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Studies on the Constituents of Asclepiadaceae Plants. L.[1] Two New Oligoglycosides, Cynanchoside C_2 and Cynanchoside C_1, from *Cynanchum caudatum* Max.

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Two new steroidal oligoglycosides, named cynanchoside C_2 (**1a**) and cyanchoside C_1 (**2a**), were isolated from the rhizome of *Cynanchum caudatum* Max. The structures of **1a** and **2a** were elucidated by the application of ^{13}C-nuclear magnetic resonance spectroscopy and chemical reactions.

Keywords——*Cynanchum caudatum*; Asclepiadaceae; steroidal oligoglycosides; cynanchoside C_2; cynanchoside C_1; D-cymarose; D-oleandrose; cynanchogenin; ^{13}C-NMR; PRFT method

The rhizome of *C. caudatum* Max. (Asclepiadaceae) has a high content of C/D-*cis*-polyoxypregnane oligoglycosides. This crude glycosides mixture has immunopotential activities.[2]

This paper describes the isolation and structures of cynanchoside C_2[3] (**1a**) and cynanchoside C_1 (**2a**) from this plant source.

These two new oligoglycosides were isolated by the procedure shown in Chart 1. The crude glycosides fraction, extracted by the same procedure as before,[4] was treated with hexane–benzene (1 : 1, v/v). The hexane–benzene (1 : 1) soluble portion was chromatographed on polyamide [eluent, AcOEt–hexane (1 : 19, v/v)] then rechromatographed on silica gel [eluent,

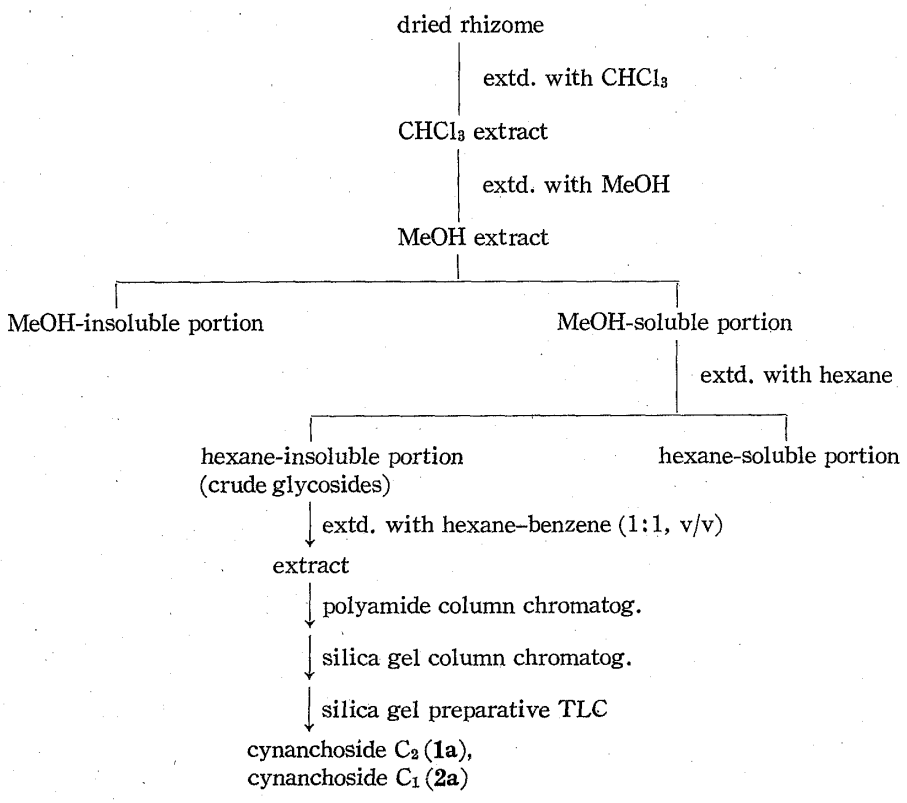

Chart 1

$CHCl_3$–MeOH (9:1, v/v) and benzene–acetone (4:1, v/v)]. The benzene–acetone (4:1) eluate was purified by silica gel preparative thin-layer chromatography (TLC), affording two new steroidal oligoglycosides named cynanchoside C_2 (**1a**), amorphous, mp 132.5—135.5°C, $[\alpha]_D$ −14.6° (c=1.0, $CHCl_3$), $C_{49}H_{78}O_{15}$, and cynanchoside C_1(**2a**), amorphous, mp 123.5—129°C, $[\alpha]_D$ +30.4° (c=0.25, $CHCl_3$), $C_{49}H_{78}O_{15}\cdot 1/2H_2O$.

