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CARDENOLIDE GLYCOSIDES OF *STROPHANTHUS DIVARICATUS*

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Key Word Index—*Strophanthus divaricatus*; Apocynaceae; cardenolides; cardiac glycosides.

Abstract—The isolation and structures of 12 cardenolide glycosides from the leaves, stems and roots of *Strophanthus divaricatus* is described.

INTRODUCTION

Strophanthus divaricatus Hook and Arn. is indigenous to the southern coastal area of China, but it is cultivated in the southern islands of Japan. The cardiac glycosides of the seeds were first investigated by Chu[1]. From 1954–1959, eight monosides including divaricoside, divostroside, caudoside, caudostroside, sinoside, sinostroside, ψ-caudoside, and ψ-caudostroside were isolated from the seeds, and their structures were determined [2, 3]. The isolation of glucosyl divaricoside and gentiobiosyl divaricoside, along with other known monosides from the seeds, were also reported by Chinese investigators[4]. This paper deals with the cardiac glycosides from the leaves, stems and roots of this plant.

RESULTS AND DISCUSSION

When the methanol percolates of the plant organs were successively extracted with hexane, benezene, chloroform and then *n*-butanol, three less polar glycosides were obtained from the benzene layer and another nine glycosides were obtained from the chloroform and *n*-butanol layers, after polystylene and silica gel column chromatographies. The glycosides were identified by ^{1}H NMR, ^{13}C NMR and mass spectrometry and in some cases, an aglycone and a component sugar were identified by hydrolysis. The glycosides thus obtained are shown in the formulae and in Tables 1 and 2. Twelve glycosides in total were isolated, 10 from the leaves, seven from the roots and two from the stems. The glycosides from the roots and the stems are similar to those of the leaves, except for the presence of sarmutogenin glucosyloleandroside (**5**) and glucosyl-diginoside (**8**) in the roots. Sarmentogenin and dambonitol were also isolated from the stems.

The major glycosides are principally the same in the three organs examined as those of the seeds, sarmentogenin α-L-oleandroside (divaricoside) (**2**), α-L-diginoside (divostroside) (**3**), and glucosides of **2** and **3** (**7** and **9**, respectively). Among them, **9** has not been obtained from the seeds. Not only **2** and **3** but also sarmutogenin 2, 6-dideoxy-3-*O*-methyl-hexosides were found to be present as their glucosides (**5** and **8**) except for the case of decoside (**1**). Whereas, glycosides with 6-deoxy-3-*O*-methyl-hexose (sarnovide **4** and musaroside **6**) and 6-deoxy-hexoses (Lokundjoside **10**, sarmentoloside **11**, and sarhamnoloside **12**) were present only as monosides. These glycosides comprise five aglycones including decogenin, sarmentogenin, sarmutogenin, bipindogenin, and sarmentologenin, in which, sarmentogenin is a major cardenolide, and five deoxysugars; L-oleandrose, L-diginose, D-digitalose, L-rhamnose, and 6-deoxy-L-talose, while, four cardenolides and two deoxy-sugars have been found from the seeds; sarmentogenin, sarmutogenin, sinogenin (11α-hydroxy-12-oxodigitoxigenin), caudogenin(12α-hydroxy-11-oxodigitoxigenin), L-oleandrose, and L-diginose [2,3]. Although **1** was synthesized from caudoside [5], no isolation from a natural source has been reported. Among four biosides, **5**, **8** and **9** were newly isolated in this study.

In order to study the chemotaxonomy of *Strophanthus*, it is worth noting that cardenolide glycosides occur not only in the seeds but also in other organs of the plants.

EXPERIMENTAL

General. Mps are uncorr. NMR spectra were recorded at 400 and 100 MHz in C_5D_5N using TMS as int. std. For TLC, PC and silica gel CC, the following solvent systems were used. 1: $CHCl_3$–MeOH–H_2O (7:3:1, 7:3:2 etc., bottom layer); 2: EtOAc–MeOH–H_2O (4:1:0.5, 9:1:0.1); 3:hexane–EtOAc–EtOH(6:10:1, 4:4:5); 4: hexane–EtOAc (1:3); 5: C_6H_6–Me_2CO (2:1); 6: *n*-BuOH–HOAc–H_2O (4:1:5, top layer); 7: *n*-BuOH–EtOH–H_2O (5:1:4, top layer). For the detection of chromatographic spots, a 1:1 mixture of 2% 3,5-dinitrobenzoic acid in MeOH and 2N NaOH (Kedde's reagent) or 10% H_2SO_4 (for cardenolides) and aniline hydrogen phthalate in H_2O–*n*-BuOH (for sugars) were sprayed.

