CORCHORUSOSIDES A, B, C, D, AND E, NEW CARDIOTONIC OLIGOGLYCOSIDES FROM THE SEEDS OF *CORCHORUS OLITORIUS* L. (MOROHEIYA)

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Abstract — The methanolic extract of the seeds of *Corchorus olitorius* L. (Moroheiya) was found to show inhibitory effect against Na^+,K^+-ATPase and positive inotropic activity in the guinea pig isolated atria. Through bioassay-guided separation from the methanolic extract, new cardenolide oligoglycosides called corchorusosides A, B, C, D, and E were isolated together with six known cardenolide oligoglycosides. The structures of new corchorusosides were determined on the basis of chemical and physicochemical evidence. All cardenolide oligoglycosides from the seeds showed potent inhibitory activity against Na^+,K^+-ATPase, which was equivalent to those of digitoxin and ouabain. The methanolic extract, glycoside fraction, and principal glycoside showed potent acute toxicity by intraperitoneal administration, whereas they showed little acute toxicity by oral administration. Furthermore, by means of HPLC quantitative analysis of the cardiotonic oligoglycosides, it was found that the glycosides mainly distributed in the seeds , while the edible parts such as fresh young leaves and stems contained only trace amount.

An annual herbaceous plant *Corchorus olitorius* L. (Tiliaceae), whose young aerial parts such as the leaves and stems are commonly called "moroheiya" in Japanese, are extensively consumed as a vegetable and health food. As chemical constituents of this plant, several cardenolide oligoglycosides and mucilages have been characterized from the seeds,[1] whereas triterpenes, sterols, and flavonoids were isolated from the leaves and roots.[2] Recently, the mass communication media in Japan gave prominence to the death accident of cattle resulted from feeding the withered aerial parts with seeds of *C. olitorius*, and finally it was concluded that the cattle death was attributable to the cardenolide oligoglycosides in the seeds without detailed examination of their cardiotonic activity, acute toxicity, and content.

In the course of our studies on the bioactive principles of medicinal foodstuffs,[3] we have characterized three ionone glycosides, corchoionosides A, B, and C, with inhibitory activities on the histamine release from rat peritoneal excudate cells and six fatty acids, corchorifatty acids A, B, C, D, E, and F, with inhibitory activity on the NO production in cultured mouse peritoneal macrophages from the young leaves of *C. olitorius*.[4] As a continuing study of "moroheiya," we have found that the methanolic extract from the seeds of *C. olitorius* showed inhibitory effects against Na^+,K^+-adenosine triphosphatase (Na^+,