1a and **2a** gave positive Keller–Kiliani reactions, indicating them to contain 2-deoxy sugar residues.

Mild acid hydrolysis of **1a** afforded cynanchogenin[5] (**3**), cymarose (**4a**), and oleandrose (**5a**), which were identical with authentic samples as determined by TLC and gas–liquid chromatography (GLC).

In addition to the above results, the ultraviolet (UV), infrared (IR), ^1H- and ^{13}C-nuclear magnetic resonance (PMR and CMR) spectra of **1a** established **1a** as a glycoside of **3** in which 3β-OH carries two moles of cymarose and one mole of oleandrose (see "Experimental" and Table I).[6]

The partially relaxed Fourier transform (PRFT) method[7] was applied to the CMR spectral measurement of **1a**, for determining the sugar sequence of **1a**. Among the signals due to sugar carbons, a set of signals with longer spin-lattice relaxation time (T_1=0.20 s, δ 72.9, 76.1, 81.3,

Table I. ^{13}C-NMR Chemical Shifts of **1a**, **2a**, **3**, **4b**, **4c**, **5b**, and **5c**

	Aglycone moieties			Sugar moieties			Methyl glycosides		
	1a	**2a**	**3**		**1a**	**2a**			
C- 1	38.9	38.9	39.2		Cymarose			**4b**	**4c**
C- 2	29.8	29.8	31.9	C-1	96.3	96.1	C-1	97.8	99.4
C- 3	77.9[a]	77.8[b]	71.5	C-2	37.2	37.3	C-2	31.9	35.1
C- 4	39.2	39.2	43.1	C-3	77.7[a]	77.5[b]	C-3	76.6	78.5
C- 5	139.3	139.1	140.2	C-4	83.3	83.2	C-4	73.3	74.0
C- 6	119.1	118.9	118.4	C-5	68.9	68.8	C-5	65.3	71.0
C- 7	34.1	34.2	34.1	C-6	18.5	18.9[c]	C-6	18.5	18.9
C- 8	74.5	74.4	74.5	C-3-OMe	58.8	58.7	C-1-OMe	54.8	56.0
C- 9	44.7	44.7	44.7				C-3-OMe	56.7	57.8
C-10	37.5	37.4	37.4		Cymarose				
C-11	25.0	25.0	25.0	C-1	100.3	100.2		**5b**	**5c**
C-12	72.2	72.2	72.3	C-2	37.2	37.3	C-1	98.7	101.0
C-13	55.7	55.6	55.6	C-3	77.6[a]	77.7[b]	C-2	35.1	36.6
C-14	87.4	87.3	87.4	C-4	83.1	82.9	C-3	79.0	81.3
C-15	35.1	35.1	35.1	C-5	68.9	68.8	C-4	76.6	76.2
C-16	21.7	21.8	21.7	C-6	18.5	18.6[c]	C-5	68.4	72.6
C-17	60.5	60.5	60.5	C-3-OMe	58.8	58.7	C-6	18.4	18.4
C-18	15.8	15.9	15.8				C-1-OMe	54.3	56.0
C-19	18.1	18.2	18.3		Oleandrose	Cymarose	C-3-OMe	57.0	56.9
C-20	209.0	208.5	209.0	C-1	102.0[e]	100.2[f]			
C-21	32.0	32.0	32.0	C-2	37.0	35.8		**5d**[d]	**5c**[d]
C- 1′	165.9	165.6	166.0	C-3	81.3[e]	78.6[f]	C-1	98.5	100.5
C- 2′	114.2	114.0	114.1	C-4	76.1[e]	73.9[f]	C-2	34.1	35.0
C- 3′	165.0	164.8	165.1	C-5	72.9[e]	70.8[f]	C-3	78.4	80.5
C- 4′	38.1	38.0	38.0	C-6	18.6	18.5[c]	C-4	76.2	78.1
C- 5′	20.9	20.9	20.9	C-3-OMe	57.0	59.9	C-5	67.5	75.5
C- 6′	20.9	20.9	20.9				C-6	18.1	17.8
C- 7′	16.4	16.4	16.4				C-1-OMe	54.7	56.2
							C-3-OMe	56.6	56.4

a—*c*) The assignments are interchangeable within the same column for shifts having the same superscript.
d) Spectra recorded in $CDCl_3$ solution. TMS was used as an internal standards for spectra.
e) T_1=0.20 s.
f) T_1=0.17 s.
Unless otherwise stated, spectra were recorded in pyridine-d_5 solution.

