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Carbohydrates from *Cynanchum otophyllum*

Yi-Bin Zhao,† Yue-Mao Shen, Hong-Ping He, Yan-Mei Li, Quan-Zhang Mu and Xiao-Jiang Hao*

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming, Yunnan 650204, PR China

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Abstract—Four new carbohydrates were isolated from the acidic hydrolysis part of the ethyl acetate extract of *Cynanchum otophyllum* Schneid (Asclepiadaceae). Their structures were determined as methyl 2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-*ribo*-hexopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-α-L-*ribo*-hexopyranoside (**1**), methyl 6-deoxy-1,3-di-*O*-methyl-β-D-*ribo*-hexosyl-(1→4)-2,6-dideoxy-3-*O*-methyl-α-D-*arabino*-hexopyranoside (**2**), methyl 2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-α-L-*ribo*-hexopyranoside (**3**), and 2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranosyl-(1→4)-2,6-dideoxy-3-*O*-methyl-α-D-*arabino*-hexopyranosyl-(1→4)-2,6-dideoxy-3-*O*-methyl-β-D-*lyxo*-hexopyranose (**4**), respectively, by spectral methods.

Keywords: *Cynanchum otophyllum*; Hydrolysate; Carbohydrates

1. Introduction

Cynanchum otophyllum Schneid, Qingyangshen, is a plant of the genus *Cynanchum* L. (Asclepiadaceae), and a traditional Chinese medicine distributed extensively over Southwest China. Pharmacodynamic and clinical experiments have established that the chloroform extract and the ethyl acetate extract of the rhizome are particularly effective against epilepsy and chronic hepatitis.[1–7] 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.[8] From the rhizome of *C. otophyllum*, Mu et al. isolated nine constituents including two C_{21} steroidal saponins and digitoxose.[9–11] Consequently, Mu and co-workers developed *C. otophyllum* into three novel medicines (Patents of China: ZL 98 1 18938.5, ZL 98 1 18173.2, and ZL 96 1 11270.0). 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 separate. To study these 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 acidic hydrolysis part of the ethyl acetate extract (the total saponins) of the rhizome of *C. otophyllum*, they isolated four new carbohydrates: methyl 2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-*ribo*-hexopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-α-L-*ribo*-hexopyranoside (**1**), methyl 6-deoxy-1,3-di-*O*-methyl-β-D-*ribo*-hexosyl-(1→4)-2,6-dideoxy-3-*O*-methyl-α-D-*arabino*-hexopyranoside (**2**), methyl 2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-α-L-*ribo*-hexopyranoside (**3**), and 2,6-dideoxy-3-*O*-

* Corresponding author. Tel.: +86-871-5219684; fax: +86-871-51502-27; e-mail addresses: zhaoyibin@263.net (Y.-B.Z.); xjhao@mail.kib.ac.cn (X.-J.H.)
† Present address: Pharmaceutical Department, Kunming General Hospital, Chengdu Military Command, Kunming, Yunan, P.R. China.

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methyl-β-D-*arabino*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-α-D-*arabino*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-D-*lyxo*-hexopyranose (**4**). Considering their structures, they are fragments of corresponding C_{21} steroidal saponins.

2. 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 methyl, 3 methylene, and 12 methine groups. The anomeric carbon resonances at δ_C 97.9 d, 100.3 d, and 102.2 d revealed the presence of three sugar residues. In Table 1, the proton at δ 4.65 d correlated with the signal at δ 97.9 d in the HMQC, and had a correlation with H-2^I in the ^1H–^1H COSY. The assignment for C-2^I (δ 33.2 t) was obtained from the correlation with H-2^I (δ 1.77 m and 2.21 d) 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 76.6 d) was obtained. In this case, the carbons at δ 97.9 d, 33.2 t, 76.6 d, 82.2 d, 63.3 d, and 18.0 q, were determined to be the carbons of the sugar residue I by ^1H–^1H COSY and HMQC. The methoxy group (δ 54.9 q, MeO-1^I) was located by the correlation of the resonance of δ 3.26 s, with C-1^I in the HMBC spectrum. MeO-3^I (57.8 q) was located by the correlation of the signal of δ 3.49 m, with C-3^I in the HMBC. The ^{13}C NMR data of the carbons of the sugar were compared with those in the literature,[12] and the sugar were determined to be methyl 6-deoxy-3-*O*-methyl-α-L-*ribo*-hexopyranoside (methyl α-L-cymaropyranoside). C-4^I was found to be at δ 82.2 d, and its corresponding proton in the HMQC, 3.50 m, had a long-range correlation with δ 100.3 d in the HMBC. Consequently, the O-4^I was linked with the sugar unit whose anomeric carbon (C-1^{II}) was at δ 100.3. 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 the unit II were determined. The data were compared with those in the literature,[12] and the moiety II was determined to be 6-deoxy-3-*O*-methyl-β-D-*ribo*-hexopyranosyl (β-D-cymaropyranosyl). Since C-4^{II} was apparent at δ 83.2 d, and it showed a long-range correlation with the proton at δ 4.72 d in the HMBC, so O-4^{II} was linked with the sugar unit III with the anomeric carbon at δ 102.2. This sugar was determined to be 2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranosyl (β-D-oleandropyranosyl) (Table 1). Since all of the ^{13}C NMR data of this sugar were those in the literature,[12] and there was no remaining sugar, it was designated the terminal sugar moiety. Therefore, **1** was elucidated as methyl 2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-*ribo*-hexopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-α-L-*ribo*-hexopyranoside (Fig. 1).