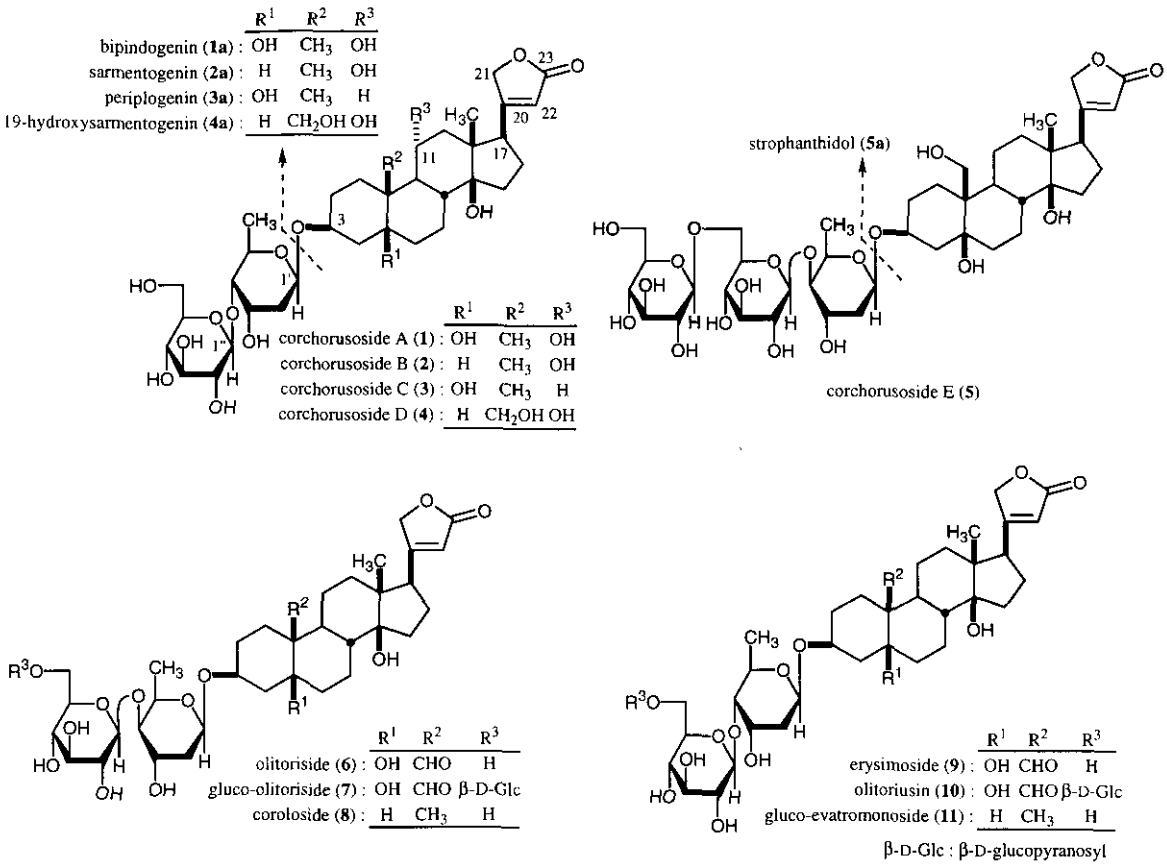

Figure 1

K^+-ATPase) and positive inotropic effect on the guinea pig isolated atria. From the methanolic extract, new cardenolide oligoglycosides called corchorusosides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) were isolated through the bioassay-guided separation using Na^+,K^+-ATPase inhibitory effect together with six known cardenolide oligoglycosides, olitoriside (**6**),[5] gluco-olitoriside (**7**),[5] coroloside (**8**),[6] erysimoside (**9**),[1b] olitoriusin (**10**),[1b] and gluco-evatromonoside (**11**).[8] This communication deals with the structures of **1**—**5** and the inhibitory activity of all cardenolide oligoglycosides from the seeds on Na^+,K^+-ATPase. In addition, we describe the acute toxicity of the methanolic extract, 1-butanol-soluble portion, and the principal glycoside (**6**) and the distribution of the glycosides in plant.

The methanolic extract and 1-butanol-soluble portion from the seeds showed inhibitory activity on Na^+,K^+-ATPase and positive inotropic activity. The 1-butanol-soluble portion was separated by normal silica gel column chromatography ($CHCl_3$-MeOH-H_2O) to give the cardenolide fraction, which was subjected to ODS silica gel column chromatography (MeOH-H_2O) and HPLC (YMC-Pack ODS-A, MeOH-H_2O) to give corchorusoside A (**1**, 0.006% from the seeds), B (**2**, 0.004%), C (**3**, 0.002%), D (**4**, 0.001%), and E (**5**, 0.010%).