102.0) than others can be assigned to a terminal sugar, β-D-oleandrose, on the basis of a comparison with the ^{13}C-chemical shifts of methyl α- and β-D-cymaropyranosides (**4b** and **4c**) and methyl α- and β-D-oleandropyranosides (**5b** and **5c**).[8]

From these results, the sugar sequence in **1a** was determined to be as shown in Chart 2.

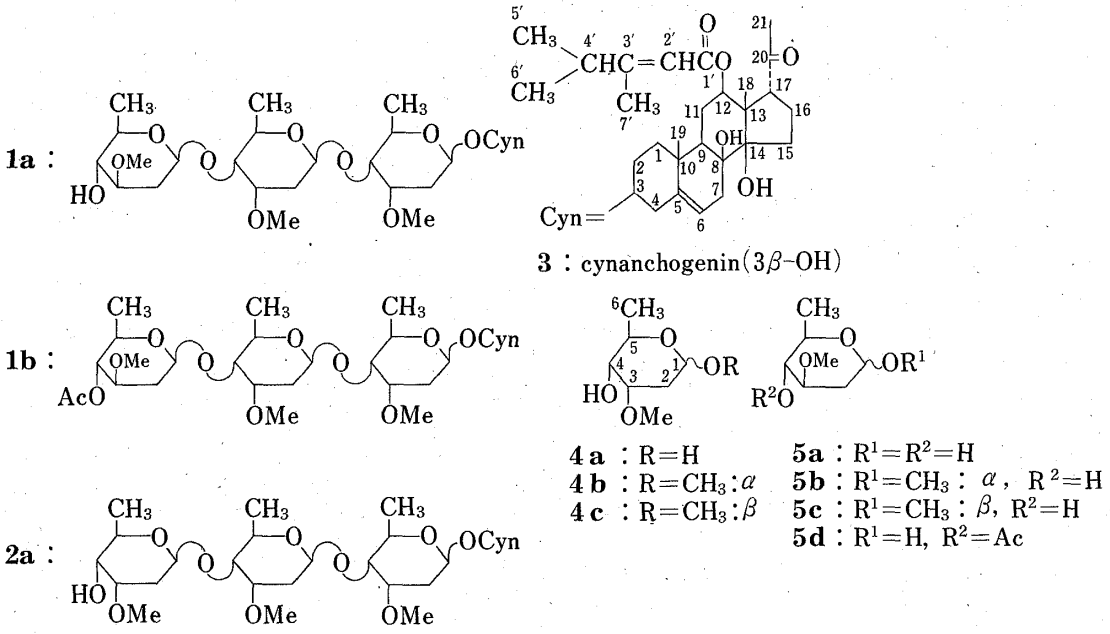

Chart 2

This conclusion was supported by the conversion of **1a** into acetyl cynanchoside C_2 (**1b**) followed by acid hydrolysis, that is, the final products in this series of reactions were 4-*O*-acetyl oleandrose (**5d**), cymarose (**4a**), and cynanchogenin (**3**).

The anomeric configurations of each sugar were assigned as all β on the basis of the coupling constants (each br d, J=9 Hz) for the three anomeric protons (δ 4.53, 4.79, 4.88) in the PMR spectrum recorded at 200 MHz.

^{13}C-Chemical schifts of the signals due to the sugar carbons supported this conclusion in comparison with ^{13}C-chemical shifts of **4c** and **5c**.

The above considerations led us to assign the structure of cynanchoside C_2 as cynanchogenin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside (**1a**), and the ^{13}C signals of **1a** were assigned as shown in Table I.

Mild acid hydrolysis of **2a** afforded **3** and **4a**. The UV, IR, PMR, and CMR (Table I) spectra of **2a** established that **2a** is a glycoside of **3** in which 3β-OH carries three moles of cymarose.

