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### Antitumor Active Glycosides from Condurango Cortex

Antitumor active condurangoglycosides A<sub>0</sub> and C<sub>0</sub> were isolated from Condurango Cortex. Their structures were established by chemical and spectral evidences.

**Keywords**—*Marsdenia cundurango*; Asclepiadaceae; pregnane; ester glycoside; condurangoglycosides A<sub>0</sub> and C<sub>0</sub>, antitumor activity; <sup>13</sup>C-NMR

The crude drug Condurango Cortex, bark of *Marsdenia cundurango* REICH. (Asclepiadaceae) native to north-western part of South America, has been used as an aromatic bitter stomachic in popular medicine, and also used against cancer or syphilis in folk remedy though no report has been found on such effects.<sup>1)</sup> According to the reports on the antitumor screening by CCNSC, the extract of this plant was evaluated failure against Sarcoma-180, Adenocarcinoma 755, Humansarcoma HSI, and KB systems.<sup>2)</sup>

Ahsan *et al.* reported that a polyoxypregnane glycoside, amplexoside-A, from a Asclepiadaceae plant *Asclepias amplexicaulis* showed a cancer inhibitory activity in the KB assay.<sup>3)</sup> Generally, Asclepiadaceae plant is abundant in the esterified polyoxypregnane glycosides,<sup>4)</sup> which therefore, promise sources for anti-tumor agents.

On the constituents of cortex condurango, Tschesche and his co-workers studied the structures of condurango glycosides A (3), A<sub>1</sub> (4), C (5), and C<sub>1</sub> (6).<sup>5)</sup> We wish to describe in this paper that the separation and structural determinations of two new condurangoglycosides A<sub>0</sub> (1) and C<sub>0</sub> (2) from the anti-tumor active points of view.

The crude glycoside mixture of condurango obtained by a usual procedure was separated into three fractions using a silica gel column chromatography. One of the fractions which still consisted of at least eight compounds by a high performance liquid chromatography (HPLC) examination showed a strong activity against a solid type Ehrlich carcinoma.<sup>6)</sup> The two new glycosides A<sub>0</sub> (1) and C<sub>0</sub> (2) were obtained from this fraction by a combined HPLC system (Waters prep-500 on silica gel followed by semi preparative scale columns of Wako-gel LC5H and Merck Lichroprep. RP-8).

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Condurangoglycoside **1**, amorphous white powder, mp 170–174°,  $[\alpha]_D +43.9^\circ$  ( $c=0.62$ , MeOH) exhibited positive Keller-Kiliani (K.K.) and Liebermann-Burchard (L.B.) reactions, which indicated the presence of 2-deoxy sugar and steroidal moieties in the molecule. The proton nuclear magnetic resonance ( $^1\text{H-NMR}$ ), infrared absorption (IR), and ultraviolet absorption (UV) spectra of **1** denoted the presence of an acetyl and a cinnamoyl ester moieties on a polyoxypregnane with C-20 oxo group as following:  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 0.96 (3H, s, C-19  $\text{CH}_3$ ), 1.11 (3H, s, C-18  $\text{CH}_3$ ), 1.23 (3H, d,  $J=6$  Hz), 1.25 (3H, d,  $J=6$  Hz), 1.29 (3H, d,  $J=6$  Hz), 1.86 (3H, s,  $-\text{OAc}$ ), 2.15 (3H, s, C-21  $\text{CH}_3$ ), 3.39 (3H, s,  $-\text{OCH}_3$ ), 3.44 (3H, s,  $-\text{OCH}_3$ ), 3.61 (3H, s,  $-\text{OCH}_3$ ), 4.80 (1H, d,  $J=10$  Hz, C-12 $\alpha$ -H), 5.34 (1H, t,  $J=10$  Hz, C-11 $\beta$ -H), 6.46 and 7.78 (2H, ABq,  $J=16$  Hz,  $-\text{C}-\text{CH}=\text{CH}-\text{Ph}$ ), 7.45 (3H, m), and 7.60 (2H, m). IR  $\nu_{\text{max}}^{\text{Nujol}}$

