

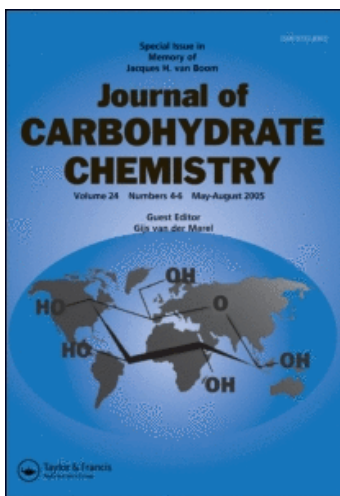
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Isolation and Structural Study on Carbohydrates from *Cynanchum otophyllum* and *Cynanchum paniculatum*

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Three new carbohydrates were isolated from the acidic hydrolysis part of the ethyl acetate extract of *Cynanchum otophyllum* Schneid (Asclepiadaceae) and one new carbohydrate from the ethyl acetate extract of *Cynanchum paniculatum* Kitagawa. Their structures were determined as methyl 2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- β -D-*arabino*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranoside (**1**), ethyl 2,6-dideoxy-3-*O*-methyl- β -D-*ribo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -L-*lyxo*-hexopyranoside (**2**), methyl 2,6-dideoxy-3-*O*-methyl- α -L-*ribo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- β -D-*lyxo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranoside (**3**), and 2,6-dideoxy-3-*O*-methyl- β -D-*ribo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranose (**4**), respectively, by spectral methods.

Keywords *Cynanchum otophyllum*; *Cynanchum paniculatum*; Spectroscopy; Hydrolysate; Carbohydrates

INTRODUCTION

Cynanchum otophyllum Schneid (Qingyangshen) and *Cynanchum paniculatum* Kitagawa are two plants of the genus *Cynanchum* L. (Asclepiadaceae)

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and two traditional Chinese medicines distributed extensively over southwest China and most areas of China, respectively. Pharmacodynamic and clinical experiments have established that the chloroform extract and the ethyl acetate extract of the rhizome of *C. otophyllum* are particularly effective against epilepsy and chronic hepatitis.^[1–8] Since 1984, Qingyangshen tablets (the total saponins of *C. otophyllum*) have been manufactured by Lijiang Pharmaceutical Co., Yunnan Baiyao Group, Lijiang, Yunnan, China. The steroidal constituents from the genus *Cynanchum* L. have been reported.^[9] From the rhizome of *C. otophyllum*, Mu et al. isolated nine constituents including two C₂₁ steroidal saponins and digitoxose.^[10–12] Moreover, Mu and coworkers developed *C. otophyllum* into three patents.^[13–15] For maintaining the lead in the research into *C. otophyllum*, the authors carried out further investigations, which were very important. However, most compounds in the total saponins were difficult to be separated. To study the compounds, the authors used the acidic hydrolysis reaction universal in the research on saponins to obtain secondary saponins that are easy to separate. From the total saponins (the ethyl acetate extract of the rhizome of *C. otophyllum*) and their acidic hydrolysis part, the authors isolated four new carbohydrates^[16] and three new C₂₁ steroidal saponins.^[17,18] Seven constituents were isolated from the rhizome.^[19] Furthermore, this article reports three new carbohydrates obtained from the same acidic hydrolysis part: methyl 2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- β -D-*arabino*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranoside (**1**), ethyl 2,6-dideoxy-3-*O*-methyl- β -D-*ribo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -L-*lyxo*-hexopyranoside (**2**), and methyl 2,6-dideoxy-3-*O*-methyl- α -L-*ribo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- β -D-*lyxo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranoside (**3**).

Since 100 g crude aglycones was obtained from 500g total glycosides, the acidic hydrolysis reaction was not complete. Consequently, some C₂₁ steroidal glycosides were hydrolyzed to produce methyl glycosides, such as **1** and **3**, in the presence of methanol (as solvent), while some oligosaccharides (oligosaccharides were a little part of the total glycosides of *C. otophyllum*), such as **2**, did not change. Thus, compound **2** was an ethyl glycoside in the presence of methanol, and a natural product. Considering their structures, compounds **1** and **3** were fragments of corresponding C₂₁ steroidal saponins.

