Cardenolide Glycosides from the Roots of *Apocynum cannabinum*

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Steroidal constituents from the roots of *Apocynum cannabinum* L. were investigated. (20*S*)-, (20*R*)-18,20-Epoxycymarin and (20*S*)-18,20-epoxyapocannoside were isolated along with cannogenin, strophanthidin and cannogenol glycosides, including D-cymaroside, D-oleandroside, D-digitoxoside and D-digitaloside, and their glucosyl, cellobiosyl or gentiobiosyl glycosides. Two pregnanes, neridienone A and 6,7-didehydrocortexone, were obtained, accompanied with cardenolides.

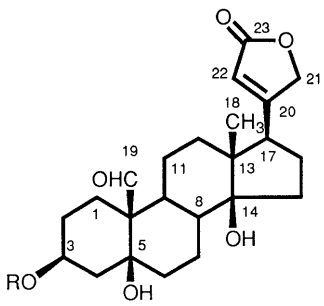
Keywords *Apocynum cannabinum*; 18,20-epoxystrophanthidin; cannogenin trioside; cardenolide; neridienone A; 6,7-didehydrocortexone

Apocynum cannabinum L. (Apocynaceae) is indigenous to the American continent, and the presence of major cardenolide monosides, cymarin, apocannoside and cynocannoside has already been described.[1] Previously, we studied pregnanes[2] and cardenolide glycosides[3] from the roots of *Apocynum venetum* L. var. *basikurumon* HARA (Japanese name, basikurumon). In order to compare the steroidal constituents of the two species, minor steroidal constituents of *A. cannabinum* were investigated.

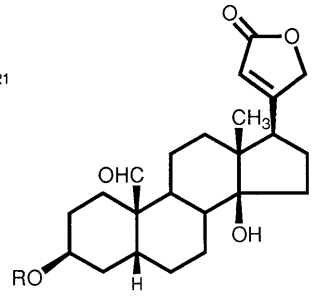
Cardenolide glycosides were fractionated by a combination of reversed-phase and normal-phase column chromatographies and preparative HPLC to isolate 14 glycosides (**4**—**17**) including 8 new glycosides (**10**—**17**), along with strophanthidin (**1**), cannogenin (**2**) and cannogenol (**3**). The identification of the glycosides was carried out by analysis of the ^{1}H- and ^{13}C-NMR spectra and by NMR, HPLC and TLC comparisons with authentic glycosides. The glycosides were classified into five groups according to their aglycones, strophanthidin, cannogenin, cannogenol, (20*R*/*S*)-18,20-epoxy-strophanthidin and (20*S*)-18,20-epoxycannogenin.

Strophanthidin glycosides (**4**—**6**) were identified as β-D-cymaroside (**4**, cymarin),[1] β-D-digitoxoside (**5**, helveticoside)[4] and β-D-glucosyl-β-D-cymaroside (**6**, k-strophanthin-β).[3] Three of cannogenin glycosides (**7**—**9**) were assigned as β-D-cymaroside (**7**, apocannoside),[1] β-D-oleandroside (**8**, cynocannoside)[1] and β-D-glucosyl-β-D-cymaroside (**9**, apobioside).[5] Compound **10** was characterized as cannogenin-β-D-glucosyl-β-D-digitaloside by comparison of the NMR signals with those of the compound with the same sugar moiety from *Nerium*.[6]

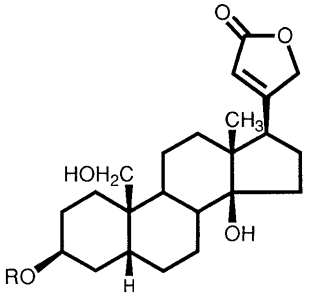
Cannogenin triosides (**11**—**13**) afforded the same molecular formula, $C_{42}H_{64}O_{18}$ in FAB-MS. Compounds **11** and **12**, showing the same polarity on TLC and column



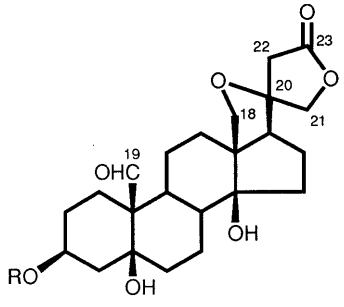
1 : R=H
4 : R=e
5 : R=a
6 : R=f



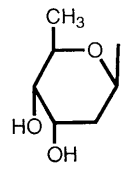
2 : R=H **10** : R=d
7 : R=e **11** : R=g
8 : R=b **12** : R=c
9 : R=f **13** : R=h



