HETEROCYCLES, Vol. 59, No. 2, 2003, pp. 805 - 809, Received, 20th November, 2002

TWO NEW CARDENOLIDE GLYCOSIDES FROM *STREBLUS ASPER*

Yoshio Hano,[1]* Patima Juma,[1] Zeper Abliz,[2] Han-Dong Sun,[3] and Taro Nomura[1]

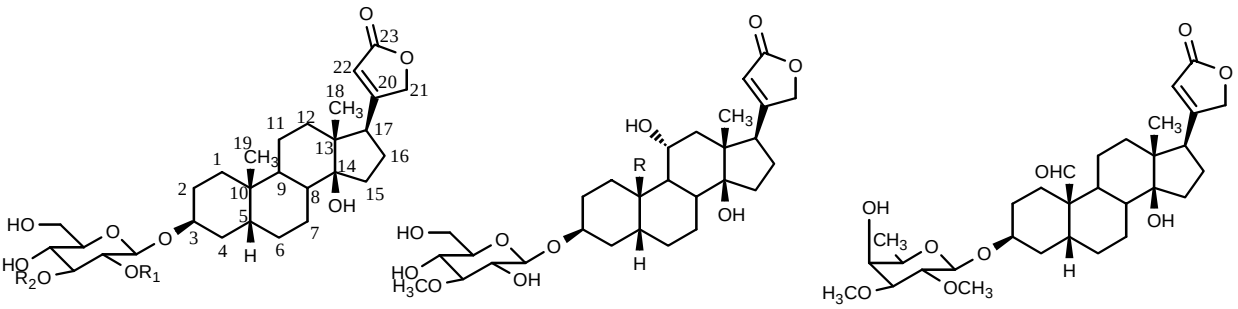
[1]Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan, e-Mail: hano@phar.toho-u.ac.jp, [2]Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 1, Xian Nong Tan Street, Beijing, 100050, P. R. China, [3]Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Kunming 650204, Yunnan, P. R. China

Abstract – Two new cardenolide glycosides, digitoxigenin-3-*O*-β-(3'-*O*-methyl)glucopyranoside (**1**) and 11, 19-dihydroxydigitoxigenin-3-*O*-β-(3'-*O*-methyl)glucopyranoside (**2**) were isolated from the underground part of *Streblus asper*, along with three known cardenolide glycosides. The structures of the new compounds were elucidated on the basis of the spectroscopic evidence.

Streblus asper (Moraceae) is distributed mainly over the region of Southeast Asia and China,[1] and has been used for heart disease in the Ayurvedic medicinal system.[1,2] More than twenty cardiac glycosides have been isolated from the root bark and stem bark of this plant.[3-6] In continuation of our survey on the Moraceae plants, we examined the constituents of the root bark of the title plant to lead the isolation of two new cardenolide glycosides (**1**) and (**2**) together with three known cardiac glycosides, strebloside (**3**), asperoside (**4**), and sarmethoside (**5**).[3-6] We report here the isolation and characterization of the new cardenolide glycosides (**1**) and (**2**).

Methanol soluble part of aqueous ethanol extract of the root bark of *S. asper* was subjected to column chromatography on Diaion HP-20 eluting successively with *n*-hexane, chloroform, ethyl acetate, and methanol. The chloroform soluble fraction was chromatographed on a silica gel column and then further purified by preparative TLC to afford compound (**1**), together with two known cardiac glycosides, strebloside (**3**) and asperoside (**4**) (Figure 1). Analogous procedures of the ethyl acetate soluble fraction afforded compound (**2**) and a known compound, sarmethoside (**5**) (Figure 1).

This paper is dedicated to the memory of 75th birthday of Dr. Yuichi Kanaoka, Professor Emeritus Hokkaido University.