$\text{cm}^{-1}$ : 3350 (br.), 1740, 1700, 1630, 1255, 1235, 1140, 1070, 870, 820. UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm ( $\epsilon$ ): 217 ( $2.33 \times 10^4$ ), 223 ( $2.10 \times 10^4$ ), 280 ( $3.39 \times 10^4$ ). Mild acid hydrolysis of **1** afforded an aglycone, which was identified as condurangenin A (**7**) by a comparison with authentic sample in the chromatographic behaviors and the spectral data.<sup>7)</sup> From the sugar fraction of the hydrolysate, cymarose (**9**), pachybiose (**10**)<sup>8)</sup> and a new trisaccharide (**11**),  $[\alpha]_D +17.4^\circ$  ( $c=1.83$ , MeOH): acetate mp 165–169°, and its methyl glycoside (**12**), were detected on a silica gel thin-layer chromatography. The trisaccharide (**11**) was hydrolysed into pachybiose (**10**) and glucose (**13**) by a  $\beta$ -glycosidase mixture prepared from a kind of snail (*Fruticicola gainesi*). These results and the signals of four anomeric carbons on the  $^{13}\text{C-NMR}$  spectrum of **1** designated that this glycoside contained four sugar components in order of cymarose (**9**), oleandrose (**14**), 6-deoxy-3-O-methylallose (**15**) and glucose (**13**) from the aglycone side. The  $^1\text{H-NMR}$  spectrum of **11** (pyridine- $d_5$ )  $\delta$ : 1.56 (3H, d,  $J=8$  Hz), 1.62 (3H, d,  $J=8$  Hz), 3.50 (3H, s), 3.81 (3H, s), 4.92 (1H, d,  $J=8$  Hz), 5.26 (1H, d,  $J=8$  Hz), 5.56 (1H, br. s), showed  $\beta$ -glycosyl linkages of both 6-deoxy-3-O-methyl-allose (**15**) and glucose (**13**).

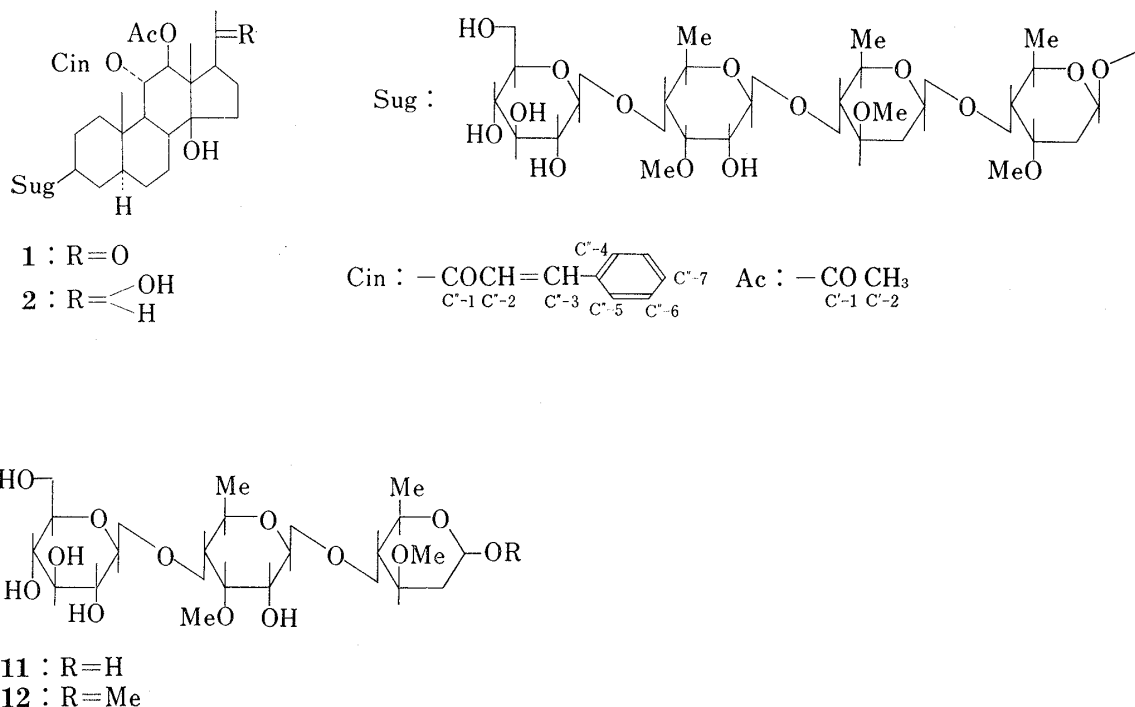


Chart 1

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TABLE I. Carbon Chemical Shifts of Glycosides and Aglycones