To investigate the differences of constituents between *C. otophyllum* and *C. paniculatum*, we separated the ethyl acetate extract of *C. paniculatum*, and obtained one new carbohydrate: 2,6-dideoxy-3-*O*-methyl- β -D-*ribo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranose (**4**). This was a natural product, but it might also be a fragment of corresponding C₂₁ steroidal saponins.

RESULTS AND DISCUSSION

Carbohydrate **1** was obtained as a white powder. The molecular formula was determined as $C_{22}H_{40}O_{10}$ by HRFABMS. The ^{13}C NMR and DEPT spectra showed 7 methyls, 3 methylenes, and 12 methines. The anomeric carbon resonances at δ_C 99.5, 102.2, and 100.4 revealed the presence of three sugar residues. In Table 1, the proton at δ 4.87 correlated with the signal at δ 99.5 in the HMQC, and had a correlation with H-2^I in the 1H - 1H COSY. The assignment for C-2^I (δ 36.3) was obtained from the correlation with H-2^I (δ 1.82; 2H) in the HMQC. These protons had correlations with H-1^I and H-3^I in the 1H - 1H COSY, from which C-3^I (δ 76.2) was obtained. In this case, the carbons at δ 99.5, 36.3, 76.2, 83.1, 69.0, and 18.4 were determined to be the carbons of the sugar residue I by 1H - 1H COSY and HMQC. The methoxy group (δ 56.0, MeO-1^I) was located by the correlation of the resonance of δ 3.27, with C-1^I in the HMBC spectrum. MeO-3^I (58.6) was located by the correlation of the signal of δ 58.6, with H-3^I in the HMBC. The ^{13}C NMR data of the carbons of the sugar were compared with those in the literature,^[20] and the sugar was determined to be methyl 2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranoside

Table 1: NMR data for carbohydrate **1** in C_5D_5N

Carbon	^{13}C	$^1H^a$	1H - 1H COSY	HMBC
1-O-Me-α-D-Ole				
C-1 ^I	99.5 d	4.87 d (10.0)	H-2 ^I	C-2 ^I ; MeO-1 ^I
C-2 ^I	36.3 f	1.82 m; 2H	H-1 ^I ; H-3 ^I	—
C-3 ^I	76.2 d	3.47 m	H-2 ^I ; H-4 ^I	C-5 ^I ; C-6 ^I ; MeO-3 ^I
C-4 ^I	83.1 d	3.47 m	H-3 ^I ; H-5 ^I	C-5 ^I ; C-6 ^I
C-5 ^I	69.0 d	4.23 m	H-4 ^I ; H-6 ^I	—
C-6 ^I	18.4 q	1.48 m; 3H	H-5 ^I	—
MeO-1 ^I	56.0 q	3.27 s; 3H	—	C-1 ^I
MeO-3 ^I	58.6 q	3.57 s; 3H	—	—
β-D-Ole				
C-1 ^{II}	102.2 d	4.76 d (10.0)	H-2 ^{II}	C-4 ^{II} ; C-2 ^{II}
C-2 ^{II}	37.2 f	2.57 m; 2H	H-1 ^{II} ; H-3 ^{II}	C-3 ^{II}
C-3 ^{II}	81.4 d	3.47 m	H-2 ^{II} ; H-4 ^{II}	C-5 ^{II} ; C-6 ^{II}
C-4 ^{II}	83.2 d	3.47 m	H-3 ^{II} ; H-5 ^{II}	C-3 ^{II} ; C-5 ^{II} ; C-6 ^{II} ; C-1 ^{III}
C-5 ^{II}	72.9 d	3.62 m	H-4 ^{II} ; H-6 ^{II}	—
C-6 ^{II}	18.5 q	1.56 m; 3H	H-5 ^{II}	C-5 ^{II}
MeO-3 ^{II}	57.0 q	3.45 m; 3H	—	C-3 ^{II}
α-D-Ole				
C-1 ^{III}	100.4 d	5.10 d (10.0)	H-2 ^{III}	C-4 ^{III} ; C-2 ^{III} ; C-4 ^{III}
C-2 ^{III}	36.8 f	1.85 m; 2H	H-1 ^{III}	C-1 ^{III}
C-3 ^{III}	77.8 d	4.04 m	H-4 ^{III}	C-1 ^{III} ; MeO-3 ^{III}
C-4 ^{III}	76.3 d	3.53 m	H-3 ^{III} ; H-5 ^{III}	C-6 ^{III}
C-5 ^{III}	69.1 d	4.18 m	H-4 ^{III} ; H-6 ^{III}	—
C-6 ^{III}	18.7 q	1.54 m; 3H	H-5 ^{III}	C-3 ^{III} ; C-4 ^{III} ; C-5 ^{III}
MeO-3 ^{III}	58.8 q	3.56 s; 3H	—	C-3 ^{III}