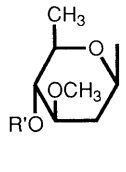
3 : R=H
14 : R=f



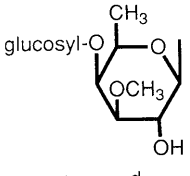
15 : R=e (20*S*)
16 : R=e (20*R*)



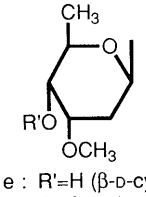
a : β-D-digitoxose



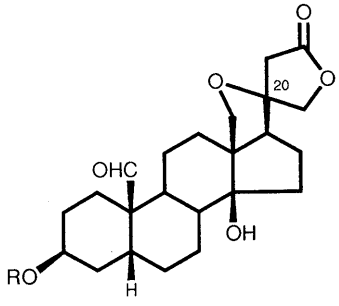
b : R'=H (β-D-oleandrose)
c : R'=β-cellobiosyl



d



e : R'=H (β-D-cymarose)
f : R'=β-D-glucosyl
g : R'=β-cellobiosyl
h : R'=β-gentiobiosyl



17 : R=e (20*S*)

Chart 1

TABLE I. ^{13}C-NMR Data for **10**—**16** and **17**, δ ppm from TMS in C_5D_5N

C	10	11	12	13	14	15	16	17
1	28.5	28.5	28.5	28.5	27.3[a]	24.4[a]	24.9[a]	28.4
2	25.5	25.5	25.5	25.5	27.0[a]	25.2[a]	25.2[a]	24.8[a]
3	73.4	72.4	72.3	72.3	73.3	75.0	75.0	72.4
4	29.6	29.7	29.7	29.9	30.8	35.7	35.7	29.9
5	29.8	30.1	30.1	30.1	29.9	73.7	73.8	30.1
6	22.2	22.3	22.3	22.3	21.7[b]	36.8[b]	36.8[b]	22.5
7	21.9	21.9	22.0	22.0	21.8[b]	18.6	18.6	22.1
8	42.2	42.3	42.3	42.3	41.9	44.4	44.4	44.8
9	35.1	35.1	35.1	35.1	35.8	39.3	39.6	35.4
10	51.2	51.3	51.3	51.3	39.8	55.3	55.2	51.2
11	21.2	21.2	21.2	21.2	24.6	24.8[a]	25.7[a]	23.6
12	39.8	39.8	39.7	39.8	40.4	37.6[c]	37.9	37.6[b]
13	50.1	50.1	50.0	50.1	50.1	59.2	60.1	60.1
14	84.3	84.3	84.3	84.3	84.8	82.6	82.4	82.4
15	32.5	32.5	32.5	32.5	32.9	35.3	35.2	35.6
16	27.2	27.2	27.2	27.2	27.0[a]	25.7[a]	23.1	25.8[a]
17	51.3	51.3	51.3	51.3	51.5	56.3	58.0	56.5
18	16.0	16.0	16.0	16.0	16.2	71.6	71.7	71.7
19	206.5	206.6	206.6	206.7	65.4	208.0	208.0	206.6
20	175.8	175.7	175.7	175.8	175.9	88.9	87.5	89.0
21	73.7	73.6	73.6	73.7	73.6	76.8	74.6	76.9
22	117.7	117.9	117.7	117.7	117.6	37.8[c]	41.2	37.9[b]
23	174.4	174.4	174.3	174.4	174.4	176.3	175.7	176.4
1′	103.4	96.9	98.3	97.0	96.6	97.6	97.6	96.9
2′	71.2[a]	37.0	37.5	37.1	37.0	36.3[b]	36.3[b]	36.1
3′	85.5	78.0[a]	79.7	78.4[a]	78.3[c]	78.7	78.6	78.9
4′	76.6	83.4	83.6	83.6	83.3	73.8	73.8	74.1
5′	70.5[a]	69.3	71.5	69.4	69.3	71.1	71.0	70.9
6′	17.7	18.7	18.9	18.7	18.7	18.9	18.8	19.0
–OMe	58.9	58.7	57.0	58.8	58.5	58.0	58.0	58.0
1″	105.4	106.1[b]	104.2[a]	106.5[b]	106.4			
2″	76.0	74.7[c]	74.7[b]	75.2	75.3			
3″	78.5[b]	76.5[d]	76.9[c]	78.4[a]	78.3[c]			
4″	71.9	81.3	81.7	72.0[c]	71.8			
5″	78.3[b]	76.3[d]	76.2[c]	77.0	78.2[c]			
6″	63.1	62.4	62.4	70.8	63.0			
1‴		104.9[b]	104.9[a]	105.7[b]				
2‴		74.9[c]	75.2[b]	75.2				
3‴		78.4[a]	78.4[d]	78.3[a]				
4‴		71.5	71.9	71.7[c]				
5‴		78.2[a]	78.2[d]	78.2[a]				
6‴		63.1	62.4	62.8				

a—*d*) Signal assignments may be interchangeable in each column.