Extraction and isolation. Plants were cultivated at the Tanegashima Experimental Station and harvested in November 1982. The leaves, stems and roots were dried in the shade (dry wt 3.2, 3.4 and 1.2 kg, respectively). Each plant material was powdered and percolated with MeOH. The MeOH solns were concd *in vacuo*, dil with H_2O and filtered. The filtrates were extd with hexane, C_6H_6, $CHCl_3$ and then *n*-BuOH. The $CHCl_3$ and *n*-BuOH exts were then passed through MCI-gel (Mitsubishi: CHP-20P) and eluted with H_2O–MeOH. Fractions containing cardenolide glycosides were then chromatographed on silica gel columns with solvents 1 and 2, repeatedly.

Cardenolide glycosides from leaves. From the C_6H_6 fraction

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Table 1. ^{1}H Chemical shifts of cardenolide glycosides, δ(ppm) from TMS in C_5D_5N

Compound	3α-H	18, 19-H_3	21-H_2	22-H	1'-H	6'-H_3	3'-OMe	Others
1	4.12 *s* (*br*)	1.19 *s*	5.08 *dd* (19, 1)	6.30 *s* (*br*)	5.23 *s* (*br*)	1.61 *d* (6)	3.47 *s*	4.36 *dd* (9, 5) 17α-*H*
		1.37 *s*	5.26 *dd* (19, 1)					3.60 *t* (9) 4'-H
2*	3.90 *s* (*br*)	0.89 *s*	4.75 *dd* (18, 2)	5.85 *t* (2)	4.95 *s* (*br*)	1.24 *d* (6)	3.40 *s*	
		1.05 *s*	5.00 *dd* (18, 2)					
3*		0.88 *s*	4.74 *dd* (18, 1)	5.84 *t* (1)	4.97 *s* (*br*)	1.24 *d* (6)	3.39 *s*	
		1.05 *s*	5.00 *dd* (18, 1)					
4	4.45 *s* (*br*)	1.09 *s*	5.03 *d* (18)	6.11 *s* (*br*)	4.77 *d* (7)	1.55 *d* (6)	3.58 *s*	4.10 *m* 11β-H
		1.10 *s*	5.29 *d* (18)					4.38 *dd* (9, 7) 2'-H
								3.50 *dd* (9, 3) 3'-H
								4.07 *d* (3) 4'-H
								3.76 *q* (6) 5'-H
7		1.10 *s*	5.01 *d* (18)	6.10 *s* (*br*)	5.17 *s* (*br*)	1.57 *d* (6)	3.40 *s*	5.21 *d* (9) 1"-H
		1.13 *s*	5.30 *d* (18)					
9		1.10 *s*	5.20 *s* (*br*)	6.09 *s* (*br*)	5.10 *s* (*br*)	1.65 *d* (7)	3.55 *s*	4.97 *d* (7) 1"-H
		1.21 *s*						
5		1.07 *s*	5.02 *d* (18)	6.23 *s* (*br*)	5.12 *s* (*br*)	1.58 *d* (6)	3.42 *s*	4.45 *s* 12α-H
		1.32 *s*	5.31 *d* (18)					5.22 *d* (9) 1"-H
6	4.32 *s* (*br*)	1.07 *s*	5.07 *d* (18)	6.26 *s* (*br*)	4.72 *d* (8)	1.55 *d* (6)	3.57 *s*	3.81 *dd* (9, 5) 17α-H
		1.28 *s*	5.23 *d* (18)					4.42 *s* 12α-H
								4.37 *dd* (9, 8) 2'-H
								3.49 *dd* (9, 2) 3'-H
								4.06 *d* (2) 4'-H
								3.74 *q* (6) 5'-H
8	4.28 *s* (*br*)	1.10 *s*	5.09 *d* (17)	6.27 *s* (*br*)	5.20 *d* (3)	1.67 *d* (6)	3.57 *s*	3.82 *dd* (9, 5) 17α-H
		1.41 *s*	5.25 *d* (17)					4.46 *s* 12α-H
								4.99 *d* (8) 1"-H
10	4.53 *s* (*br*)	1.13 *s*	5.04 *d* (18)	6.13 *s* (*br*)	5.50 *s* (*br*)	1.62 *d* (6)		4.19 *m* 11β-H
		1.31 *s*	5.28 *d* (18)					3.01 *dd* (9, 5) 17α-H
11	4.37 *s* (*br*)	1.15 *s*	5.05 *d* (18)	6.12 *s* (*br*)	5.52 *s* (*br*)	1.50 *d* (6)		3.01 *dd* (9, 5) 17α-H
			5.29 d (18)					4.45 *d* (11) 19-Ha
								4.62 *d* (11) 19-Hb
12	4.53 *s* (*br*)	1.15 *s*	5.04 *d* (18)	6.12 *s* (*br*)	5.51 *s* (*br*)	1.62 *d* (6)		3.01 *dd* (9, 5) 17α-H
			5.28 *d* (18)					4.41 *d* (11) 19-Ha
								4.57 *d* (11) 19-Hb