^aCoupling constants are in Hz.

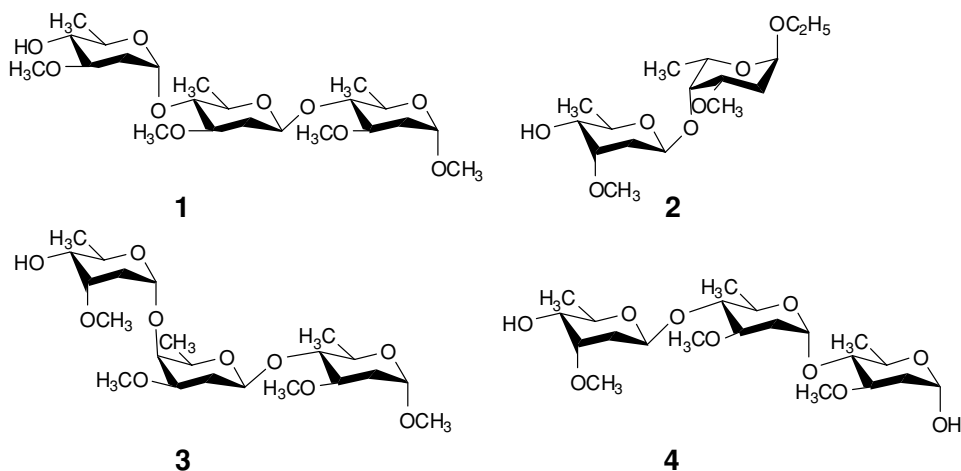


Figure 1: Structures of carbohydrates **1**, **2**, **3**, and **4**.

(methyl α -D-oleandropyranoside). C-4^I was found to be at δ 83.1, and in the HMBC, it showed a long-range correlation with the proton at δ 4.76, which was correlated with the carbon at δ 102.2 in the HMQC. Consequently, the O-4^I was linked with the sugar unit whose anomeric carbon (C-1^{II}) was at δ 102.2. On the basis of the correlations between the protons in the ^1H - ^1H COSY and the long-range correlation of MeO- in the HMBC in Table 1, all of the ^{13}C NMR data of unit II were determined. The data were compared with those in the literature,^[20] and the moiety II was determined to be 2,6-dideoxy-3-*O*-methyl- β -D-*arabino*-hexopyranosyl (β -D-oleandropyranosyl). Since C-4^{II} was apparent at δ 83.2, and it showed a long-range correlation with the proton at δ 5.10 in the HMBC, O-4^{II} was linked with the sugar unit III with the anomeric carbon at δ 100.4. This sugar was determined to be 2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranosyl (α -D-oleandropyranosyl) (Table 1). Since all of the ^{13}C NMR data of this sugar were those in the literature^[20] and there was no remaining sugar, it was the terminal sugar moiety. Therefore, **1** was elucidated as methyl 2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- β -D-*arabino*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranoside (Fig. 1).