TABLE II. ^{1}H-NMR Data for **10**—**16** and **17**, δ ppm from TMS in C_5D_5N (J (Hz) in Parentheses)

H	10	11	12	13	14	15	16	17
3	4.31 (br s)		4.23 (br s)	4.19 (br s)	4.26 (br s)	4.32 (br s)	4.33 (br s)	4.24 (br s)
17	2.79 (dd, 9, 5)	2.79 (dd, 9, 5)	2.80 (dd, 8, 5)	2.79 (dd, 9, 5)	2.79 (dd, 9, 5)			
18	1.09 (s)	1.10 (s)	1.11 (s)	1.10 (s)	1.02 (s)	3.55 (d, 9)	3.55 (d, 9)	3.65 (d, 9)
						4.47 (d, 9)	4.48 (d, 9)	4.54 (d, 9)
19	9.49 (s)	9.68 (s)	9.72 (s)	9.67 (s)	3.75 (d, 10)	10.39 (s)	10.38 (s)	9.69 (s)
					4.14 (d, 10)			
21	5.02 (dd, 18, 2)	5.02 (dd, 18, 1)	5.03 (dd, 18, 1)	5.03 (dd, 18, 2)	5.02 (dd, 18, 1)	4.14 (d, 10)	4.59 (d, 10)	4.14 (d, 10)
	5.28 (dd, 18, 1)	5.29 (dd, 18, 1)	5.30 (dd, 18, 1)	5.29 (dd, 18, 1)	5.30 (dd, 18, 1)	4.42 (d, 10)	4.71 (d, 10)	4.43 (d, 10)
22	6.12 (br s)	6.13 (br s)	6.14 (br s)	6.13 (br s)	6.11 (br s)	3.00 (2H, s)	2.77 (d, 17)	3.00 (2H, s)
							2.84 (d, 17)	
1′	4.68 (d, 8)	5.16 (br d, 10)	4.74 (br d, 10)	5.17 (dd, 10, 2)	5.22 (dd, 9, 2)	5.12 (dd, 10, 2)	5.13 (dd, 10, 2)	5.15 (dd, 10, 2)
3′	3.53 (dd, 9, 3)	4.08 (q, 2)		4.45 (q, 3)	4.10 (br s)	3.70 (q, 3)	3.70 (q, 3)	3.76 (q, 3)
4′	4.31 (br s)	3.65 (dd, 9, 3)	3.70 (t, 9)	3.68 (dd, 9, 3)	3.69 (dd, 9, 3)	3.52 (dd, 9, 3)	3.53 (br d, 9)	3.56 (dd, 9, 3)
6′	1.59 (d, 6)	1.63 (d, 6)	1.73 (d, 6)	1.57 (d, 6)	1.65 (d, 6)	1.51 (d, 6)	1.51 (d, 6)	1.54 (d, 6)
–OMe	3.67 (s)	3.52 (s)	3.47 (s)	3.63 (s)	3.50 (s)	3.41 (s)	3.40 (s)	3.46 (s)
1″,1‴	5.12 (d, 8)	5.02 (d, 8)	5.07 (d, 8)	4.84 (d, 8)	4.93 (d, 8)			
		5.17 (d, 8)	5.16 (d, 8)	5.09 (d, 8)				