The anomeric configurations of the sugars were assigned as all β on basis of the CMR spectra as follows.

The PRFT method was applied to the CMR spectrum of **2a**, in order to differentiate between ^{13}C signals due to terminal cymarose and others in the sugar chain. A set of signals with longer T_1 (T_1=0.17 s, δ 70.8, 73.9, 78.6, 100.2) than others can be assigned to a terminal sugar, β-D-cymarose, on the basis of a comparison with the ^{13}C-chemical shifts of **4b** and **4c**.

The anomeric configurations of the remaining cymarose moieties were also assigned as β. This conclusion was confirmed by comparison of the CMR spectra of **1a** and **2a**. The sugar "sequence" of the central and inner (attached directly to the aglycone) sugars in **1a** and **2a** was identical, and the ^{13}C-chemical shifts of signals due to two moles of cymarose in **1a** were in agreement with those of the corresponding sugars in **2a** (Table I).

On the basis of the arguments mentioned above, we concluded that the structure of cynanchoside C_1 is cynanchogenin 3-*O*-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**2a**) (Chart 2), and the ^{13}C signals of **2a** were assigned as shown in Table I.

Experimental

Melting points were taken on a Kofler hot stage and are uncorrected. Optical rotation were measured with a JASCO DIP-4 digital polarimeter at room temperature. IR spectra were recorded with a Hitachi 215 grating infrared spectrophotometer. UV spectra were taken on a Shimadzu UV-220 double-beam spectrophotometer. Field desorption (FD) mass spectra were obtained with a JEOL JMS-OISG-2 machine. ^1H- and ^{13}C-NMR spectra were recorded with JEOL FX-100 and FX-200 spectrometers, and tetramethylsilane was used as an internal standard for spectra run in pyridine-d_5.

Polyamide column chromatography was carried out on Polyamide C-200 (WAKO), and silica gel column chromatography was performed on Kieselgel 60 (Merck). TLC was carried out on silica gel PF_{254} (Type 60, Merck) and precoated TLC plates silica gel $60F_{254}$ (Merck), using the following solvent systems: (A) $CHCl_3$–MeOH–H_2O (10: 1: 1, under phase), (B) hexane–$CHCl_3$–acetone (19: 19: 1), (C) hexane–$CHCl_3$–MeOH (15: 14:1), (D) $CHCl_3$–MeOH (20: 1), (E) CH_2Cl_2–benzene–MeOH (6: 6: 1), (F) $CHCl_3$–MeOH (9: 1). Gas-liquid chromatographic analyses were carried out with a Shimadzu GC-3BF. All samples were injected as their trimethyl silyl ether (TMS) derivatives. L-Rhamnose TMS derivative was used as an internal standard. When a sugar showed more than one peak, the highest peak was compared with that of L-rhamnose. Analytical conditions were as follows. Column: 1.5% OV-17 (2.1 m) on Shimalite W. Column temperature: 85°C. N_2 flow: 1.0 kg/cm^2.

Isolation of Cynanchoside C_2 (1a) and Cynanchoside C_1 (2a) from *C. caudatum*——The dried rhizomes (50 kg) of *C. caudatum* were extracted with $CHCl_3$ and the $CHCl_3$ extract was treated with MeOH, followed by hexane, to yield a mixture of crude glycosides (2.7 kg) according to the procedure described before.[4]

The crude glycosides (200 g) were extracted with hexane–benzene (1: 1, v/v, 10 l), and the extract (40 g) was subjected to column chromatography on polyamide (400 g). The non-polar glycoside mixture was eluted with ethyl acetate–hexane mixture (1: 19, v/v). Preliminary fractionation of the mixture containing **1a** and **2a** (10.1 g) by silica gel TLC (solvent system A) followed by silica gel column chromatography with $CHCl_3$–MeOH mixture, the MeOH content of which was increased gradually from 1 to 12% (v/v), and with benzene–acetone (4: 1, v/v) mixture gave the glycosides **2a** and **1a**, in that order. **1a** and **2a** were purified by repeated silica gel TLC (solvent systems B, C, and D).