Carbohydrate **2** was obtained as a white powder, $\text{C}_{16}\text{H}_{30}\text{O}_7$ from HRESIMS. Two anomeric carbons were observed (δ_{C} 98.1 and 95.4) revealing the presence of two sugar residues. The sugar at δ 98.1 was determined to be ethyl 2,6-dideoxy-3-*O*-methyl- α -L-*lyxo*-hexopyranoside (ethyl α -L-diginopyranoside) in the same way as was previously assigned (Table 2). Since H-4^I (δ 3.80) had a long-range correlation with the resonance at δ 95.4 in the HMBC, and C-4^I was at δ 78.3, O-4^I was linked with the sugar whose anomeric carbon was at δ 95.4. This sugar was determined to be 2,6-dideoxy-3-*O*-methyl- β -D-*ribo*-hexopyranosyl (β -D-cymaropyranosyl) (Table 2).

Table 2: NMR data for carbohydrate **2** in C₅D₅N

Carbon	¹³ C	¹ H ^a	¹ H- ¹ H COSY	HMBC
1-O-Et- α -L-Digin				
C-1 ^I	98.1 <i>d</i>	4.98 m	H-2 ^I	CH ₂ O-1 ^I
C-2 ^I	35.7 <i>t</i>	1.75 m; 2H	H-1 ^I ; H-3 ^I	C-1 ^I
C-3 ^I	74.5 <i>d</i>	3.82 s	H-2 ^I ; H-4 ^I	C-5 ^I ; C-6 ^I
C-4 ^I	78.3 <i>d</i>	3.80 m	H-3 ^I ; H-5 ^I	C-1 ^{II}
C-5 ^I	69.4 <i>d</i>	4.21 m	H-4 ^I ; H-6 ^I	—
C-6 ^I	18.9 <i>q</i>	1.53 m; 3H	H-5 ^I	C-3 ^I ; C-4 ^I ; C-5 ^I
CH ₂ O-1 ^I	64.2 <i>t</i>	3.47 m; 3.97 m	H-Me-EtO-1 ^I	C-1 ^I ; Me-EtO-1 ^I
Me-EtO-1 ^I	15.5 <i>q</i>	1.13 m; 3H	H-CH ₂ O-1 ^I	CH ₂ O-1 ^I
MeO-3 ^I	58.2 <i>q</i>	3.43 m; 3H	—	C-3 ^I
β -D-Cym				
C-1 ^{II}	95.4 <i>d</i>	5.18 d (9.5)	H-2 ^{II}	C-2 ^{II} ; C-3 ^{II}
C-2 ^{II}	35.7 <i>t</i>	1.82 m; 2H	H-1 ^{II} ; H-3 ^{II}	C-1 ^{II}
C-3 ^{II}	78.8 <i>d</i>	3.76 m	H-2 ^{II} ; H-4 ^{II}	C-1 ^{II} ; C-6 ^{II}
C-4 ^{II}	74.2 <i>d</i>	3.54 m	H-3 ^{II} ; H-5 ^{II}	—
C-5 ^{II}	71.1 <i>d</i>	4.09 m	H-4 ^{II} ; H-6 ^{II}	—
C-6 ^{II}	19.0 <i>q</i>	1.51 m; 3H	H-5 ^{II}	C-4 ^{II} ; C-5 ^{II}
MeO-3 ^{II}	58.2 <i>q</i>	3.48 m; 3H	—	C-3 ^{II}

^aCoupling constants are in Hz.

Since all of the ¹³C NMR data of this sugar were those in the literature,^[20] and there was no remaining sugar, it was assigned as the terminal sugar moiety. Therefore, **2** was elucidated as ethyl 2,6-dideoxy-3-*O*-methyl- β -D-*ribo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -L-*lyxo*-hexopyranoside (Fig. 1).