Carbohydrate **2** was obtained as a white powder. $C_{16}H_{32}O_8$ from HRFABMS. Two anomeric carbons

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

Carbon	^{13}C	^1H[a]	^1H–^1H COSY	HMBC
1-*O*-Me-α-L-Cym				
C-1^I	97.9 d	4.65 d (4.4)	H-2^I	C-3^I; C-5^I; MeO-1^I
C-2^I	33.2 t	1.77 m; 2.21 d (2.8)	H-1^I; H-3^I	—
C-3^I	76.6 d	3.94 m	H-2^I; H-4^I	—
C-4^I	82.2 d	3.50 m	H-3^I; H-5^I	C-6^I; C-1^{II}
C-5^I	63.3 d	4.44 m	H-4^I; H-6^I	—
C-6^I	18.0 q	1.28 d (6.4); 3H	H-5^I	C-4^I; C-5^I
MeO-1^I	54.9 q	3.26 s; 3H	—	C-1^I
MeO-3^I	57.8 q	3.49 m; 3H	—	C-3^I
β-D-Cym				
C-1^{II}	100.3 d	5.11 d (7.6)	H-2^{II}	C-4^I
C-2^{II}	36.7 t	1.81 d (3.6); 2.28 m	H-1^{II}; H-3^{II}	—
C-3^{II}	77.8 d	4.01 m	H-2^{II}; H-4^{II}	—
C-4^{II}	83.2 d	3.46 m	H-3^{II}; H-5^{II}	C-1^{II}
C-5^{II}	69.0 d	4.18 m	H-4^{II}; H-6^{II}	—
C-6^{II}	18.6 q	1.40 m; 3H	H-5^{II}	C-4^{II}; C-5^{II}
MeO-3^{II}	58.8 q	3.52 s; 3H	—	C-3^{II}
β-D-Ole				
C-1^{III}	102.2 d	4.72 d (1.6)	H-2^{III}	C-4^{II}
C-2^{III}	37.2 t	1.70 m; 2.51 m	H-1^{III}; H-3^{III}	C-3^{III}; C-4^{III}
C-3^{III}	81.4 d	3.44 m	H-2^{III}	
C-4^{III}	76.2 d	3.45 m	—	—
C-5^{III}	72.9 d	3.56 m	H-6^{III}	—
C-6^{III}	18.7 q	1.54 m; 3H	H-5^{III}	C-4^{III}; C-5^{III}
MeO-3^{III}	57.1 q	3.42 s; 3H	—	C-3^{III}

[a] Coupling constants are in hertz.

