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A New Furostanol Saponin from the Water-extract of *Dioscorea nipponica* Mak., the Raw Material of the Traditional Chinese Herbal Medicine Wei Ao Xin

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Abstract: 26-*O*-*β*-D-Glucopyranosyl-furost-5(6),20(22)-dien-3*β*,26-diol **1**, a new furostanol saponin, was isolated from the water-extract of *Dioscorea nipponica* Mak., the raw material of a traditional Chinese herbal medicine Wei Ao Xin. The structure of **1** was determined on the basis of its spectral data especially by NMR spectroscopy. The result provides the first example of naturally occurring furostanol saponins with a single saccharide chain at the C-26 position.

Keywords: 26-*O*-*β*-D-Glucopyranosyl-furost-5(6),20(22)-dien-3*β*,26-diol, furostanol saponin, steroidal saponin, structure, NMR, *Dioscorea nipponica*, Dioscoreaceae.

Dioscorea nipponica Mak. (Dioscoreaceae)[1] is a traditional Chinese medicinal herb (Chuanlong Shuyu in Chinese name) and its water extract is used as the raw material of Wei Ao Xin, a cardiovascular drug, which has been used for treatment of certain cardiovascular diseases such as coronary heart disease, hyperlipaemia and angina, *etc*[2,3]. From the water extract of *Dioscorea nipponica* Mak., we have now isolated a new furostanol saponin, 26-*O*-*β*-D-glucopyranosyl-furost-5(6),20(22)-dien-3*β*,26-diol **1**. In this communication, the isolation and structure determination of **1** were described.

Water extract (2.9 kg, dried powder) of *D. nipponica* Mak. was extracted with methanol to obtain a methanol-soluble part (26.4 g). The methanol-soluble part (26.4 g) was separated by vacuum liquid chromatography on a SYNTHWARE™ glass vacuum column (5 x 30 cm, Tianjin Synthware Glass Instruments Co., Tianjin, China) over silica gel H (Qingdao Haiyang Chemical group Co., China) using $CHCl_3$-MeOH (CM, in a stepwise gradient manner) as eluting solvent to give six fractions, Fr.-1 ($CHCl_3$ eluent), Fr.-2 (CM 99:1 eluent), Fr.-3 (195.8 mg, CM 92:8 eluent), Fr.-4 (CM 80:20 eluent), Fr.-5 (CM 70:30 eluent) and Fr.-6 (MeOH eluent) respectively. Fr.-3 was recrystallized from MeOH to give pure **1** (163 mg) as colorless needles.

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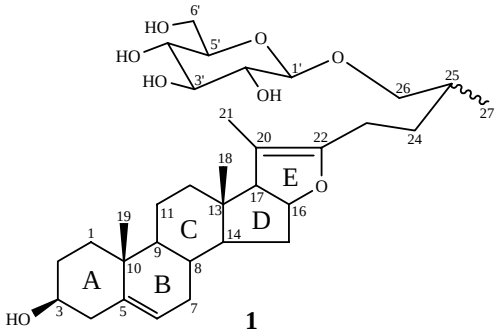
Figure 1 Chemical Structure of **1**



1

Compound **1**, mp 246-248°C, $[\alpha]_D^{20}$ -13 (*c* 0.03, pyridine), showed characteristic colors typical for furostanol saponins in both Ehrlich reagent test and Liebermann-Burchard test[4], suggesting that **1** is a furostanol saponin. It gave *quasi*-molecular ion peaks at *m/z* 600 $[M+Na]^+$ and 577 $[M+H]^+$ in the positive-ion ESI-MS and its molecular formula could be determined to be $C_{33}H_{52}O_8$ by high-resolution ESI-MS measurement (measured 577.3724, calcd. for $C_{33}H_{53}O_8$ $[M+H]^+$ 577.3734). Another significant ion peak at *m/z* 415 $[(M+H)-162]^+$ in the ESI-MS, responsible for the loss of a terminal hexose residue ($C_6H_{10}O_5$, 162), suggested the presence of a hexose moiety in **1**. This was further supported by very strong absorptions at 3317 and 1050 cm^{-1} in the IR (KBr) spectrum of **1**.

The ^1H NMR spectrum of **1** in methanol-d_4 solution showed signals ascribable to a *sec*-methyl (δ0.94 *d*, *J* = 7.0 Hz) and three *tert*-methyl (δ0.71, 1.03 and 1.60, each *s*) groups together with an olefinic (δ5.34, *m*) and an anomeric (δ4.22 *d*, *J* = 7.7 Hz) protons as well as some methylene and methine groups typical for a sugar residue and steroid skeleton (**Table 1**). In parallel, the ^{13}C NMR spectrum of **1** in methanol-d_4 solution showed signals due to thirty three carbons (**Table 1**) including four olefinic (δ105.2, 122.2, 142.3, 152.9) and an anomeric (δ104.5) carbons, further supporting **1** to be a furostanol monoglycoside[5].

Detailed analysis of the ^1H and ^{13}C NMR spectra of **1** with the aid of DEPT, PFG ^1H-^1H COSY and PFG HMQC techniques enabled us to deduce all of partial structures related to the proton spin systems in **1**. For instance, a proton spin system consisted of H_2-1 (δ1.07 and 1.86), H_2-2 (δ1.48 and 1.79), H-3 (δ3.38) and H_2-4 (δ2.21 and 2.24) in **1** could be elucidated by tracing their correlation peaks, starting from the oxygenated methine proton H-3 (δ3.38), in the PFG ^1H-^1H COSY spectrum. The results of DEPT and PFG HMQC spectra can give the information of the partial structure related in A-ring. Similarly, the monohexose moiety was readily demonstrated and evidenced to be an O-β-D-glucopyranosyl residue ($^4C_1$) by the chemical shift, splitting pattern and coupling constant of each proton on the sugar residue (**Table 1**)[6].