Carbohydrate **3** was obtained as a white powder, C₂₂H₄₀O₁₀ from HRFABMS. The anomeric carbon resonances at δ_C 99.5, 100.4, and 98.2 revealed the presence of three sugar residues. The sugar at δ 99.5 was determined to be methyl 2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranoside (methyl α -D-oleandropyranoside) in the same way as was previously assigned (Table 3). C-4^I was found to be at δ 83.1, and in the HMBC, it displayed a long-range correlation with the proton at δ 5.06, which itself was correlated with the carbon at δ 100.4 in the HMQC. Consequently, the O-4^I was linked with the sugar unit whose anomeric carbon (C-1^{II}) was at δ 100.4. This sugar was determined to be 2,6-dideoxy-3-*O*-methyl- β -D-*lyxo*-hexopyranosyl (β -D-diginopyranosyl) (Table 3). Since the resonance of C-4^{II} was at δ 74.1, the O-4^{II} was linked with the remaining sugar unit III. This sugar was determined to be 2,6-dideoxy-3-*O*-methyl- α -L-*ribo*-hexopyranosyl (α -L-cymaropyranosyl) (Table 3). C-4^{III} was apparent at δ 73.7, and there was no remaining sugar, so it was assigned as the terminal sugar moiety. Therefore, **3** was elucidated as methyl 2,6-dideoxy-3-*O*-methyl- α -L-*ribo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- β -D-*lyxo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranoside (Fig. 1).

Table 3: NMR data for carbohydrate **3** in C₅D₅N

Carbon	¹³ C	¹ H ^a	¹ H- ¹ H COSY	HMBC
1-O-Me-α-D-Ole				
C-1 ^I	99.5 <i>d</i>	4.88 d (2.0)	H-2 ^I	C-2 ^I ; MeO-1 ^I
C-2 ^I	36.2 <i>t</i>	1.75 m; 2.21 m	H-1 ^I ; H-3 ^I	—
C-3 ^I	77.7 <i>d</i>	4.03 m	H-2 ^I ; H-4 ^I	—
C-4 ^I	83.1 <i>d</i>	3.48 m	H-3 ^I ; H-5 ^I	C-5 ^I ; C-6 ^I
C-5 ^I	69.1 <i>d</i>	4.15 m	H-4 ^I ; H-6 ^I	—
C-6 ^I	18.5 <i>q</i>	1.35 t (5.2); 3H	H-5 ^I	C-4 ^I ; C-5 ^I
MeO-1 ^I	56.1 <i>q</i>	3.44 m; 3H	—	C-1 ^I
MeO-3 ^I	58.5 <i>q</i>	3.52 d (0.6); 3H	—	C-3 ^I
β-D-Digin				
C-1 ^{II}	100.4 <i>d</i>	5.06 m	H-2 ^{II}	C-4 ^{II} ; C-5 ^{II}
C-2 ^{II}	35.9 <i>t</i>	1.79 m; 2.37 d (13.6)	H-1 ^{II} ; H-3 ^{II}	—
C-3 ^{II}	78.8 <i>d</i>	3.73 d (2.8)	H-2 ^{II} ; H-4 ^{II}	—
C-4 ^{II}	74.1 <i>d</i>	3.52 m	H-3 ^{II} ; H-5 ^{II}	—
C-5 ^{II}	70.9 <i>d</i>	4.10 m	H-4 ^{II} ; H-6 ^{II}	—
C-6 ^{II}	19.0 <i>q</i>	1.54 s; 3H	H-5 ^{II}	C-4 ^{II} ; C-5 ^{II}
MeO-3 ^{II}	58.1 <i>q</i>	3.44 m; 3H	—	C-3 ^{II}
α-L-Cym				
C-1 ^{III}	98.2 <i>d</i>	5.01 m	H-2 ^{III}	—
C-2 ^{III}	36.4 <i>t</i>	1.83 m; 2H	H-1 ^{III} ; H-3 ^{III}	—
C-3 ^{III}	75.7 <i>d</i>	3.73 d (2.8)	H-2 ^{III} ; H-4 ^{III}	—
C-4 ^{III}	73.7 <i>d</i>	3.52 m	H-3 ^{III} ; H-5 ^{III}	—
C-5 ^{III}	64.2 <i>d</i>	4.00 m	H-4 ^{III} ; H-6 ^{III}	—
C-6 ^{III}	15.5 <i>q</i>	1.15 s; 3H	H-5 ^{III}	C-5 ^{III}
MeO-3 ^{III}	58.4 <i>q</i>	3.52 m; 3H	—	—

^aCoupling constants are in Hz.