*$CDCl_3$ as solvents.
Coupling constants (J in Hz) are given in parentheses.

(12.1g), **1** (75 mg), **2** (270 mg), and **3** (330 mg) were isolated. From $CHCl_3$ (10.5 g) and *n*-BuOH (63.1 g) extracts, **4** (430 mg), **6** (195 mg), **7** (1.56 g), **9** (600 mg), **10** (140 mg), **11** (300 mg), and **12** (27 mg) were isolated.

Compound **1**. Prisms from hexane–EtOAc, mp 180–185°, $[\alpha]_D^{22}$ −31.2° (MeOH; *c* 0.65), λ_{max}^{MeOH} nm (log ε): 214(4.079), 286(3.763). FABMS *m/z*: 569 $[M+Na]^+$, 403, 385, 145.

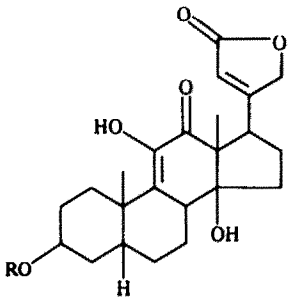
Compound **2**. Aggregates from hexane–EtOAc, after CC with solvent 3(6:10:1), mp 192–195°, $[\alpha]_D^{22}$ −30.9° (MeOH; *c* 0.67), FDMS *m/z*: 535 $[M+H]^+$, 490, 389, 145. On reflux of **2** (50 mg) with 0.05 N H_2SO_4–50% EtOH for 1 hr, the aglycone was obtained as long needles by crystallization from hexane–EtOAc, mp 263–268°, which was in good agreement with authentic sarmentogenin (mmp, TLC). The component sugar was identified as L-oleandrose in comparison with an authentic sample (PC, solvent 7).

Compound **3**. Crystalline powder, mp 195–199°, $[\alpha]_D^{22}$ −54.5° (MeOH; *c* 0.56), FDMS *m/z*: 535 $[M+H]^+$, 490, 389, 145. On hydrolysis with 0.05 N H_2SO_4–50% EtOH, it gave sarmentogenin (TLC) and L-diginose (PC, solvent 7).

Compound **4**. Solid, $[\alpha]_D^{22}$ +7.8° (MeOH; *c* 0.80), FABMS *m/z*: 551 $[M+H]^+$, 391, 373, 355, 337. On hydrolysis with 1% HCl in Me_2CO at room temp for 2 days (Mannich's condition), sarmentogenin and D-digitalose were identified by comparison with authentic samples (TLC; PC, solvent 7).