Corchorusoside A (**1**), a white powder, $[\alpha]_D^{24}$ +12.8°, $C_{35}H_{54}O_{14}$, UV (MeOH) : 218 nm (log ε 4.2), IR (KBr) : 3453, 2940, 1747, 1072 cm^{-1}, showed quasimolecular ion peak at *m/z* 721 $(M+Na)^+$ in the positive-ion FAB-MS, whereas a quasimolecular ion peak at *m/z* 697 $(M-H)^-$ in addition to fragment ion peaks at *m/z* 535 $(M-C_6H_{11}O_5)^-$ and *m/z* 405 $(M-C_{11}H_{21}O_8)^-$ were observed in the negative-ion FAB-MS. Methanolysis of **1** with 9% HCl-dry MeOH liberated bipindogenin

Table 1. ^{13}C-NMR Data for Corchorusosides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**)

	1[a]	2[a]	3[a]	4[b]	5[b]		1[a]	2[a]	3[a]	4[b]	5[b]
1	27.7	33.6	26.1	27.8	24.5	Dig-1'	97.3	96.5	97.4	96.5	
2	27.9	28.3	26.5	31.6	26.2	2'	39.0	39.3	39.0	39.2	
3	76.4	73.6	75.9	73.4	75.3	3'	67.7	67.8	67.7	67.8	
4	35.8	31.2	35.5	28.1	35.9	4'	83.6	84.0	83.6	84.0	
5	74.2	38.8	73.7	42.6	76.1	5'	69.1	68.9	69.2	68.9	
6	35.8	27.6	35.5	27.4	35.9	6'	18.7	18.9	18.7	18.8	
7	24.4	22.2	24.4	21.9	20.3	Boi-1'					98.2
8	40.7	41.3	41.1	32.7	40.9	2'					35.2
9	45.4	42.4	39.3	41.2	39.5	3'					66.3
10	42.5	37.0	41.2	41.0	43.7	4'					76.4
11	67.9	67.8	22.1	68.4	22.3	5'					69.6
12	50.5	50.5	40.0	50.5	40.4	6'					17.7
13	50.2	50.2	50.0	50.4	50.1	Glc-1''	106.0	105.9	106.0	105.8	103.2
14	84.3	84.3	84.7	84.7	85.0	2''	75.1	75.2	75.2	75.1	74.6
15	33.7	33.6	33.2	33.4	32.8	3''	78.4	78.4	78.5	78.4	78.4
16	27.3	27.3	27.3	27.4	27.3	4''	71.7	71.7	71.7	71.6	72.0
17	51.1	51.3	51.4	51.3	51.4	5''	78.3	78.2	78.3	78.2	77.4
18	17.7	17.6	16.2	17.8	16.3	6''	62.7	62.7	62.7	62.6	70.6
19	17.8	24.4	17.3	66.8	64.8	Glc-1'''					105.6
20	175.2	175.3	175.8	175.5	175.9	2'''					75.3
21	73.7	73.7	73.7	73.8	73.8	3'''					78.5
22	117.7	117.7	117.8	117.6	117.7	4'''					71.7
23	174.4	174.4	174.5	174.5	174.5	5'''					78.4
						6'''					62.8

pyridine-d_5, a : 68 MHz, b : 125 MHz

(**1a**)[9] together with methyl digitoxoside and methyl glucoside, while acid hydrolysis of **1** furnished D-digitoxose and D-glucose, which were identified by GLC analysis of their trimethylsilylthiazolidine derivative.[10] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[11] of **1** indicated the presence of a bipindogenin part [δ 1.10, 1.32 (both s, 18, 19-H$_3$), 4.14 (m, 11-H), 4.35 (m, 3-H), 5.01, 5.25 (both d, *J*=18.1 Hz, 21-H$_2$), 6.09 (br s, 22-H)], β-D-digitoxopyranosyl moiety [δ 1.59 (d, *J*=6.3 Hz, 6'-H$_3$), 3.65 (dd, *J*=2.6, 9.6 Hz, 4'-H), 4.68 (m, 3'-H), 5.39 (dd-like, 1'-H)], and β-D-glucopyranosyl moiety [δ 4.95 (d, *J*=7.9 Hz, 1''-H)]. The HMBC experiment of **1** showed long-range correlations between the 1''-proton and the 4'-carbon and between the 1'-proton and the 3-carbon. Finally, comparisons of the ^{13}C-NMR data with those of **9** and **11** led us formulate the structure of corchorusoside A (**1**).