Carbohydrate **4** was obtained as a white powder, C₂₁H₃₈O₁₀ from HRFABMS. Three anomeric carbons were observed (δ_C 99.5, 99.4, and 92.8) revealing the presence of three sugar residues. The sugar at δ 99.5 was determined to be 2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranose (α -D-oleandropyranose) (Table 4). The resonance of C-4^I was at δ 89.4, and its corresponding proton in the HMQC (δ 4.14) had a correlation with H-6^{II} in the NOESY. Consequently, O-4^I was linked with the sugar unit II. This sugar was determined to be 2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranosyl (α -D-oleandropyranosyl) (Table 4). The signal of C-4^{II} was at δ 88.5. Thus, O-4^{II} was linked with the remaining sugar unit whose anomeric carbon was at δ 92.8. This sugar was determined to be 2,6-dideoxy-3-*O*-methyl- β -D-*ribo*-hexopyranosyl (β -D-cymaropyranosyl) (Table 4). Since C-4^{III} was apparent at δ 74.6, and there was no remaining sugar, it was determined to be the terminal sugar moiety. Therefore, **4** was elucidated as 2,6-dideoxy-3-*O*-methyl- β -D-*ribo*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-3-*O*-methyl- α -D-*arabino*-hexopyranose (Fig. 1).

Table 4: NMR data for carbohydrate **4** in C₅D₅N

Carbon	¹³ C	¹ H ^a	¹ H- ¹ H COSY	NOESY	HMBC
<i>α</i> -D-Ole					
C-1 ^I	99.5 <i>d</i>	5.98 t (4.8)	H-2 ^I	H-2 ^I	—
C-2 ^I	41.4 <i>t</i>	2.28 d (1.6); 2.39 s	H-1 ^I ; H-3 ^I	H-3 ^I ; H-6 ^I ; H-MeO-3 ^I	C-1 ^I ; C-3 ^I
C-3 ^I	82.2 <i>d</i>	4.45 m	H-2 ^I	H-2 ^I	—
C-4 ^I	89.4 <i>d</i>	4.14 m	H-5 ^I	H-6 ^I	C-1 ^I ; C-3 ^I
C-5 ^I	68.8 <i>d</i>	4.17 m	H-6 ^I	H-6 ^I	C-1 ^I ; C-3 ^I
C-6 ^I	19.5 <i>q</i>	1.51 dd (6.4, 12.4); 3H	H-5 ^I	H-5 ^I	—
MeO-3 ^I	56.9 <i>q</i>	3.35 s; 3H	—	H-3 ^I ; H-5 ^I ; H-6 ^I	—
<i>α</i> -D-Ole					
C-1 ^{II}	99.4 <i>d</i>	5.89 d (5.2)	H-2 ^{II}	H-2 ^{II}	C-3 ^{II} ; C-4 ^{II}
C-2 ^{II}	40.3 <i>t</i>	2.24 s; 2.34 s	H-1 ^{II} ; H-3 ^{II}	H-3 ^{II} ; H-6 ^{II} ; H-MeO-3 ^{II}	C-1 ^{II} ; C-3 ^{II}
C-3 ^{II}	82.0 <i>d</i>	4.26 m	H-2 ^{II}	H-2 ^{II}	C-1 ^{II}
C-4 ^{II}	88.5 <i>d</i>	4.40 dd (1.6, 3.2)	H-5 ^{II}	H-6 ^{II}	—
C-5 ^{II}	68.1 <i>d</i>	4.05 s	H-4 ^{II}	H-6 ^{II}	—
C-6 ^{II}	20.4 <i>q</i>	1.47 s; 3H	—	H-4 ^{II} ; H-5 ^{II}	C-4 ^{II} ; C-5 ^{II}
MeO-3 ^{II}	57.2 <i>q</i>	3.35 s; 3H	—	H-2 ^{II} ; H-3 ^{II} ; H-6 ^{II}	C-3 ^{II}
<i>β</i> -D-Cym					
C-1 ^{III}	92.8 <i>d</i>	5.48 d (9.2)	H-2 ^{III}	H-2 ^{III} ; H-5 ^{III}	C-2 ^{III}
C-2 ^{III}	37.3 <i>t</i>	1.88 m; 2.47 m	H-1 ^{III} ; H-3 ^{III}	H-3 ^{III} ; H-4 ^{III}	C-1 ^{III} ; C-3 ^{III} ; C-4 ^{III}
C-3 ^{III}	79.3 <i>d</i>	3.79 d (2.8)	H-2 ^{III} ; H-4 ^{III}	H-2 ^{III}	C-1 ^{III} ; C-4 ^{III}
C-4 ^{III}	74.6 <i>d</i>	3.58 s	H-3 ^{III} ; H-5 ^{III}	H-6 ^{III}	C-5 ^{III}
C-5 ^{III}	71.2 <i>d</i>	4.12 m	H-4 ^{III} ; H-6 ^{III}	H-1 ^{III} ; H-6 ^{III}	—
C-6 ^{III}	20.4 <i>q</i>	1.47 s; 3H	H-5 ^{III}	H-4 ^{III} ; H-5 ^{III}	—
MeO-3 ^{III}	58.2 <i>q</i>	3.46 s; 3H	—	H-2 ^{III}	C-3 ^{III}