Cynanchoside C_2 (**1a**): 74 mg. mp 132.5—135.5°C. $[\alpha]_D$ −14.6° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{49}H_{78}O_{15}$: C, 64.90; H, 8.61. Found: C, 64.56; H, 8.64. FD-MS m/z: 906 (M^+). UV λ_{max}^{MeOH} nm (ε): 222 (14600). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570 (OH), 1705 and 1640 (C=CH–C=O). ^1H-NMR (in $CDCl_3$) δ: 1.08 (6H, d, J= 7 Hz, C-5′- and C-6′-CH_3), 1.12 (3H, s, C-19-CH_3), 1.56 (3H, s, C-18-CH_3), 2.15 (3H, s, C-7′-CH_3), 2.17 (3H, s, C-21-CH_3), 5.39 (1H, br s, C-6-H), 5.54 (1H, br s, C-2′-H), 1.25 (6H, d, J=6 Hz, sugar C-6-CH_3), 1.35 (3H, d, J=6 Hz, sugar C-6-CH_3), 3.42 (3H, s, sugar C-3-OCH_3), 3.48 (6H, s, sugar C-3-OCH_3), 4.53 (1H, br d, J= 9 Hz, sugar C-1-H), 4.79 (1H, br d, J=9 Hz, sugar C-1-H), 4.88 (1H, br d, J=9 Hz, sugar C-1-H).

Cynanchoside C_1 (**2a**): 30 mg. mp 123.5—129°C. $[\alpha]_R$ +30.4° (c=0.25, $CHCl_3$). *Anal.* Calcd for $C_{49}H_{78}O_{15}\cdot 1/2H_2O$: C, 64.26; H, 8.63. Found: C, 64.26; H, 8.68. FD-MS m/z: 906 (M^+). UV λ_{max}^{MeOH} nm (ε): 224 (12300). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1700 and 1640 (C–CH–C–O). ^1H-NMR (in $CDCl_3$) δ: 1.06 (6H, d, J=7 Hz, C-5′- and C-6′-CH_3), 1.12 (3H, s, C-19-CH_3), 1.54 (3H, s, C-18-CH_3), 2.13 (3H, s, C-7′-CH_3), 2.15 (3H, s, C-21-CH_3), 5.35 (1H, br m, C-6-H), 5.51 (1H, br s, C-2′-H), 1.22 (3H, d, J=6 Hz, sugar C-6-CH_3), 1.25 (3H, d, J=6 Hz, sugar C-6-CH_3), 1.27 (3H, d, J=6 Hz, sugar C-6-CH_3), 3.42 (3H, s, sugar C-3-OCH_3), 3.44 (3H, s, sugar C-3-OCH_3), 3.46 (3H, s, C-3-OCH_3), 4.60—4.87 (4H, m).

Mild Acid Hydrolysis of Cynanchoside C_2 (1a)——**1a** (4 mg) was hydrolyzed with 0.05 N H_2SO_4 in 50% MeOH (10 ml) at 75°C for 1.5 h. After removal of MeOH under reduced pressure, H_2O (5 ml) was added to the reaction mixture, and the whole was warmed at 60°C for 3 h. The acidic solution was neutralized with 1% $Ba(OH)_2$.

Cynanchogenin (**3**), *Rf* 0.47, cymarose (**4a**), *Rf* 0.42, and oleandrose (**5a**), *Rf* 0.39, were identified by TLC comparison (solvent system E) with authentic samples, moreover, **4a** and **5a** were detected by GLC. TMS-sugar relative retention times (min): 0.22 (D-oleandrose), 0.28 (D-cymarose).

Acid Hydrolysis of Acetyl Cynanchoside C_2 (1b)——Ac_2O (4 ml) was added to a solution of **1a** (3.4 mg) in pyridine (4 ml), and the mixture was allowed to stand for 15 h at room temperature. The product (**1b**) was hydrolyzed with 0.05 N H_2SO_4 in 50% MeOH (2 ml) at 70°C for 1 h. H_2O (2 ml) was added to the reaction mixture, followed by the removal of MeOH under reduced pressure. The aqueous solution was warmed at 60°C for 3 h. The reaction mixture was worked up as described above. Cynanchogenin (**3**), *Rf* 0.47, cymarose (**4a**), *Rf* 0.42, and 4-*O*-acetyl oleandrose (**5d**), *Rf* 0.67 were identified by TLC comparison (solvent system F) with authentic samples, and the absence of oleandrose (**5a**) was confirmed at the same time.

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References and Notes

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