The structures of corchorusosides B (**2**),[12] C (**3**),[13] D (**4**),[14] and E (**5**)[15] were elucidated in the same way. By the acid hydrolysis and methanolysis, **2**, **3**, and **4** gave sarmentogenin (**2a**),[9] periplogenin (**3a**),[16] and 19-hydroxysarmentogenin (**4a**),[17] respectively, together with D-digitoxose and D-glucose or their methyl glycosides, while **5** yielded strophanthidol (**5a**)[18] together with D-boivinose and D-glucose or their methyl glycosides in a 1 : 2 ratio. On the basis of detailed examination of their ^{1}H- and ^{13}C-NMR (Table 1) spectra[11] including HMBC experiment, which showed long-range correlations between the following protons and carbons (**2**, **3**, and **4** : 1''-H and 4'-C, 1'-H and 3-C; **5** : 1'''-H and 6''-C; 1''-H and 4'-C, 1'-H and 3-C). The structures of corchorusosides B (**2**), C (**3**), D (**4**), and E (**5**) were characterized as shown.

Table 2 shows inhibitory activity of the extract, fractions, corchorusosides (**1**—**5**), and six known cardenolide oligoglycosides (**6**—**11**) against Na^+,K^+-ATPase, which has been usually used as a indication of cardiotonic activity. All cardenolide oligo-

Table 2. Inhibitory Activity of the MeOH Extract, 1-Butanol-soluble Portion, Water-soluble Portion, Glycoside Fraction, and Glycosides (**1**—**11**) from the Seeds of *C. olitorius* against Na^+,K^+-ATPase

Sample	IC_{50}
MeOH extract	4.3 μg/ml
1-butanol-soluble portion	1.9 μg/ml
water-soluble portion	120 μg/ml
glycoside fraction	0.37 μg/ml
corchorusoside A (**1**)	1.1×10^{-6} M
corchorusoside B (**2**)	6.3×10^{-7} M
corchorusoside C (**3**)	4.5×10^{-7} M
corchorusoside D (**4**)	1.3×10^{-6} M
corchorusoside E (**5**)	9.0×10^{-7} M
olitoriside (**6**)	5.7×10^{-7} M
gluco-olitoriside (**7**)	8.3×10^{-7} M
corotoside (**8**)	2.7×10^{-7} M
erysimoside (**9**)	7.7×10^{-7} M
olitoriusin (**10**)	8.1×10^{-7} M
gluco-evatromonoside (**11**)	2.1×10^{-7} M
digitoxin	2.2×10^{-7} M
ouabain (G-strophanthin)	5.3×10^{-7} M

The incubation mixture contained 50 mM imidazole-HCl buffer (pH 7.2), 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA-2Na, 1 mg/mL Na^+,K^+-ATPase (from dog kidney, Sigma), 4.6 mM ATP·3Na as a substrate, and test sample in a total volume of 0.2 mL. The reaction was initiated by the addition of ATP at 37 °C. After 30 min of incubation, the reaction was stopped by the addition of 800 μL of 20% TCA. Phosphate was measured by the method Fiske and Subbarow.[20]

glycosides (**1**—**11**) from the seeds of *C. olitorius* were found to show potent inhibitory activity against Na^+,K^+-ATPase, which were practically equivalent to those of digitoxin and ouabain.

The methanolic extract, 1-butanol-soluble portion, and the principal glycoside (**6**) showed potent acute toxicity (LD_{50} : 542 mg/kg, 223 mg/kg, and 65.2 mg/kg, respectively) by intraperitoneal administration in mice, whereas they showed little acute toxicity by oral administration (LD_{50} : the extract and fraction > 2000 mg/kg, **6** > 500 mg/kg). The distribution of the cardiotonic glycosides in plant was clarified by HPLC quantitative analysis.[19] The cardiotonic oligoglycosides were found to exist in the matured seeds at high content, but they disappeared within two weeks after germination of the seeds and the edible parts such as the young leaves and stems contained only trace amount.