^aCoupling constants are in Hz.

EXPERIMENTAL

General Methods

FABMS and ESIMS were performed on a VG AutoSpec-3000 spectrometer. Bruker Am-400 and DRX-500 instruments were used to record ¹H NMR and 2D NMR spectra (400 MHz) and ¹³C NMR. Pyridine-*d*₅ (C₅D₅N) was the solvent and the internal standard at rt. Column chromatography (CC) was carried out on silica gel. Silica gel (200–300 mesh) for column chromatography and silica gel plate (GF-254) for thin layer chromatography were the products of Qingdao Haiyang Chemical Group Co., Qingdao, China.

Materials

The rhizomes of *C. otophyllum* and *C. paniculatum* were bought from a drug market in Kunming. They were identified by Dr. Yue-Mao Shen and two voucher specimens (KUN No. 0776933 and 0307938) were deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

Extraction and Isolation

The dried powder of the rhizome of *C. otophyllum* (40 kg) was extracted with EtOH (120 L). The extract was evaporated, was extracted with EtOAc (6 L), and was defatted with petroleum ether (1.4 L). The extract was the total saponins (0.70 kg) (the above completed at the processing factory of the Institute). A part of the total saponins (500 g) was dissolved in 2.25 L of 1:2 MeOH-0.025 mol/L H₂SO₄ in a water bath at 70°C. After 2 h, Ba(OH)₂ solution was added until pH 7; BaSO₄ was filtered. The solution was dried up to give the crude aglycones (100 g).