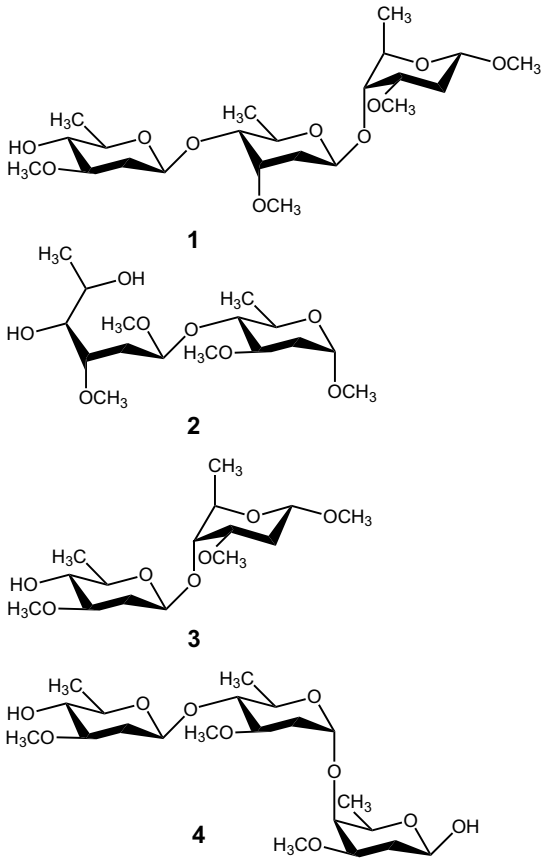

Figure 1. Structures of carbohydrates **1**, **2**, **3**, and **4**.

were observed (δ_C 106.4 d and 99.6 d) revealing the presence of two sugar residues. The sugar at δ 106.4 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 2). Since the resonance of C-4^{I} was at δ 89.7 d, so O-4^{I} was linked with the sugar whose anomeric carbon was at δ 99.6. This sugar was determined to be 6-deoxy-1,3-di-*O*-methyl-β-D-*ribo*-hexosyl (1-*O*-methyl-β-D-cymarosyl) (Table 2). Since all of the ^{13}C NMR data of this sugar were those in the literature,[12] and there was no remaining sugar, so it was assigned as the terminal sugar moiety. Therefore, **2** was elucidated as methyl 6-deoxy-1,3-di-*O*-methyl-β-D-*ribo*-hexosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-α-D-*arabino*-hexopyranoside (Fig. 1).

Carbohydrate **3** was obtained as a white powder. $C_{15}H_{28}O_7$ from HRFABMS. The anomeric carbon resonances at δ_C 97.9 d, and 101.9 d revealed the presence of two sugar residues. The sugar at δ 97.9 was determined to be methyl 6-deoxy-3-*O*-methyl-α-L-*ribo*-hexopyranoside (methyl α-L-cymaropyranoside) in the same way as was previously assigned (Table 3). C-4^{I} was found to be at δ 82.2 d, and its corresponding proton in the HMQC, 3.58 m, had a long-range correlation with δ 101.9 d in the HMBC. Consequently, the O-4^{I} was linked with the sugar unit whose anomeric carbon (C-1^{II}) was at δ 101.9. This sugar was determined to be 2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranosyl (β-D-oleandropyranosyl) (Table 3). Since the resonance of C-4^{II} was at δ 76.2, 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-β-D-*arabino*-hexopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-α-L-*ribo*-hexopyranoside (Fig. 1).