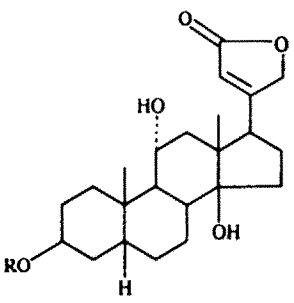
Compound **6**. Aggregates from hexane–EtOAc, mp 230–232°, $[\alpha]_D^{22}$ +31.2° (MeOH; *c* 0.67), FABMS *m/z*: 565 $[M+H]^+$, 405, 387, 369. On hydrolysis with 1% HCl in Me_2CO, sarmutogenin (mp 225–230°) and digitalose (PC, solvent 7) were identified. The structure of sarmutogenin was confirmed by NMR and FABMS. ^{1}H NMR: δ 1.09, 1.44 (3H each *s*, H-18, 19), 3.01 (1H, *d*, J = 13 Hz, H-9), 3.83 (1H, *dd*, J = 9, 5 Hz, H-17), 4.32 (1H, *br s*, H-3α), 4.45 (1H, *s*, H-12α), 5.08, 5.24 (1H each, *dd*, J = 18, 1 Hz, H-21), 6.26 (1H, *br s*, H-22). FABMS *m/z*: 405 $[M+H]^+$, 387, 180, 135.

Compound **7**. Prisms from $CHCl_3$–MeOH–H_2O, mp 165–175°, $[\alpha]_D^{21}$ −58.3° (MeOH; *c* 0.77), FABMS *m/z*: 719 $[M+Na]^+$. (Found: C, 59.3; H, 7.9. $C_{36}H_{56}O_{13}\cdot 2H_2O$ requires: C, 59.0; H, 8.3%). On hydrolysis of **7** with snail digestive juice at 38° for



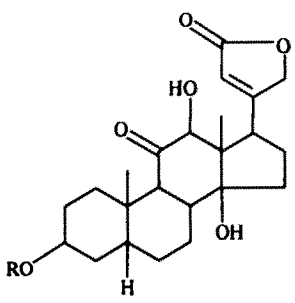
Decogenin (R = H)

1 R = L-Oleandrosyl



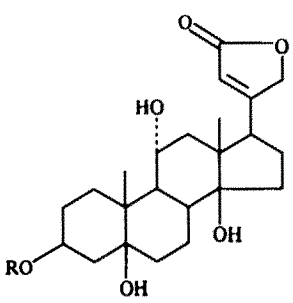
Sarmentogenin (R = H)

2 R = L-Oleandrosyl
3 R = L-Diginosyl
4 R = D-Digitalosyl
7 R = D-Glucosyl-L-oleandrosyl
9 R = D-Glucosyl-L-diginosyl



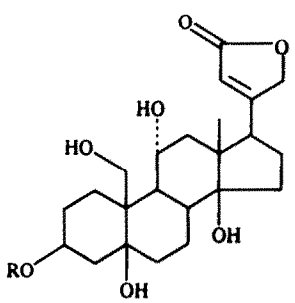
Sarmutogenin (R = H)

5 R = D-Glucosyl-L-oleandrosyl
6 R = D-Digitalosyl
8 R = D-Glucosyl-L-diginosyl



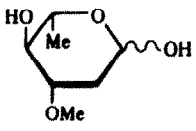
Bipindogenin (R = H)

10 R = L-Rhamnosyl

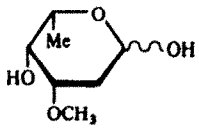


Sarmentologenin (R = H)

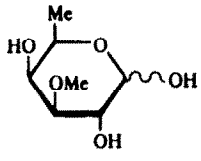
11 R = 6-Deoxy-L-talosyl
12 R = L-Rhamnosyl



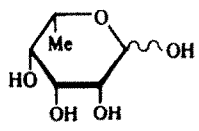
L-Oleandrose



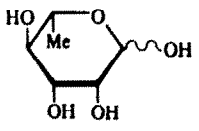
L-Diginose



D-Digitalose



6-Deoxy-L-talose



L-Rhamnose

Table 2. ^{13}C Chemical shifts of cardenolide glycosides, δ(ppm) from TMS in C_5D_5N