chromatography, were isolated by HPLC. Based on the ^{1}H- and ^{13}C-NMR spectra, the sugar moieties of **11** and **12** were composed of two glucose residues and one 2,6-dideoxy-3-*O*-methylhexose. Since the ^{13}C-NMR signals of the C-6 carbinols in the two glucose residues were observed at almost the same chemical shifts (δ 62.4, 63.1 in **11**, 62.4×2 in **12**), gentiobiose was ruled out. 2,6-Dideoxy-3-*O*-methylhexose in **11** was identified as D-cymarose by comparison of the NMR signals with those of **6**. All ^{13}C-NMR signals due to the sugar moiety of **11** were in good agreement with those of strophanthidin-β-cellobiosyl-β-D-cymaroside, previously obtained from basikurumon.[3] In **12**, the presence of D-oleandrose was confirmed besides a cellobiosyl unit by comparison of the ^{1}H- and ^{13}C-NMR signals with those of **8**. Thus, the structure of **11** was characterized as cannogenin-β-cellobiosyl-β-D-cymaroside and that of **12** as cannogenin-β-cellobiosyl-β-D-oleandroside. Compound **13** had the same component sugars as those of **11**. In contrast to **11** and **12**, however, the signals of C-6 in the two glucose residues were observed at δ 70.8 and 62.8 and the presence of a gentiobiosyl residue was indicated. Accordingly, the structure was assigned as cannogenin-β-gentiobiosyl-β-D-cymaroside.

Compound **14** showed signals due to one angular methyl and an A,B-quartet (δ 3.75 and 4.14, 1H each, J=10 Hz) due to a primary carbinol, but no formyl proton, suggesting the aglycone to be cannogenol. In a comparison of the ^{13}C-NMR signals, the sugar moiety of **14** showed the same signal pattern as those of **6**. The molecular formula from FAB-MS was consistent with the NMR considerations. Therefore, **14** was characterized as cannogenol-β-D-glucosyl-β-D-cymaroside.

Compounds **15** and **16** showed the same molecular formula, $C_{30}H_{44}O_{10}$, and the component sugar was identified as D-cymarose based on the NMR spectra. Therefore, the aglycone was considered to have the molecular formula, $C_{23}H_{32}O_6$. While the NMR spectra showed similar patterns to each other, neither angular methyl protons nor olefinic H-22 appeared. Instead, one formyl proton signal assignable to H-19 was observed. The characteristic signals of three sets of methylene groups at δ 3.55, 4.47 (H-18), 4.14, 4.42 (H-21), 3.00×2 (H-22) in **15**, 3.55, 4.48 (H-18), 4.59, 4.71 (H-21) and 2.77, 2.84 (H-22) in **16**, coincided with those of (20*S*)- and (20*R*)-18,20-epoxydigitoxigenin glycosides, respectively, from *Thevetia*.[7] The ^{13}C signals due to rings A and B were in good agreement with those of **4**, and those from rings C and D of **15** and **16** were identical with those of (20*S*)- and (20*R*)-18,20-epoxydigitoxigenin glycosides, respectively. Thus, **15** and **16** were determined to be (20*S*)- and (20*R*)-isomers of 18,20-epoxycymarin, respectively.

Compound **17** also showed a duplicated pattern of ^{1}H- and ^{13}C-NMR signals concerning the 18,20-epoxide structure of **15**. However, signals for rings A and B as well as the sugar moiety were identical with those of **7**. The structure of **17** was therefore determined to be (20*S*)-18,20-epoxycannogenin-β-D-cymaroside.

Two pregnanes, neridienone A[2,8] and 6,7-didehydrocortexone,[2,9] were isolated from the less polar fraction of the methanol percolate.

Cannogenin glycosides were the major glycosides in the roots of *A. cannabinum*, although they were not obtained from the roots of basikurumon. It is noteworthy that (20*S*)-epoxides were predominant in this plant material unlike *Thevetia neriifolia*,[7] in which (20*R*)-18,20-epoxydigitoxigenin-α-L-thevetoside was predominant over the (20*S*)-isomer. Biogenesis of the 18,20-epoxycardanolides remains to be studied. Two free pregnanes, neridienone A and 6,7-didehydrocortexone were obtained as in the case of basikurumon.[2] However, teikagenin glycosides,[10] pregnane glycosides obtained together with neridienone A from basikurumon, were not detected in this study.

Experimental

The melting points were measured on a hot stage apparatus and recorded uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 spectrometer in pyridine-d_5. Chemical shifts are given in δ values referred to internal tetramethylsilane, and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br s=broad singlet, dd=doublet of doublets. FAB-MS was recorded on a JEOL HX-110 spectrometer. Optical rotations were measured on a JASCO DIP 360 polarimeter. For silica gel column chromatography and TLC, the following solvent systems were applied: solvent 1, $CHCl_3$–MeOH–H_2O (7:2:2—7:3:1.2, bottom layer); solvent 2, EtOAc–MeOH–H_2O (7:1:1—4:1:1, top layer); solvent 3, benzene–acetone; solvent 4, hexane–EtOAc. Spray reagents: 1, Kedde's reagent (1:1 mixture of 2% 3,5-dinitrobenzoic acid in MeOH and 2 N NaOH); 2, dilute H_2SO_4.