Table 2. NMR data for carbohydrate **2** in C_5D_5N

Carbon	^{13}C	^{1}H[a]	^{1}H–^{1}H COSY	NOESY	HMBC
1-*O*-Me-α-D-Ole					
C-1^{I}	106.4 d	5.20 dd (2.8, 2.8)	H-2^{I}	H-2^{I}; H–MeO-1^{I}	C-4^{I}; MeO-1^{I}
C-2^{I}	39.7 t	2.22 dd (1.2, 4.4); 2.28 dd (3.2, 3.6)	H-1^{I}; H-3^{I}	H-1^{I}; H-3^{I}	C-1^{I}; C-3^{I}; C-4^{I}
C-3^{I}	82.5 d	4.38 m	H-2^{I}; H-4^{I}	H-2^{I}; H–MeO-3^{I}	C-1^{I}; C-5^{I}; MeO-3^{I}
C-4^{I}	89.7 d	4.10 dd (1.6, 2.4)	H-3^{I}; H-5^{I}	H-6^{I}	C-1^{I}; C-3^{I}; C-5^{I}; C-6^{I}
C-5^{I}	68.4 d	3.97 m	H-4^{I}; H-6^{I}	H-6^{I}	C-3^{I}; C-4^{I}
C-6^{I}	21.2 q	1.48 dd (3.2, 3.2); 3H	H-5^{I}	H-4^{I}; H-5^{I}; H–MeO-1^{I}; H–MeO-3^{I}	C-4^{I}; C-5^{I}
MeO-1^{I}	55.3 q	3.35 s; 3H	—	H-1^{I}; H-5^{I}	C-1^{I}
MeO-3^{I}	56.7 q	3.32 s; 3H	—	H-3^{I}; H-6^{I}; H-1^{II}	C-3^{I}
1-*O*-Me-β-D-Cymarosyl					
C-1^{II}	99.6 d	4.82 dd (2.0, 7.6)	H-2^{II}	H-2^{II}; H-5^{II}; H–MeO-1^{II}	C-2^{II}; MeO-1^{II}
C-2^{II}	35.3 t	1.71 dd	(1.6, 2.4); 2.28 dd (3.2, 3.6)	H-1^{II}; H-3^{II} H-1^{II}; H-3^{II}; H–MeO-1^{II}	C-1^{II}; C-3^{II}; C-4^{II}; C-6^{II}
C-3^{II}	78.7 d	3.72 dd (3.2, 3.2)	H-2^{II}; H-4^{II}	H-2^{II}; H–MeO-3^{II}	—
C-4^{II}	74.2 d	3.49 m	H-3^{II}; H-5^{II}	H-1^{II}; H-2^{II}; H-3^{II}; H-5^{II}; H-6^{II}	C-5^{II}
C-5^{II}	71.1 d	4.03 dd (2.8, 3.2)	H-4^{II}; H-6^{II}	H-1^{II}; H-6^{II}	C-1^{II}; C-4^{II}
C-6^{II}	19.1 q	1.48 dd (3.2, 3.2); 3H	H-5^{II}	H-2^{II}; H-4^{II}; H-5^{II}	C-4^{II}; C-5^{II}
MeO-1^{II}	56.2 q	3.49 m; 3H	—	H-1^{II}	C-1^{II}
MeO-3^{II}	57.9 q	3.43 s; 3H	—	H-2^{II}; H-3^{II}	C-3^{II}

[a]Coupling constants are in hertz.

Table 3. NMR data for carbohydrate **3** in C_5D_5N

Carbon	^{13}C	^{1}H[a]	^{1}H–^{1}H COSY	HMBC
1-*O*-Me-α-L-Cym				
C-1^{I}	97.9 d	4.68 d (4.5)	H-2^{I}	C-3^{I}; C-5^{I}; MeO-1^{I}
C-2^{I}	33.2 t	1.82 t (4.0); 2H	H-1^{I}; H-3^{I}	—
C-3^{I}	76.5 d	3.96 dd (3.1, 3.2)	H-2^{I}; H-4^{I}	C-1^{I}; C-4^{I}; C-5^{I}; MeO-3^{I}
C-4^{I}	82.2 d	3.58 m	H-3^{I}; H-5^{I}	C-3^{I}; C-5^{I}; C-6^{I}; C-1^{II}
C-5^{I}	63.3 d	4.49 dd (3.0, 6.0)	H-4^{I}; H-6^{I}	C-1^{I}; C-3^{I}; C-4^{I}
C-6^{I}	18.1 q	1.38 d (6.5); 3H	H-5^{I}	C-4^{I}; C-5^{I}
MeO-1^{I}	54.9 q	3.28 s; 3H	—	C-1^{I}
MeO-3^{I}	57.7 q	3.55 d (1.0); 3H	—	C-3^{I}
β-D-Ole				
C-1^{II}	101.9 d	4.78 d (9.5)	H-2^{II}	C-4^{I}; C-2^{II}
C-2^{II}	37.1 t	2.52 dd (2.0, 10.5); 2H	H-1^{II}; H-3^{II}	C-1^{II}; C-3^{II}; C-4^{II}
C-3^{II}	81.5 d	3.46 m	H-2^{II}; H-4^{II}	C-4^{II}; C-5^{II}; C-6^{II}; MeO-3^{II}
C-4^{II}	76.2 d	3.45 m	H-3^{II}; H-5^{II}	C-3^{II}; C-5^{II}; C-6^{II}
C-5^{II}	72.9 d	3.58 m	H-4^{II}; H-6^{II}	C-1^{II}; C-6^{II}
C-6^{II}	18.7 q	1.55 m; 3H	H-5^{II}	C-4^{II}; C-5^{II}
MeO-3^{II}	57.0 q	3.42 m; 3H	—	C-3^{II}

[a]Coupling constants are in hertz.