1 : R_1 = H, R_2 = CH_3 **2** : R = CH_2OH **3**

4 : R_1 = R_2 = CH_3 **5** : R = CH_3

Figure 1 Cardenolide glycosides from the under part of *Streblus asper*

Compound (**1**) was obtained as a colorless amorphous powder and was shown to have the molecular formula $C_{30}H_{46}O_9$ from a pseudomolecular ion peak at m/z 551.3236 in the HRFABMS spectrum. A carbonyl stretching at 1735 cm^{-1} in the IR spectrum and the absorption maximum at 216 nm in the UV spectrum led to an assumption that compound (**1**) has a butenolactone ring system. The ^{13}C-NMR spectrum of **1** exhibited thirty carbon signals, which consist of three methyl, eleven methylene, eleven methine, and five quaternary carbons in DEPT spectrum (Table 1). In the ^{1}H-NMR spectrum, characteristic proton signals for a butenolactone ring system resonated at δ 4.84, 4.98 (each d, *J* = 18) and 5.87 (1H, br s) (Table 2). Three methyl signals were those of two tertiary methyl groups [δ 0.86, 0.93 (each 3H, s)] and a methoxy group [δ 3.66 (3H, s)] (Table 2). The ^{1}H-^{1}H COSY spectrum of **1** revealed presence of a glucose moiety from a sequence of the proton signals [δ 4.34 (1H, d, *J* = 7), 3.43 (1H, dd, *J* = 7 and 9), 3.19 (1H, t, *J* = 9), 3.53 (1H, d, *J* = 9), 3.26 (m), 3.77, 3.88 (each 1H, dd, *J* = 4 and 12)]. These data suggested compound (**1**) to be a cardenolide glycoside analogous to asperoside (**4**). Clarification of the C-H connectivity by the HMBC and HMQC spectra followed by comparison of the ^{13}C-NMR chemical shifts of **1** with those of asperoside (**4**) to indicate that **1** has a digitoxigenin as an aglycon moiety and a glucose residue with a methyl ether (Table 1). Glycosyl linkage at C-3 position was supported by ^{3}J correlation of the anomeric proton with C-3 at 74.2 in the HMBC spectrum and by NOE correlation between the anomeric proton and C-3-H at 4.03 (Figure 2). Furthermore, the methyl ether in the glucose moiety was placed at C-3' position based on ^{3}J correlation of the methyl protons with C-3' at 85.5 (Figure 2). These spectral data gave the structure of compound (**1**) to be digitoxigenin-3-*O*-(3'-*O*-methyl)glucopyranoside. Positive Cotton curve at 239 nm in the CD spectrum of **1** exhibited the same stereochemistry as that of asperoside (**4**) with digitoxigenin as the aglycon. Thus, the structure of compound (**1**) was established by digitoxigenin-3-*O*-β-(3'-*O*-methyl)glucopyranoside.

Table 1 ^{13}C-NMR chemical shifts of **1**, **2**, and **4**

Carbon No.	**1**[a]		**4**[a]	**2**[b]	
C-1	30.1	CH_2	30.3	26.1	CH_2
2	26.5	CH_2	26.6	26.3	CH_2
3	74.2	CH	73.8	73.7	CH
4	29.9	CH_2	29.6	29.9	CH_2
5	36.6	CH	35.2	30.7	CH
6	26.5	CH_2	26.7	26.7	CH_2
7	21.3	CH_2	21.4	20.9	CH_2
8	41.7	CH	41.9	41.4	CH
9	35.7	CH	36.4	40.3	CH
10	35.1	C	35.9	40.0	C
11	21.1	CH_2	21.2	67.7	CH
12	39.9	CH_2	40.0	49.3	CH_2
13	49.5	C	49.6	49.7	C
14	85.3	C	85.6	84.4	C
15	33.1	CH_2	33.2	32.1	CH_2
16	26.8	CH_2	26.9	26.6	CH_2
17	50.8	CH	50.9	50.5	CH
18	15.7	CH_3	15.8	16.3	CH_3
19	23.6	CH_3	23.9	65.2	CH_2
20	174.5	C	174.5	176.3	C
21	73.4	CH_2	73.4	73.9	CH_2
22	117.6	CH	117.8	116.6	CH
23	174.5	C	174.4	175.7	C
1'	101.2	CH	101.3	100.9	CH
2'	74.5	CH	83.9	73.5	CH
3'	85.5	CH	85.8	86.4	CH
4'	70.2	CH	70.6	69.8	CH
5'	75.1	CH	74.7	76.4	CH
6'	62.7	CH_2	62.9	61.4	CH_2
2'-OMe			60.9		
3'-OMe	60.5	CH_3	60.4	59.6	CH_3

Solvent: [a]$CDCl_3$ [b]CD_3OD

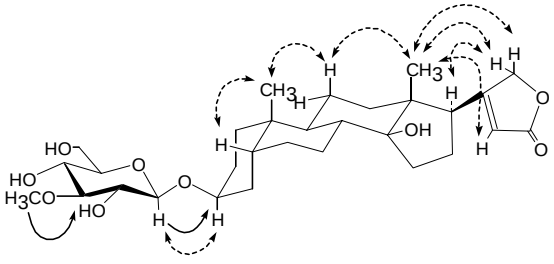
Table 2 ^{1}H-NMR data of **1** ($CDCl_3$)