	1	Sar.*	2	3	4	7	9	5	6	8	10	11	12
C-1	32.8	33.1[a]	33.9[a]	33.9[b]	33.6[a]	33.5[a]	33.9[b]	30.0[b]	30.3[a]	30.8[b]	27.7[b]	27.3	27.4
C-2	29.9	29.9	28.1	28.2	28.3	27.8	28.1	27.6[c]	27.6[b]	27.2[c]	28.1[b]	27.3	27.4
C-3	71.9	66.5	72.8	72.5	74.9	72.8	72.7	71.7[a]	73.8	71.8[a]	75.1	75.1	74.7
C-4	30.6	34.9	31.1	31.1	31.3	30.9	31.2	30.7[b]	30.6[a]	30.5[b]	35.8[a]	36.1	36.1
C-5	38.1	38.4	38.9	38.9	38.4	38.8	38.9	37.8	37.4	37.9	74.3	76.0	76.1
C-6	25.9	27.3[b]	27.3[b]	27.3[c]	27.3[b]	27.3[b]	27.3[c]	27.1[c]	27.4[b]	27.6[c]	35.3[a]	36.1	36.1
C-7	22.0	22.3	22.2	22.3	22.2	22.2	22.2	22.9	23.1	23.1	24.5	24.5	24.4
C-8	40.4	42.2	42.3	42.3	43.6	42.3	42.3	43.5	43.6	43.6	40.7	40.5	40.5
C-9	135.0	41.3	41.3	41.4	41.3	41.3	41.3	46.5	46.4	46.4	45.3	45.5	45.5
C-10	41.3	37.3	37.0	37.1	36.9	36.9	37.0	35.1	35.1	35.2	42.4	45.1	45.1
C-11	142.1	67.8	67.7	67.7[a]	67.7	67.7	67.7[a]	210.4	210.4	210.5	67.9	68.5	68.5
C-12	199.8	50.5	50.5	50.6	50.5	50.5	50.6	79.9	79.8	79.8	50.6	50.7	50.6
C-13	60.7	50.3	50.3	50.3	50.2	50.3	50.3	60.9	60.9	60.9	50.2	50.4	50.4
C-14	82.6	84.3	84.3	84.3	84.3	84.2	84.2	83.5	83.6	83.6	84.2	84.7	84.7
C-15	33.8	33.6[a]	33.6[a]	33.7[b]	33.5[a]	33.9[a]	33.6[b]	33.6	33.6	33.6	33.6	33.3	33.2
C-16	27.0	27.7[b]	27.7[b]	27.8[c]	27.6[b]	27.7[b]	27.7[c]	26.6[c]	26.6[b]	26.6[c]	27.3[b]	27.3	27.4
C-17	43.6	51.2	51.3	51.3	51.3	51.3	51.3	44.9	45.1	45.0	51.1	51.2	51.2
C-18	15.8	17.6	17.6	17.7	17.6	17.6	17.7	10.9	10.9	11.0	17.7[c]	17.9	17.9
C-19	18.6	24.5	24.5	24.5	24.2	24.5	24.5	24.1	23.9	24.2	17.8[c]	64.9	64.9
C-20	174.0	175.5	175.4	175.4	175.3	175.4	175.4	175.1	175.0	175.0	175.3	175.4	175.4
C-21	73.7	73.7	73.7	73.8	73.7	73.7	73.8	73.8	73.7	73.7	73.8	73.9	73.8
C-22	118.8	117.5	117.7	117.7	117.7	117.7	117.7	118.0	118.0	118.1	117.7	117.7	117.7
C-23	174.0	174.6	174.5	174.5	174.4	174.5	174.5	174.4	174.3	174.4	174.4	174.5	174.5
C-1′	96.1		96.2	96.4	103.5	95.8	96.5	95.7	103.4	96.5	100.7	101.4	100.7
C-2′	36.2		36.3	31.4	70.9	35.9	32.6	35.7	70.7	32.4	70.5	67.4[a]	70.6
C-3′	79.3		79.4	76.0	85.1	79.2	74.6	79.1	85.0	74.6	73.9	73.8	73.7
C-4′	77.2		77.2	67.1	68.8	82.6	75.3	82.5	68.6	75.2	72.6	68.4[a]	72.6
C-5′	69.1		69.1	67.8[a]	71.1	67.7	67.6[a]	67.6	71.1	67.6	72.9	72.4	72.9
C-6′	18.6		18.6	17.7	17.4	18.6	17.8	18.5	17.4	17.8	18.5	17.3	18.5
3′-OMe	57.1		57.1	55.0	57.2	56.9	55.7	56.8	57.1	55.7			
C-1″						105.2	105.4	105.1		105.3			
C-2″						75.9	75.5	75.9		75.5			
C-3″						78.3	78.5	78.2		78.5			
C-4″						71.9	71.8	71.9[a]		71.7[a]			
C-5″						78.3	78.3	78.2		78.2			
C-6″						63.0	62.9	62.9		63.0			

*Sar. = sarmentogenin.

[a–c]Signal assignments in each column may be reversed.