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11. The ^{1}H- and ^{13}C-NMR spectra of **1**, **2**, **3**, **4**, and **5** were assigned with the aid of homo- and hetero-correlation spectroscopy (^{1}H-^{1}H, ^{1}H-^{13}C COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuculear multiple bond connectivity (HMBC) experiments.

12. Corchorusoside B (**2**) : A white powder, $[\alpha]_D^{26}$ +12.9° (*c*=0.9, MeOH). High-resolution positive-ion FAB-MS : Calcd for $C_{35}H_{54}O_{13}Na$ $(M+Na)^+$: 705.3462; Found: 705.3474. UV λ_{max}^{MeOH} nm (log ε) : 217 (4.2). IR (KBr) cm^{-1} : 3410, 2934, 1741, 1072. ^{1}H-NMR (270 MHz, pyridine-d_5) δ : 1.08, 1.14 (3H each, both s, 18, 19-H_3), 1.62 (3H, d, *J*=5.9 Hz, 6'-H_3), 3.68 (1H, dd, *J*=2.6, 9.6 Hz, 4'-H), 4.06 (1H, m, 11-H), 4.28 (1H, m, 3-H), 4.30 (1H, dd, *J*=5.3, 11.6 Hz, 6"-H), 4.44 (1H, dd, *J*=2.6, 11.6 Hz, 6"-H), 4.74 (1H, br d, *J*=2.8 Hz, 3'-H), 4.96 (1H, d, *J*=7.6 Hz, 1"-H), 4.99, 5.25 (1H each, both d, *J*=18.0 Hz, 21-H_2), 5.41 (1H, dd, *J*=1.7, 7.9 Hz, 1'-H), 6.08 (1H, br s, 22-H). ^{13}C-NMR (68 MHz, pyridine-d_5) δc : given in Table 1. Negative-ion FAB-MS (*m/z*) : 681 $(M-H)^-$, 519 $(M-C_6H_{11}O_5)^-$. Positive-ion FAB-MS (*m/z*): 705 $(M+Na)^+$, 683 $(M+H)^+$.

13. Corchorusoside C (**3**) : a white powder, $[\alpha]_D^{24}$ +20.6° (*c*=0.5, MeOH). High-resolution positive-ion FAB-MS : Calcd for $C_{35}H_{54}O_{13}Na$ $(M+Na)^+$: 705.3462; Found: 705.3479. UV λ_{max}^{MeOH} nm (log ε) : 217 (4.1). IR (KBr) cm^{-1} : 3431, 2934, 1741, 1072. ^{1}H-NMR (270 MHz, pyridine-d_5) δ : 1.02, 1.07 (3H each, both s, 18, 19-H_3), 1.63 (3H, d, *J*=6.3 Hz, 6'-H_3), 3.66 (1H, dd, *J*=2.3, 9.2 Hz, 4'-H), 4.28 (1H, m, 3-H), 4.30, 4.46 (1H each, both dd-like, 6"-H_2), 4.97 (1H, d, *J*=7.6 Hz, 1"-H), 5.01, 5.23 (1H each, both d, *J*=17.5 Hz, 21-H_2), 5.35 (1H, dd-like, 1'-H), 6.12 (1H, br s, 22-H). ^{13}C-NMR (68 MHz, pyridine-d_5) δc : given in Table 1. Negative-ion FAB-MS (*m/z*): 681 $(M-H)^-$, 519 $(M-C_6H_{11}O_5)^-$, 389 $(M-C_{12}H_{21}O_8)^-$. Positive-ion FAB-MS (*m/z*): 705 $(M+Na)^+$, 683 $(M+H)^+$.