Table 1 600 MHz ^{1}H and 150 MHz ^{13}C NMR data for **1** in methanol-d_4 [a)]

Positions	δ_H (J in Hz)	^{1}H-^{1}H COSY[b)]	δ_C	HMBC[c)]	
				$^{2}J_{CH}$	$^{3}J_{CH}$
1	1.07 td (13.5, 3.5)	2	38.5 t		19
	1.86 dt (13.5, 3.5)	2			
2	1.48 m; 1.79 m	1, 3	32.3 t		4
3	3.38 m	2, 4	72.4 d	4	1
4	2.21 m; 2.24 m	3	43.0 t		6
5	——		142.3 s	4	19
6	5.34 m	7 (δ2.01)	122.2 d		4
7	1.59 m; 2.01 m	6, 8	33.3 t		9
8	1.59 m	7, 9	32.6 d		6
9	0.97 td (11.4, 5.0)	8, 11, 14	51.7 d		19
10	——		37.8 s	19	4, 6
11	1.54 m; 1.57 m	9, 12	22.1 t		
12	1.28 td (12.6, 5.0)	11	40.7 t		17, 18
	1.82 dt (12.6, 3.5)				
13	——		44.4 s	18	15, 16
14	1.04 m	8, 15	56.3 d	15	18
15	1.40 td (13.2, 5.7)	14, 16	35.1 t		
	2.15 m	14, 16			
16	4.71 m	15, 17	85.6 d	15	
17	2.49[d)] d (10.3)	16, 21	65.6 d		15, 18, 21
18	0.71 (3H) s		14.5 q		17
19	1.03 (3H) s		19.9 q		
20	——		105.2 s	17, 21	
21	1.60[e)] (3H) s	17	11.9 q		17
22	——		152.9 s	23	17, 21, 24
23	2.09 m; 2.12 m	24	24.1 t	24	
24	1.24 m; 1.63 m	23	32.0 t	23	27
25	1.76 m	26, 27	34.1 d	24, 27	23
26	3.38 m; 3.70 dd (9.5, 6.6)	25	75.8 t	25	1', 24, 27
27	0.94 (3H) d (7.0)	25	17.3 q		26
1'	4.22 d (7.7)	2'	104.5 d	2'	26
2'	3.17 dd (8.8,7.7)	1', 3'	75.2 d	3'	
3'	3.33 br t (8.8)	2', 4'	78.1 d	2', 4'	
4'	3.27 dd (9.5, 8.8)	3', 5'	71.7 d	3'	
5'	3.23 m	4', 6'	77.9 d	4'	3'
6'	3.66 dd (11.7, 5.5)	5'	62.8 t		
	3.85 dd (11.7, 2.0)				

a) Signal assignments were based on the results of PFG ^{1}H-^{1}H COSY, PFG HMQC, and PFG HMBC spectroscopy. Multiplicities of the carbon signals were determined by the DEPT method and are indicated as s (singlet), d (doublet), t (triplet) and q (quartet), respectively. b) Numbers in the column indicate the protons that showed correlations with the proton(s) on the line in the PFG ^{1}H-^{1}H COSY spectrum. All of the methylene protons showed correlation peaks respectively with own geminal-coupling counterpart in the PFG ^{1}H-^{1}H COSY spectrum. c) Numbers in the column indicate the protons that correlated with the carbon on the line through two ($^{2}J_{CH}$) and three ($^{3}J_{CH}$) bonds, respectively, in the PFG HMBC spectrum measured under the condition optimized with long-range J_{CH} = 8 Hz. d) and e) Weak but significant correlation peaks due to allylic coupling between 17-H and 21-H$_3$ were detected in the PFG ^{1}H-^{1}H COSY spectrum.

The total structure of **1** was determined by HMBC correlations between the corresponding C and H in the partial structure. In the PFG HMBC spectrum, H_3-19 showed long-range correlations with C-1, C-10, C-9 and C-5, while the olefin proton H-6 correlated with C-4, C-10 and C-8. Thus, the A/B ring connection could be deduced and the olefin group should be located at C-5 and C-6. Furthermore, H_3-18 correlated with C-12, C-13, C-14 and C-17, while H_3-21 with C-17, C-20 and C-22. The latter C-22 further correlated with H-17 and H_2-23, and the former C-20 with H-17 and H_2-21. These correlations demonstrated the C/D/E ring junction, the location of another olefin group at C-20 and C-22, and the connection of C-22 and C-23. Finally, the glucose residue in **1** could be located at C-26 by the HMBC correlation between H-1' and C-26.

From the above mentioned evidence, the chemical structure of **1** could be determined as 26-*O*-*β*-D-glucopyranosyl-furost-5(6),20(22)-dien-3*β*,26-diol.

Compound **1** is a new frostanol saponin, belonging to the steroidal saponins, which was isolated from *Dioscorea nipponica* Mak. in the present study. Frostanol saponins are a well-known class of steroidal saponins and all members of this class ordinarily carry multi-saccharide chains. Frostanol saponin carrying a single saccharide chain at the C-26 position has not been obtained from the nature so far, because the glycoside bond at C-26 is more readily cleaved by enzymatic hydrolysis than the other glycoside bonds in the frostanol saponins in the natural condition. The present result provides the first example of naturally occurring furostanol saponins with a single saccharide chain at the C-26 position, which provides also the first evidence for the existence of frostanol with a single saccharide chain as the C-26 glycoside in nature.

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