosyl-(1 → 4)-2,6-dideoxy-β-*D*-ribo-hexopyranosyl]oxy}-8,14,17-trihydroxy-20-oxopregn-5-en-12-yl 4-Hydroxybenzoate*; **4**). Colorless, amorphous powder. $[\alpha]_D^{20} = -36$ ($c = 0.3$, MeOH). UV (MeOH): 212 (3.44), 255 (4.18). IR (KBr): 3453, 2933, 1712, 1610, 1450. ^{1}H-NMR *Tables 3* and *4*. ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 1085.5305 ($[M+Na]^+$, $C_{55}H_{82}NaO_{20}^+$; calc. 1085.5297).

Wilfoside E (=*(3α,12β,14β,17α)-3-{[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*-arabino-hexopyranosyl-(1 → 4)-2,6-dideoxy-β-*D*-ribo-hexopyranosyl]oxy}-8,14,17-trihydroxy-20-oxopregn-5-en-12-yl 4-Hydroxybenzoate*; **5**). Colorless, amorphous powder. $[\alpha]_D^{20} = -56$ ($c = 0.3$, MeOH). UV (MeOH): 212 (3.41), 255 (4.15). IR (KBr): 3448, 2933, 1712, 1610, 1450. ^{1}H-NMR *Tables 3* and *4*. ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 1229.6079 ($[M + Na]^+$, $C_{62}H_{94}NaO_{23}^+$; calc. 1229.6084).

Wilfoside F (=*(3α,12β,14β,17α)-3-{[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*-arabino-hexopyranosyl-(1 → 4)-2,6-dideoxy-β-*D*-ribo-hexopyranosyl]oxy}-8,14,17-trihydroxy-20-oxopregn-5-en-12-yl (2*E*)-3,4-Dimethylpent-2-enoate*; **6**). Colorless, amorphous powder. $[\alpha]_D^{20} = -58$ ($c = 0.3$, MeOH). UV (MeOH): 225 (0.79), 272 (4.76). IR (KBr): 3446, 1714, 1641. ^{1}H-NMR: *Tables 3* and *4*. ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 1219.6611 ($[M + Na]^+$, $C_{62}H_{100}NaO_{22}^+$; calc. 1219.6604).

Wilfoside G (=*(1*R*)-1-{(1*R*,2*S*)-2-[(1*S*)-1-(Acetyloxy)ethyl]-2-hydroxy-1-methyl-5-oxocyclopentyl}-2-[(1*R*,6*S*,8a*R*)-6-{[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}-8a-methyl-2-oxo-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-yl]ethyl (2*E*)-3-Phenylprop-2-enoate*; **7**). Colorless, amorphous powder. $[\alpha]_D^{20} = -58$ ($c = 0.3$, MeOH). UV (MeOH): 217 (2.95), 272 (3.61), 278 (3.64). IR (KBr): 3448, 2933, 1716, 1637, 1450. ^{1}H-NMR: *Tables 3* and *4*. ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 1151.5781 ($[M+Na]^+$, $C_{60}H_{88}NaO_{20}^+$; calc. 1151.5767).

Wilfoside H (=*(1*R*)-1-{(1*R*,2*S*)-2-[(1*S*)-1-(Acetyloxy)ethyl]-2-hydroxy-1-methyl-5-oxocyclopentyl}-2-[(1*R*,6*S*,8a*R*)-6-{[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}-8a-methyl-2-oxo-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-yl]ethyl (2*E*)-3-Phenylprop-2-enoate*; **8**). Colorless, amorphous powder. $[\alpha]_D^{20} = -19$ ($c = 0.3$, MeOH). UV (MeOH): 218 (2.94), 273 (3.62), 278 (3.63). IR (KBr): 3448, 2933, 1733, 1714, 1637, 1450. ^{1}H-NMR: *Tables 3* and *4*. ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 985.5150 ($[M+H]^+$, $C_{53}H_{77}O_{17}^+$; calc. 985.5161).

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