14. Corchorusoside D (**4**) : a white powder, $[\alpha]_D^{25}$ -5.1° (*c*=0.3, MeOH). High-resolution positive-ion FAB-MS : Calcd for $C_{35}H_{54}O_{14}Na$ $(M+Na)^+$: 721.3411; Found: 721.3409. UV λ_{max}^{MeOH} nm (log ε) : 217 (4.2). IR (KBr) cm^{-1} : 3439, 2938, 1701, 1070. ^{1}H-NMR (500 MHz, pyridine-d_5) δ : 1.10 (3H, s, 18-H_3), 1.63 (3H, d, *J*=6.1 Hz, 6'-H_3), 2.95 (1H, dd-like 17-H), 3.68 (1H, d, *J*=2.6, 9.4 Hz, 4'-H), 4.11, 4.20 (1H each, both d, *J*=11.0 Hz, 19-H_2), 4.30 (1H, dd, *J*=5.2, 11.9 Hz, 6"-H), 4.44 (1H, dd, *J*=2.1, 11.3 Hz, 6"-H), 4.32 (1H, m, 3-H), 4.37 (1H, m, 11-H), 4.71 (dd-like, 3'-H), 4.95 (1H, d, *J*=7.6 Hz, 1"-H), 5.00, 5.21 (1H each, both d, *J*=18.0 Hz, 21-H_2), 5.44 (1H, dd-like, 1'-H), 6.09 (1H, br s, 22-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δc : given in Table 1. Negative-ion FAB-MS (*m/z*): 697 $(M-H)^-$. Positive-ion FAB-MS (*m/z*): 721 $(M+Na)^+$, 699 $(M+H)^+$.

15. Corchorusoside E (**5**) : a white powder, $[\alpha]_D^{26}$ -12.1° (*c*=1.2, MeOH). High-resolution positive-ion FAB-MS : Calcd for $C_{41}H_{64}O_{19}Na$ $(M+Na)^+$: 883.3940; Found: 883.3931. UV λ_{max}^{MeOH} nm (log ε) : 218 (4.0). IR (KBr) cm^{-1} : 3453, 2936, 1736, 1081. ^{1}H-NMR (500 MHz, pyridine-d_5) δ : 1.04 (3H, s, 18-H_3), 1.64 (3H, d, *J*=6.4 Hz, 6'-H_3), 2.81 (1H, dd, *J*=5.5, 8.5 Hz, 17-H), 3.96, 4.36 (1H, each, both d-like, 19-H_2), 4.12 (1H, br s, 4'-H), 4.28 (1H, dd, *J*=5.8, 11.3 Hz, 6'-H), 4.76 (1H, dd-like, 6'-H), 4.37 (1H, m, 3-H), 4.38, 4.47 (1H each, both m, 6"'-H_2), 4.70 (1H, m, 3'-H), 4.80 (1H, d, *J*=7.6 Hz, 1"-H), 5.02, 5.28 (1H each, both d, *J*=18.0 Hz, 21-H_2), 5.12 (1H, d, *J*=7.6 Hz, 1"'-H), 5.37 (dd, *J*=0.9, 9.8 Hz, 1'-H), 6.11 (1H, br s, 22-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δc : given in Table 1. Negative-ion FAB-MS (*m/z*): 859 $(M-H)^-$, 697 $(M-C_6H_{11}O_5)^-$, 535 $(M-C_{12}H_{21}O_{10})^-$, 405 $(M-C_{18}H_{31}O_{13})^-$. Positive-ion FAB-MS (*m/z*): 883 $(M+Na)^+$, 861 $(M+H)^+$.

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19. HPLC conditions : YMC-Pack ODS-AQ 314 (300 x 6.0 mm i. d.), solvent : CH_3CN-H_2O (3 : 7, v/v); Temp. : 40 °C; flow rate : 0.7 mL/min.

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