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Studies on the Constituents of Asclepiadaceae Plants. L.¹⁾ Two New
Oligoglycosides, Cynanchoside C₂ and Cynanchoside
C₁, from *Cynanchum caudatum* MAX.

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Two new steroidal oligoglycosides, named cynanchoside C₂ (**1a**) and cynanchoside C₁ (**2a**), were isolated from the rhizome of *Cynanchum caudatum* MAX. The structures of **1a** and **2a** were elucidated by the application of ¹³C-nuclear magnetic resonance spectroscopy and chemical reactions.

Keywords—*Cynanchum caudatum*; Asclepiadaceae; steroidal oligoglycosides; cynanchoside C₂; cynanchoside C₁; D-cymarose; D-oleandrose; cynanchogenin; ¹³C-NMR; PRFT method

The rhizome of *C. caudatum* MAX. (Asclepiadaceae) has a high content of C/D-*cis*-polyoxy-pregnane oligoglycosides. This crude glycosides mixture has immunopotential activities.²⁾

This paper describes the isolation and structures of cynanchoside C₂³⁾ (**1a**) and cynanchoside C₁ (**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,

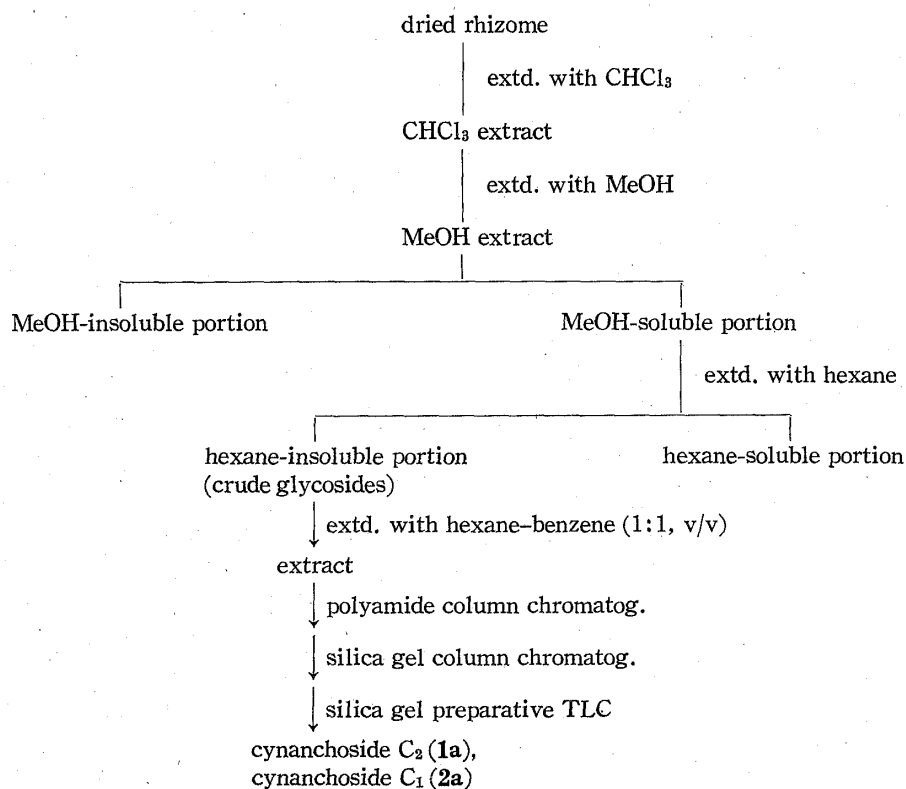


Chart 1

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, $[\alpha]_D -14.6^\circ$ ($c=1.0$, CHCl_3), $\text{C}_{49}\text{H}_{78}\text{O}_{15}$, and cynanchoside C_1 (**2a**), amorphous, mp 123.5–129°C, $[\alpha]_D +30.4^\circ$ ($c=0.25$, CHCl_3), $\text{C}_{49}\text{H}_{78}\text{O}_{15} \cdot 1/2\text{H}_2\text{O}$.

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

Mild acid hydrolysis of **1a** afforded cynanchogenin⁵⁾ (**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 $3\beta\text{-OH}$ carries two moles of cymarose and one mole of oleandrose (see "Experimental" and Table I).⁶⁾

The partially relaxed Fourier transform (PRFT) method⁷⁾ 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 ^{e)}	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_6 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.