	1	7	2	8	5
C- 1	37.3(-1.1)	38.4	37.3(-0.9)	38.2	37.3
C- 2	30.5(-2.3)	32.8	30.5(-2.3)	32.8	30.4
C- 3	79.3(+7.5)	71.8	79.2(+7.2)	72.0	79.2
C- 4	35.6(-4.1)	39.7	35.5(-4.1)	39.6	35.6
C- 5	44.8(-0.4)	45.2	44.7(-0.4)	45.1	44.8
C- 6	29.4	29.6	29.5	29.6	29.5
C- 7	28.4	28.6	28.5	28.4	28.5
C- 8	40.0	39.9	40.0	40.1	40.1
C- 9	50.2	50.3	50.4	50.3	50.4
C-10	38.0	38.1	38.0	37.9	38.0
C-11	71.8	69.9	71.9	69.8	72.0
C-12	78.5	78.5	79.8	79.6	79.8
C-13	54.8	54.9	53.9	53.4	54.0
C-14	83.9	84.0	83.4	83.4	83.5
C-15	34.0	34.1	33.1	33.1	33.1
C-16	24.4	24.5	26.7	26.7	26.8
C-17	58.3	58.4	52.8	52.8	52.8
C-18	12.4	12.7	12.5	12.6	12.5
C-19	11.6	12.0	12.5	12.6	12.5
C-20	213.6	213.4	70.3	70.2	70.3
C-21	31.9	31.7	23.7	23.6	23.7
C'-1	170.2	170.3	170.5	170.4	170.4
C'-2	21.4	21.5	21.7	21.6	21.6
C''-1	166.9	166.8	167.1	167.0	167.1
C''-2	118.2	118.0	118.7	118.6	118.7
C''-3	146.3	146.3	145.6	145.6	145.6
C''-4	130.8	130.9	130.7	130.9	130.6
C''-5	129.3	129.8	129.2	129.2	129.2
C''-6	128.4	129.4	128.6	128.6	128.6
C''-7	134.9	134.6	134.8	134.8	134.8

( ): glycosidation shifts, measured in pyridine- $d_5$ .

Condurangoglycoside C<sub>0</sub> (**2**), amorphous powder, mp 160–170°,  $[\alpha]_D +25.9^\circ$  ( $c=1.28$ , MeOH), also showed positive K.K and L.B reactions. The spectral data of **2** were quite similar to those of **1** except that **2** lacked a peak due to the methyl ketone instead of an additional doublet methyl signal at about  $\delta$  1.24 ppm in its  $^1\text{H-NMR}$  spectrum. This glycoside (**2**) gave condurangogenin C (**8**)<sup>7)</sup> and a sugar mixture which afforded identical chromatogram on TLC with that of the sugar fraction of **1**. Therefore, it is deduced that the difference between both the glycosides is attributed to the diversity at C-20 position of the pregnane aglycones. This was confirmed by a reduction of **1** with sodium borohydride to give **2**.

The terminal glucose of the glycosides was established by a treatment of **2** with the  $\beta$ -glycosidase mixture<sup>9)</sup> prepared from the aqueous extract of this plant to give deglycosyl condurangoglycoside C<sub>0</sub> (**5**), amorphous powder,  $[\alpha]_D +44.9^\circ$  ( $c=0.37$ , MeOH), of which no carbon signals due to glucose moiety were detected in the  $^{13}\text{C-NMR}$  spectrum (Table II). Quantitative analyses of the released glucose from the enzymatic hydrolysate of **2** were carried out gravimetrically and colorimetrically by Glucostat Reagent,<sup>10)</sup> showing one mole of glucose was contained. Although the elemental analyses of both the glycosides gave unsatisfactory results, their  $^{13}\text{C-NMR}$  spectra supported that the glycosides contained only one molecule of

9) The preparation of this enzyme will be reported in other paper.

10) Made by Worthington Biochemical Corporation.