Hydrogen No.	
3	4.03 br s
17	2.77 dd (9,5)
18	0.93 s
19	0.86 s
21α,β	4.98 d (18)
	4.84 d (18)
22	5.87 br s
1'	4.34 d (7)
2'	3.43 m
3'	3.19 t (9)
4'	3.53 t (9)
5'	3.36 m
6'	3.88 dd (12,4)
	3.77 dd (12,4)
3'-OMe	3.66 s

Table 3 ^{1}H-NMR data of **2** (CD_3OD)

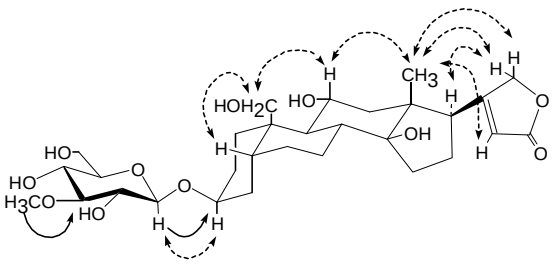
Hydrogen No.	
3	4.07 br s
11	3.78 dt (4, 10)
17	2.88 dd (9,5)
18	0.89 s
19	3.84 d (11)
	3.69 d (11)
21α,β	5.00 d (18)
	4.90 d (18)
22	5.90 br s
1'	4.31 d (7)
2'	3.25 dd (9,7)
3'	3.07 t (9)
4'	3.33 t (9)
5'	3.22 m
6'	3.82 dd (12,4)
	3.65 dd (12,4)
3'-OMe	3.63 s

Figure 2
Representative HMBC and NOE correlations of **1**



⇢ : representative NOE correations
⟶ : representative HMBC correations

Figure 3
Representative HMBC and NOE correlations of **2**



⇢ : representative NOE correations
⟶ : representative HMBC correations

Compound (**2**) was obtained as a colorless powder and gave the molecular formula $C_{30}H_{46}O_{11}$ in its HRFABMS. The IR absorption band at 1733 cm^{-1} and UV absorption maximum at 216 nm suggested the presence of a butenolactone ring system as observed in compound (**1**). The ^{13}C-NMR spectrum of **2** exhibited thirty carbon signals and the chemical shifts and DEPT data were summarized in Table 1. The 1D ^{1}H-NMR and 2D ^{1}H-^{1}H COSY spectra revealed the presence of a butenolactone ring system [δ 4.90, 5.00 (each 1H, d, *J* = 18), 5.90 (1H, br s)] and a glucose moiety [δ 4.31 (1H, d, *J* = 7), 3.25 (1H, dd, *J* = 7 and 9), 3.07 (1H, t, *J* = 9), 3.33 (1H, t, *J* = 9), 3.22 (m), 3.65, 3.82 (each 1H, dd, *J* = 4 and 12)], indicating that compound (**2**) is an analogue of **1** and **4** (Table 3). In addition, the NMR spectral data exhibited that aglycon part of **2** contains two more hydroxyl groups than digitoxigenin. Detailed analysis of the HMQC and HMBC spectra indicated that the hydroxyl groups were placed at C-11 and C-19 positions of digitoxigenin moiety, respectively, and that a methyl ether [δ 3.63 (3H, s)] was formed at C-3' position of glucose moiety (Figure 3). Glycosyl linkage at C-3 position was confirmed by significant ^{3}J correlation of the anomeric proton to C-3 position at 73.7 in HMBC spectrum (Figure 3). α-Orientation of C-11-OH was estimated by the NOESY spectrum (Figure 3) and further by ^{1}H-^{1}H spin-spin coupling constant of C-11-H (t, *J* = 10) with C-12-H_{α} and C-9-H. From the above result, the compound (**2**) was confirmed as 11, 19-dihydroxydigitoxigenin-3-*O*-β-(3'-*O*-methyl)glucopyranoside.

EXPEIRIMENTAL

General – Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. IR and UV spectra were recorded on a JASCO FTIR-3000E and a Shimadzu UV-265 spectrophotometers, respectively. NMR spectra were measured on JEOL JNM ECP-500 instruments and MS spectra were recorded on a JEOL JMS-600 and Autospec-Ultima ETOF. Wakogel C-200 and B-5F were used for column-chromatography and TLC, respectively.