Table 4. NMR data for carbohydrate **4** in C_5D_5N

Carbon	^{13}C	^{1}H[a]	^{1}H–^{1}H COSY	HMBC
β-D-Digin				
C-1^{I}	108.7 d	5.03 s	—	—
C-2^{I}	35.6 t	3.01 d (4.0); 2H	H-3^{I}	—
C-3^{I}	75.3 d	4.11 d (2.3)	H-2^{I}; H-4^{I}	—
C-4^{I}	79.0 d	3.91 d (2.0)	H-3^{I}; H-5^{I}	C-5^{I}; C-6^{I}; C-1^{II}
C-5^{I}	74.3 d	4.82 m	H-4^{I}; H-6^{I}	C-4^{I}
C-6^{I}	18.9 q	1.34 s; 3H	H-5^{I}	C-4^{I}; C-5^{I}
MeO-3^{I}	57.9 q	3.49 m; 3H	—	C-3^{I}
α-D-Ole				
C-1^{II}	99.7 d	5.14 dd (1.8, 1.8)	H-2^{II}	C-4^{I}; C-2^{II}
C-2^{II}	36.7 t	1.80 m; 2.29 m	H-1^{II}; H-3^{II}	C-1^{II}; C-4^{II}
C-3^{II}	77.7 d	4.04 m	H-2^{II}; H-4^{II}	C-1^{II}; C-5^{II}; MeO-3^{II}
C-4^{II}	82.9 d	3.49 m	H-3^{II}; H-5^{II}	C-5^{II}; C-6^{II}; C-1^{III}
C-5^{II}	69.1 d	4.17 m	H-4^{II}; H-6^{II}	—
C-6^{II}	18.7q	1.40 m; 3H	H-5^{II}	C-4^{II}; C-5^{II}
MeO-3^{II}	58.9 q	3.56 s; 3H	—	C-3^{II}
β-D-Ole				
C-1^{III}	102.2 d	4.77 d (9.0)	H-2^{III}	C-4^{II}; C-2^{III}
C-2^{III}	37.2 t	1.71 dd (8.0, 8.0); 2.52 m	H-1^{III}; H-3^{III}	C-1^{III}; C-3^{III}; C-6^{III}
C-3^{III}	81.4 d	3.47 m	H-2^{III}; H-4^{III}	C-1^{III}; C-4^{III}; C-6^{III}
C-4^{III}	76.2 d	3.47 m	H-3^{III}; H-5^{III}	C-1^{III}; C-6^{III}
C-5^{III}	73.0 d	3.59 m	H-4^{III}; H-6^{III}	—
C-6^{III}	18.5 q	1.55 m; 3H	H-5^{III}	C-4^{III}; C-5^{III}
MeO-3^{III}	57.1 q	3.46 m 3H	—	C-3^{III}

[a]Coupling constants are in hertz.

Carbohydrate **4** was obtained as a white powder. $C_{21}H_{38}O_{10}$ from HRESIMS. Three anomeric carbons were observed (δ_C 108.7 d, 99.7 d, and 102.2 d) revealing the presence of three sugar residues. The sugar at 108.7 was determined to be 2,6-dideoxy-3-*O*-methyl-β-D-*lyxo*-hexopyranose (β-D-diginopyranose) (Table 4). In the same way, O-4^{I} was linked with the sugar with the anomeric carbon at δ 99.7. This sugar was determined to be 2,6-dideoxy-3-*O*-methyl-α-D-*arabino*-hexopyranosyl (α-D-oleandropyranosyl) (Table 4). The O-4^{II} was linked with the sugar unit whose anomeric carbon was at δ 102.2. This sugar was determined to be 2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranosyl (β-D-oleandropyranosyl) (Table 4). Since C-4^{III} was apparent at δ 76.2 d, and there was no remaining sugar, so it was determined to be the terminal sugar moiety. Therefore, **4** was elucidated as 2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-α-D-*arabino*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-D- *lyxo*-hexopyranose (Fig. 1).

3. Experimental

3.1. General

FABMS and ESIMS were performed on a VG Auto-Spec-3000 spectrometer. Bruker AM-400 and DRX-500 instruments were used to record ^{1}H NMR and 2D NMR spectra (400 MHz), and ^{13}C NMR. Pyridine-d_5 (C_5D_5N) was the solvent and the internal standard at room temperature. 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.