TABLE II. Sugar Carbon Chemical Shifts

		1	2	5	11	10	16
9	1	96.1	96.0	96.1			96.3
	2	37.5	37.6	37.6			37.2
	3	77.9	77.8	77.9			77.7
	4	82.9	82.8	82.8			83.1
	5	68.9	68.8	68.8			68.9
	6	18.2	18.2	18.6			18.5
	-OMe	58.8	58.8	58.8			58.8
14	1	101.7	101.8	101.9	91.4	91.4	
	2	38.1	38.0	38.0	36.6	36.6	
	3	76.2	76.1	76.2	77.6	77.4	
	4	83.3	83.3	83.5	84.0	83.9	
	5	72.0	71.9	72.0	67.3	67.3	
	6	18.7	18.7	18.6	18.2	18.6	
	-OMe	57.2	57.3	57.2	57.3	57.2	
15	1	101.8	101.8	101.9	102.0	102.1	
	2	72.6	72.6	73.1*	72.3	73.2	
	3	83.3	83.3	83.5	83.2	83.9	
	4	83.0	83.1	74.6*	83.1	74.5	
	5	69.5	69.4	71.0*	69.5	70.9	
	6	18.8	18.8	18.9	19.1	19.2	
	-OMe	61.6	61.8	62.0	61.6	62.0	
13	1	106.4	106.5		106.4		
	2	75.4	75.4*		75.4		
	3	78.2	78.3*		78.2		
	4	72.0	71.9*		71.9		
	5	78.3	78.3*		78.2		
	6	63.1	63.0*		63.0		

\* The shifts with asterisk have the longest dipole-dipole relaxation time by PRFT measurements.

glucose, respectively. The molecular ion of the glycosides was not obtained by means of several techniques of mass spectrometry.

The carbon chemical shifts of the glycosides were assigned from the multiplicities and the correlation to those of the aglycones and sugars. Since the glycosidation shifts<sup>11)</sup> of the aglycone carbons were observed at C-2 ( $-2.3$  ppm), C-3 ( $+7.5$ ), and C-4 ( $-4.1$ ) in **1**, the sugar moiety linked to C-3 hydroxyl group of the aglycone. The terminal glucose signals are easily distinguished from other sugar signals by PRFT measurement.<sup>12)</sup> The terminal sugar of **5** was assigned to be **15** from its PRFT measurements. The glycosidation shifts at C-3 ( $-0.2$  ppm), C-4 ( $+8.5$ ), and C-5 ( $-1.6$ ) carbons of 6-deoxy-3-O-methyl allosyl moiety between **2** and **5** were also seen in the case between **11** and **10**. This shift indicated the glucose linked at C-4 of **15**. The chemical shifts attributed to oleandrosyl and cymarosyl moieties were assigned by comparison with those of cynanchoside C<sub>2</sub>(**16**) from *Cynanchum caudatum*<sup>13)</sup> under the consideration of the glycosidation shifts.

Thus, the structures of condurangoglycosides A<sub>0</sub> (**1**) and C<sub>0</sub> (**2**) were given as  $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-6-deoxy-3-O-methyl-allopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-oleandropyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-cymaropyranosyl-(1 $\rightarrow$ 3)-condurangogenin A and C, respectively. Antitumor activities and LD<sub>50</sub> were shown in the Table III.

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TABLE III. Antitumor Activities and LD<sub>50</sub> of 1 and 2

	Sarcoma 180, (T/C) (ddY mouse)	Ehrlich carcinoma (T/C) (ICR mouse)	LD <sub>50</sub> mg/kg
1	4.0	29.0	75
2	21.0	34.0	375

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### Synthesis of Non-K-region Dihydrodiols and Epoxides of Carcinogenic Dibenz[*c,h*]acridine

Dibenz[*c,h*]acridine-1,2-oxide, dibenz[*c,h*]acridine-3,4-oxide, *trans*-1,2-dihydroxy-1,2-dihydrodibenz[*c,h*]acridine and *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*c,h*]acridine, which are possible active metabolites of dibenz[*c,h*]acridine, were synthesized.

**Keywords**—arene oxide; aza-arene oxide; dibenz[*c,h*]acridine oxide; carcinogen; mutagen

For the carcinogenic polynuclear aromatic hydrocarbons including benzo[*a*]pyrene, metabolically formed arene oxides and diol epoxides have emerged as the most responsible molecules to account covalent binding to biomolecules such as nucleic acid proposed as a prerequisite for the chemical induction of cancer.<sup>1)</sup> Many aza-arenes such as dibenz[*c,h*]acridine (DBA) are known to be carcinogenic and some of them are as quite highly active as benzo[*a*]pyrene.<sup>2)</sup> They were also found in tar, urban atmosphere and tobacco smoke.<sup>3)</sup>

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