Extraction and separation

The under ground part (4 kg) of *Streblus asper* was collected in xishuangbannna, Yunnan provinece, China on August 1998 and extracted three times with ethanol under a reflux condition to afford the ethanol solution. The ethanol solution was evaporated under reduced pressure to yield a residue (316 g).

A part of the residue (100 g) was dissolved with MeOH, and then the MeOH solution was evaporated under a reduced pressure to afford a residue (52 g). The MeOH extract was subjected to column chromatography on Diaion HP-20 eluted with *n*-hexane, $CHCl_3$, EtOAc, and MeOH to afford each soluble fraction. The $CHCl_3$ soluble part (4.6 g) was chromatographed on a silica gel with $CHCl_3$-acetone gradient system to afford eleven fractions. Fr. 10 (1.3 g) eluted with 100 % acetone was rechromatographed on silica gel with $CHCl_3$-acetone step gradients, and then the fraction eluted with CHCl3- atone (2 : 3) was further purified by preparative TLC (silica gel, solvent system, benzene : MeOH = 4 : 1) to yield

compound (**1**, 20 mg), strebloside (**3**, 6 mg), and asperoside (**4**, 6 mg). The EtOAc soluble fraction (6 g) was subjected to a silica gel column chromatography with $CHCl_3$-MeOH gradient system to give five fractions. The fraction (100 mg) eluted with $CHCl_3$-MeOH (1 : 4) was rechromatographed on silica gel and further purified by preparative TLC (silica gel, solvent system, $CHCl_3$: acetone = 1 : 4) to give sarmenthoside (**5**, 43 mg). A part (7 g) of the MeOH soluble fraction (25 g) was subjected to repeated chromatography on silica gel with $CHCl_3$-MeOH step gradients, and then rechromatography of the fraction (460 mg) eluted with $CHCl_3$-MeOH (4 : 1) on silica gel followed by repeated purification with preparative TLC (silica gel, solvent system, $CHCl_3$: MeOH = 2 : 1) to give compound (**2**, 6 mg). Three known compounds, strebloside (**3**), asperoside (**4**), and sarmethoside (**5**), were identified by their physical and NMR spectroscopic evidence and by comparison of the data with those reported in the literatures.

*Digitoxigenin-3-O-β-D-(3'-O-methyl)-glucopyranoside (**1**)*

Compound (**1**) was obtained as a white powder. $[\alpha]_D^{22}$ – 7.1 ° (*c* = 0.045, MeOH). IR νmax cm^{-1}(KBr): 3478, 2950, 1735, 1625, 1446, 1361, 1076. UV λmax (MeOH) (log *ε*): 216 (4.25). CD Δ*ε* (MeOH): $\Delta\varepsilon_{239}$ +3.3. HRFABMS *m/z*: 551.3236 (MH^+, $C_{30}H_{47}O_9$ calcd for 551.3220). ^{1}H-NMR and ^{13}C-NMR (Table 1).

11, 19-Dihydroxydigitoxigenin-3-O-β-D-(3'-O-methyl)glucopyranoside (2)

Compound (**2**) was obtained as a white powder. $[\alpha]_D^{22}$ – 3.0 ° (*c* = 0.09, MeOH). IR νmax cm^{-1}(KBr): 3401, 2933, 1733, 1623, 1452, 1367, 1294, 1110, 1097, 1035. UV λmax (MeOH) (log *ε*): 216 (4.27). HRFABMS *m/z*: 567.3139 (MH^+, $C_{30}H_{47}O_{10}$ calcd for 567.3169). ^{1}H-NMR and ^{13}C-NMR (Table 1).

REFERENCES

1. B. B. Gaitonde, A. X. Vax, and J. R. Patel, *Indian J. Med. Sci*., 1964, **18**, 191.
2. M. Fiebig, C. –Y. Dun, J. M. Pezzuto, A. D. Kinghorn, and N. R. Fransworth, *J. Nat. Prod*., 1985, **48**, 981.
3. M. P. Khare, S. S. Bhatnagar, O. Shindler, and T. Reichstein, *Helv. Chim. Acta*, 1962, **45**, 1515.
4. M. P. Khare, O. Shindler, and T. Reichstein, *Helv. Chim. Acta*, 1962, **45**, 1534.
5. A. R. Manzetti and T. Reichstein, *Helv. Chim. Acta*, 1964, **47**, 2303.
6. A. R. Manzetti and T. Reichstein, *Helv. Chim. Acta*, 1964, **47**, 2320.