3.2. Materials

The rhizome of *C. otophyllum* was bought from a drug market in Kunming. It was identified by Dr. Yue-Mao Shen and a voucher specimen (KUN No 0776933) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

3.3. 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) were dissolved in 2.25 L of 1:2 MeOH–0.025 mol/L H_2SO_4 in a water bath at 70 °C. After 2 h, $Ba(OH)_2$ solution was added until pH 7; $BaSO_4$ was filtered. The solution was dried up to give the crude aglycones (100 g).

The crude aglycones (100 g) were separated into 21 fractions through column chromatography over silica gel (300 g) by elution with a gradient mixture of $CHCl_3$–MeOH from $CHCl_3$ to 100:8.5 (v/v). Fraction 7 (1.6 g, 100:1 $CHCl_3$–MeOH required) was subjected to silica gel column chromatography (71 g), eluting with $CHCl_3$–MeOH (100:1.5, 100:2), and then to silica gel column chromatography (31 g) eluting with petroleum ether–Me_2CO (10:3), and finally to silica gel column chromatography (6 g) eluting with petroleum ether–EtOAc (45:55, 2:8), to afford **1** (10 mg). Fraction 2 (7.3 g, $CHCl_3$ needed) was subjected to silica gel column chromatography (80 g) eluting with $CHCl_3$–MeOH (100:0, 100:0.5, 100:1), to silica gel column chromatography (50 g) eluted with petroleum ether–Me_2CO (10:1), to silica gel column chromatography (50 g) eluting with petroleum ether–Me_2CO (10:1.5), to silica gel column chromatography (40 g) eluted with petroleum ether–EtOAc (8:2, 75:25), and to silica gel column chromatography (18 g) eluted with $CHCl_3$–MeOH (100:0.7, 100:2, 100:5), to yield **2** (347 mg). Fraction 4 (1.8 g, $CHCl_3$) was subjected to silica gel column chromatography (78 g) eluting with $CHCl_3$–MeOH (100:0.5, 100:1), to silica gel column chromatography (27 g) eluting with petroleum ether–Me_2CO (10:3.5), and to silica gel column chromatography (5.5 g) eluting with petroleum ether–EtOAc (1:1, 3:7), to afford **3** (9 mg). Fraction 16 (1.2 g, 100:8.5) was subjected to silica gel column chromatography (73 g) eluting with $CHCl_3$–MeOH (100:8), to silica gel column chromatography (53.5 g) eluting with petroleum ether–Me_2CO (10:8), and to silica gel column chromatography (5 g) eluting with petroleum ether–EtOAc (2:8), to yield **4** (1 mg).

3.3.1. Carbohydrate 1. White powder: HRFABMS $[M-1]^-$ m/z: Calcd for $C_{22}H_{39}O_{10}$: 463.2543; Found: 463.2558; FABMS m/z (%): 463 (3.5 $[M-1]^-$), 375 (4), 342 (4), 311 (5), 283 (7), 255 (9.5), 97 (13), 80 (8); ^{1}H, ^{13}C, and 2D NMR data, see Table 1.

3.3.2. Carbohydrate 2. White powder: HRFABMS $[M-1]^+$ m/z: Calcd for $C_{16}H_{31}O_8$: 351.2019; Found: 351.1986; FABMS m/z (%): 353 (0.2 $[M+1]^+$), 330 (1.5), 298 (9), 175 (12.5), 145 (100), 113 (19.5), 101 (22.5), 71 (23), 59 (17.5); ^{1}H, ^{13}C, and 2D NMR data, see Table 2.

3.3.3. Carbohydrate 3. White powder: HRFABMS $[M+1]^+$ m/z: Calcd for $C_{15}H_{29}O_7$: 321.1913; Found: 321.1952; EIMS m/z (%): 319 (0.4 $[M-1]^+$), 288 (1.5), 257 (25), 218 (12.5), 186 (50), 158 (51.5), 145 (98.5), 127 (55.5), 113 (79.5), 101 (61.5), 87 (88.5), 71 (73), 59 (100); ^{1}H, ^{13}C, and 2D NMR data, see Table 3.

3.3.4. Carbohydrate 4. White powder: HRESIMS $[M-2+Na]^+$ m/z: Calcd for $C_{21}H_{36}NaO_{10}$: 471.2206; Found: 471.2219; FABMS m/z (%): 449 (4 $[M-2]^+$), 417 (14), 145 (100), 113 (52), 85 (15), 58 (32.5); ^{1}H, ^{13}C, and 2D NMR data, see Table 4.

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