The crude aglycones (100 g) were separated into 21 fractions (fraction 1 to fraction 21) through column chromatography over silica gel (300 g) by elution with a gradient mixture of CHCl₃-MeOH from CHCl₃ to 100:8.5 (v/v). Fraction 7 (1.6 g, 100:1 CHCl₃-MeOH required) was subjected to CC (71 g) eluting with CHCl₃-MeOH (100:1.5, 100:2), and then to CC (31 g) eluting with petroleum ether-Me₂CO (10:3), and finally to CC (5 g) eluting with petroleum ether-EtOAc (35:65), to afford **1** (1 mg, yield 0.001%). Fraction 3 (4 g, CHCl₃ needed) was subjected to CC (80 g) eluting with CHCl₃-MeOH (100:0, 100:0.5, 100:1, 100:1.5) and to CC (52 g) eluting with petroleum ether-Me₂CO (10:1.5), and seven fractions (fraction 3a to fraction 3g) were obtained. Fraction 3d (11 mg) was subjected to CC (2 g) eluting with petroleum ether-EtOAc (65:35, 4:6), to yield **2** (2 mg, 0.002%). Fraction 3c (66 mg) was subjected to CC (5.5 g) eluting with petroleum ether-EtOAc (7:3, 4:6), to afford **3** (1 mg, 0.001%).

The dried powder of the rhizome of *C. paniculatum* (27 kg) was extracted with EtOAc, and the extract was evaporated, to yield the EtOAc extract (1.5 kg). A part of the extract (70.5 g) was subjected to CC (320 g) eluting with CHCl₃-MeOH (2:0.7, 1:1), to CC (320 g) eluting with CHCl₃-MeOH (2:0.5, 1:1), to CC (320 g) eluting with CHCl₃-MeOH (75:25, 65:35, 50:50), to CC (45 g) eluted with CHCl₃-MeOH (85:15, 8:2, 75:25), to CC (45 g) eluted with CHCl₃-MeOH (8:2), and to CC (20 g) eluted with CHCl₃-MeOH (85:15), to yield **4** (140 mg, 0.20%).

Carbohydrate 1

White powder: HRFABMS [M-1]⁻*m/z*: Calcd for C₂₂H₃₉O₁₀: 463.2543; Found: 463.2580; FABMS *m/z* (%): 463 (16 [M-1]⁻), 325 (80.5), 221 (28.5), 80 (21); ¹H, ¹³C, and 2D NMR data, see Table 1.

Carbohydrate 2

White powder: HRESIMS [M+Na]⁺*m/z*: Calcd for C₁₆H₃₀NaO₇: 357.1889; Found: 357.1862; FABMS *m/z* (%): 333 (2.5 [M-1]⁺), 298 (1.5), 257 (4.5), 173 (1.5), 145 (100), 113 (31.5), 99 (9.5), 85 (13), 69 (12.5), 59 (16); ¹H, ¹³C, and 2D NMR data, see Table 2.

Carbohydrate 3

White powder: HRFABMS [M-1]⁻*m/z*: Calcd for C₂₂H₃₉O₁₀: 463.2543; Found: 463.2524; FABMS *m/z* (%): 463 (12 [M-1]⁻), 408 (35), 325 (76), 255 (100), 221 (25.5), 80 (28); ¹H, ¹³C, and 2D NMR data, see Table 3.

Carbohydrate 4

White powder: HRFABMS [M-1]⁻*m/z*: Calcd for C₂₁H₃₇O₁₀: 449.2387; Found: 449.2398; FABMS *m/z* (%): 449 (9.5 [M-1]⁻), 391 (8), 333 (8), 299 (68), 241 (59.5), 149 (100); ¹H, ¹³C, and 2D NMR data, see Table 4.

CONCLUSION

According to previous research, Qingyangshen tablets were composed of a C₂₁ steroidal aglycone moiety of the 3-OH substitution, and a sugar chain. Thus, biological activities and molecular identifications were two main aspects of the medicinal application of this plant material. The aglycone moieties of natural C₂₁ steroidal glycosides served the function of biological activities of glycosides, while the sugar chains in glycosides served the function of molecular identifications of glycosides. Carbohydrates 1–3 (2 might be a natural product as described above) showed the kinds of sugar residues in the glycosides, which were 2-deoxy-hexose. All of the linkages between them were 1→4 linkages. Since Qingyangshen tablets are particularly effective against epilepsy and chronic hepatitis, glycosides of this structure class may be particularly effective against epilepsy and chronic hepatitis, which may shed light on the activity of Qingyangshen tablets. Further studies are required to receive information regarding structure-activity relationships.

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