16 hr, **2** was observed on TLC (solvent 1). On hydrolysis of **7** with 0.05 N H_2SO_4–50% EtOH as described above, sarmentogenin was identified.

Compound **9**. Solid, $[\alpha]_D^{22}$ −32.8° (MeOH; *c* 0.67), FABMS *m/z*: 719 $[M+Na]^+$, 441, 391, 373, 355, 145. On enzymic hydrolysis with snail digestive juice, **3** was identified (TLC, solvent 1). On acid hydrolysis with 0.05 N H_2SO_4–50% EtOH, sarmentogenin was identified (TLC, solvent 4 and 5).

Compound **10**. Aggregates from hexane–EtOAc, mp 217–219°, $[\alpha]_D^{22}$ −13.3° (MeOH; *c* 0.64), FDMS *m/z*: 552 $[M]^+$, 407, 389, 372, 236, 147. On hydrolysis with Mannich's procedure, bipindogenin (mp 258–262° from hexane–EtOAc, identified by comparison of ^{13}C NMR[6]) was obtained and L-rhamnose was identified (PC, solvent 6).

Compound **11**. Solid, $[\alpha]_D^{21}$ −23.9° (MeOH; *c* 2.44), FABMS *m/z*: 569 $[M+H]^+$, 375, 357, 339, 145. On hydrolysis with Mannich's procedure, the sugar component showed a different R_f value (PC, solvent 6) in comparison with D-fucose, L-rhamnose, D-quinovose and 6-deoxy-D-allose. The aglycone (mp 250–255° from MeOH–AcOEt) was identified as sarmentologenin by NMR and FABMS. ^1H NMR: δ 1.15 (3H, *s*, H-18), 3.01 (1H, *dd*, *J* = 9, 5 Hz, H-17α), 4.41, 4.74 (1H each, *d*, *J* = 11 Hz, H-19), 4.41 (1H, *m*, H-11β), 4.49 (1H, *br s*, H-3α), 5.04, 5.28 (1H each, *dd*, *J* = 18, 1 Hz, H-21), 6.11 (1H, *br s*, H-22). FABMS *m/z*: 445 $[M+Na]^+$, 265, 137, 115.

Compound **12**. Solid, $[\alpha]_D^{21}$ −13.0° (MeOH; *c* 0.53), FABMS *m/z*: 591 $[M+Na]^+$. On hydrolysis with Mannich's procedure, the same aglycone was identified as that of **11**, and the sugar was identified as L-rhamnose (PC, solvent 6).

Cardenolide glycosides from roots. Roots were treated in the same manner as the leaves. From the C_6H_6 and $CHCl_3$ exts, **2** (180 mg) and **3** (170 mg) were obtained and identified by comparison with those from the leaves. From the BuOH ext, **5** (130 mg), **7** (1.08 g), **8** (40 mg), **9** (230 mg) and **11** (66 mg) were isolated and identified by comparison with those from the leaves, except for **5** and **8**.

Compound **5**. Prisms from EtOAc–MeOH, mp 123–128°, $[\alpha]_D^{19}$ −27.5° (MeOH; *c* 1.70), FDMS *m/z*: 710 $[M]^+$, 636, 404, 145. On hydrolysis with 0.05 N H_2SO_4–50% EtOH, the aglycone was identified as sarmutogenin (TLC, solvent 1 and 5) and the sugar

was identified as glucosyl-L-oleandrose (TLC, solvent 2).

Compound **8**. Solid, $[\alpha]_D^{19}$ −43.1° (MeOH; *c* 2.31), FABMS *m/z*: 733 (M + Na]$^+$. On hydrolysis with 0.05 N H_2SO_4–50% EtOH, sarmutogenin and glucosyl-L-diginose were identified on TLC.

Cardenolide glycosides from stems. The stems were percolated with MeOH in the same manner as the leaves, and **7** (200 mg) and **9** (120 mg) were obtained besides sarmentogenin, mp 267–269°, $[\alpha]_D^{22}$ + 17.7° (MeOH; *c* 0.33). From the *n*-BuOH layer, dambonitol (45 mg) was obtained, mp 185–187° (Found: C, 46.8; H, 7.8. $C_8H_{16}O_6$ requires: C, 46.2; H, 7.7%), and identified by comparison with an authentic sample.

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