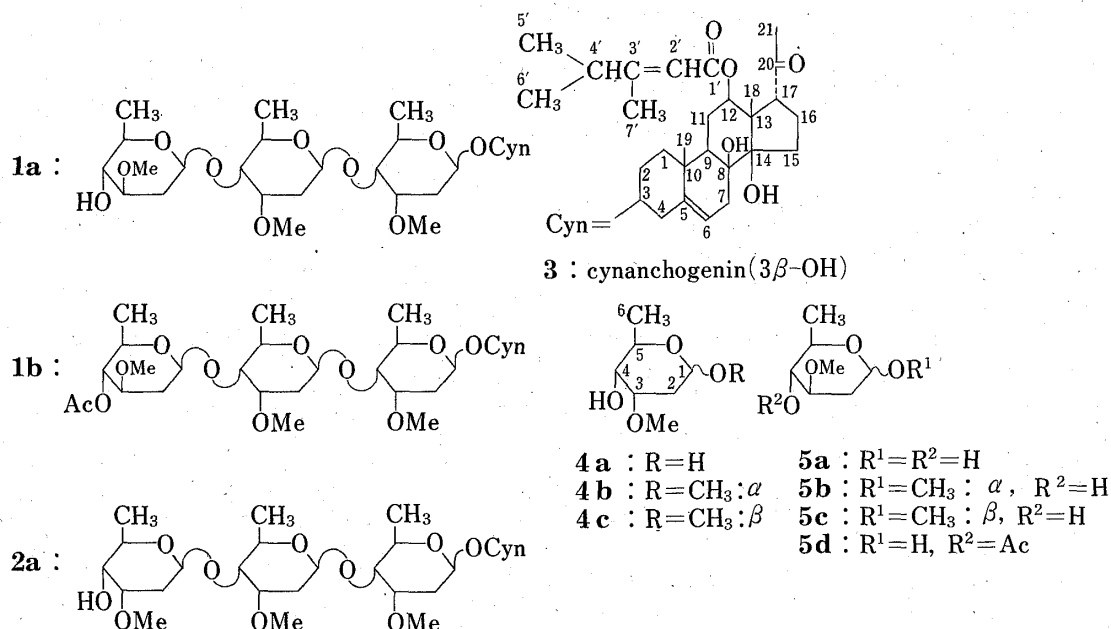


Chart 2

This conclusion was supported by the conversion of **1a** into acetyl cynanchoside **C₂** (**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 shifts 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 **C₂** as cynanchogenin 3-*O*- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 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 3 β -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 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 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 PF₂₅₄ (Type 60, Merck) and precoated TLC plates silica gel 60F₂₅₄ (Merck), using the following solvent systems: (A) CHCl_3 -MeOH-H₂O (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/cm².

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. $[\alpha]_D -14.6^\circ$ ($c=1.0$, CHCl_3). Anal. Calcd for $\text{C}_{49}\text{H}_{78}\text{O}_{15}$: C, 64.90; H, 8.61. Found: C, 64.56; H, 8.64. FD-MS m/z : 906 (M^+). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 222 (14600). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ 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'-CH₃), 1.12 (3H, s, C-19-CH₃), 1.56 (3H, s, C-18-CH₃), 2.15 (3H, s, C-7'-CH₃), 2.17 (3H, s, C-21-CH₃), 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-CH₃), 1.35 (3H, d, $J=6$ Hz, sugar C-6-CH₃), 3.42 (3H, s, sugar C-3-OCH₃), 3.48 (6H, s, sugar C-3-OCH₃), 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. $[\alpha]_R +30.4^\circ$ ($c=0.25$, CHCl_3). Anal. Calcd for $\text{C}_{49}\text{H}_{78}\text{O}_{15} \cdot 1/2\text{H}_2\text{O}$: C, 64.26; H, 8.63. Found: C, 64.26; H, 8.68. FD-MS m/z : 906 (M^+). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 224 (12300). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ 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'-CH₃), 1.12 (3H, s, C-19-CH₃), 1.54 (3H, s, C-18-CH₃), 2.13 (3H, s, C-7'-CH₃), 2.15 (3H, s, C-21-CH₃), 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-CH₃), 1.25 (3H, d, $J=6$ Hz, sugar C-6-CH₃), 1.27 (3H, d, $J=6$ Hz, sugar C-6-CH₃), 3.42 (3H, s, sugar C-3-OCH₃), 3.44 (3H, s, sugar C-3-OCH₃), 3.46 (3H, s, C-3-OCH₃), 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% $\text{Ba}(\text{OH})_2$.

Cynanchogenin (**3**), R_f 0.47, cymarose (**4a**), R_f 0.42, and oleandrose (**5a**), R_f 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**), R_f 0.47, cymarose (**4a**), R_f 0.42, and 4-*O*-acetyl oleandrose (**5d**), R_f 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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