Extraction and Isolation of Cardenolide Glycosides and Pregnanes The roots of *Apocynum cannabinum* L. were purchased from Wilcox Natural Products, Boone, North Carolina, U.S.A. in February, 1992. The roots were powdered and percolated with MeOH. The percolate was concentrated *in vacuo* to 1 l and H_2O (1 l) was added. The precipitates were filtered off and the filtrate was extracted with benzene and $CHCl_3$. The MeOH in the H_2O layer was removed by evaporation *in vacuo*. The H_2O layer was then passed through an MCI gel (Mitsubishi CHP-20) column and eluted with H_2O–MeOH, containing increasing MeOH concentrations. The 100% MeOH eluate was concentrated *in vacuo*. The residue (20.6 g) was chromatographed repeatedly on a silica gel column with solvents 1—4, and further purified by reversed-phase column chromatography on an octadecyl silica (ODS) column with MeCN–H_2O (20—30%) or by preparative HPLC on an ODS column. The benzene (5.2 g) and $CHCl_3$ (10.0 g) extracts were subjected to column chromatography principally in the same manner as in the case of the H_2O layer, and finally the following cardenolide glycosides and pregnanes were isolated: strophanthidin (**1**, 15 mg), cannogenin (**2**, 46 mg), cannogenol (**3**, 2.6 mg), cymarin (**4**, 121 mg), helveticoside (**5**, 14 mg), k-strophanthin-β (**6**, 315 mg), apocannoside (**7**, 460 mg), cynocannoside (**8**, 12 mg), apobioside (**9**, 200 mg), **10** (31 mg), **11** (17 mg), **12** (7 mg), **13** (37 mg), **14** (9 mg), **15** (32 mg), **16** (10 mg), **17** (28 mg), neridienone A (12 mg), 6,7-didehydrocortexone (114 mg).

Cannogenin-β-D-glucosyl-β-D-digitaloside (10) Solid, $[\alpha]_D^{27}$ −25.6° (c=0.54, MeOH). FAB-MS m/z: 733.3409 (Calcd for $C_{36}H_{54}O_{14}$+Na: 733.3411).

Cannogenin-β-cellobiosyl-β-D-cymaroside (11) Solid, $[\alpha]_D^{22}$ −5.6° (c=0.85, MeOH). FAB-MS m/z: 879.3995 (Calcd for $C_{42}H_{64}O_{18}$+Na: 879.3990).

Cannogenin-β-cellobiosyl-β-D-oleandroside (12) Solid, $[\alpha]_D^{23}$ −10.8° (c=0.36, MeOH). FAB-MS m/z: 879.4000 (Calcd for $C_{42}H_{64}O_{18}$+Na: 879.3990).

Cannogenin-β-gentiobiosyl-β-D-cymaroside (13) Solid, $[\alpha]_D^{25}$ −18.8° (c=0.95, MeOH). FAB-MS m/z: 879.3988 (Calcd for $C_{42}H_{64}O_{18}$+Na: 879.3990).

Cannogenol-β-D-glucosyl-β-D-cymaroside (14) Solid, $[\alpha]_D^{28}$ +21.9° (c=0.47, MeOH). FAB-MS m/z: 719.3616 (Calcd for $C_{36}H_{56}O_{13}$+Na: 719.3619).

(20*S*)-18,20-Epoxystrophanthidin-β-D-cymaroside (15) Prisms from MeOH–H_2O, mp 150—155 °C, $[\alpha]_D^{21}$ +54.8° (c=0.80, MeOH). FAB-MS m/z: 587.2835 (Calcd for $C_{30}H_{44}O_{10}$+Na: 587.2832).

(20*R*)-18,20-Epoxystrophanthidin-β-D-cymaroside (16) Prisms from EtOH–H_2O, mp 127—135 °C, $[\alpha]_D^{24}$ +9.9° (c=0.55, MeOH). FAB-MS m/z: 587.2835 (Calcd for $C_{30}H_{44}O_{10}$+Na: 587.2832).

(20*S*)-18,20-Epoxycannogenin-β-D-cymaroside (17) Prisms from MeOH, mp 218—220 °C, $[\alpha]_D^{24}$ +2.2° (c=1.39, MeOH). FAB-MS m/z: 571.2887 (Calcd for $C_{30}H_{44}O_9$+Na: 571.2883).

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