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PLANT ANTICANCER AGENTS, XLI. CARDIAC GLYCOSIDES FROM *STREBLUS ASPER*[1]

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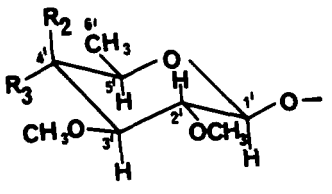
Streblus asper Lour. (Moraceae) is a small tree occurring in dry regions of India, Sri Lanka, Malaya, Vietnam and Thailand (2,3). Various parts of this tree have been used in Ayurvedic medicine for cardiac disorders, epilepsy, and edema (2). Reichstein and co-workers have isolated more than twenty cardiac glycosides from the root bark of *S. asper* and were able to structurally characterize about fifteen such compounds, mainly as a result of the application of degradative techniques (3-6). An investigation on the stem bark of this species resulted in the isolation of α-amyrin and lupeol and their acetates, as well as β-sitosterol (7).



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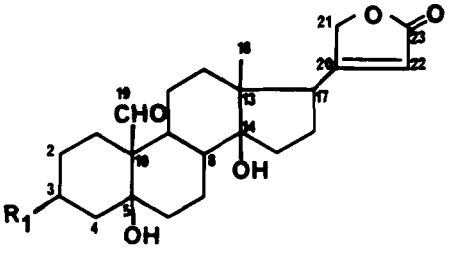

	R_1	R_2	R_3
1	G	OH	H
2	G	H	OH
3	OH	—	—
4	G	H	OAc

In a continuing search for tumor inhibitors from plants, it was found that KB cytotoxicity, determined according to published protocols (8,9), was concentrated sequentially into MeOH and CH_2Cl_2 extracts of *S. asper* stem bark. Fractionation of the CH_2Cl_2 extracts over silica gel by gravity column chromatography, and purification by preparative tlc, afforded two cytotoxic cardiac glycosides, stebloside (**1**) and mansonin (**2**), which are respectively the 2,3-di-*O*-methyl-β-D-fucopyranoside and the 2,3-di-*O*-methyl-6-deoxy-β-D-glucopyranoside of strophanthidin (**3**).

Strebloside (**1**), a compound that gave a positive test with Kedde reagent (10), was identified by direct comparison (mmp, ir, ^{1}H nmr, co-tlc) with an authenic sample isolated previously from *S. asper* stem bark (3,4). Because no ^{1}H-nmr data have thus far been reported for **1**, double irradiation studies and a two-dimensional ^{1}H-^{1}H homonuclear shift correlated (COSY) experiment (11) were conducted. In this manner, it was possible to assign the protons of the sugar portion of **1** (Table 1).

A second Kedde reagent-positive isolate, **2**, exhibited closely comparable uv, ir, and mass spectral parameters to **1**. On hydrolysis of **2** with 2 N trifluoroacetic acid, its aglycone, strophanthidin (**3**), was obtained. The identity of **2** as mansonin, obtained to date only from the seeds of *Mansonia altissima* A. Chev. (Sterculiaceae) (12-14), was confirmed

[1]For the previous paper in this series, see Fiebig, *et al.* (1).

TABLE 1. ^{1}H-nmr Chemical Shifts (δ) and Coupling Constants (Hz, in parenthesis) of Compounds **1**, **2**, and **4**[a]

Proton	1	2	4
3	4.23 bs	4.22 bs	4.21 bs
15α,β	~1.7 m	~1.7 m	~1.7 m
16α,β	~1.9 m	~1.9 m	~1.9 m
	~2.2 m	~2.2 m	~2.2 m
17	2.76 dd (9,5)	2.77 dd (9,5)	2.76 dd (9,5)
18	0.87 s	0.87 s	0.87 s
19	10.04 s	10.06 s	10.05 s
21α,β	4.97 d (18)	4.96 d (18)	4.95 d (18)
	4.81 d (18)	4.80 d (18)	4.80 d (18)
22	5.88 s	5.89 s	5.89 s
1′	4.35 d (7)	4.39 d (8)	4.40 d (8)
2′	~3.23 m	3.02 dd (8,9)	3.09 dd (8,9)
3′	~3.23 m	3.10 dd (9,9)	3.26 dd (9,9)
4′	3.83 bs	3.17 dd (9,9)	4.70 dd (9,9)
5′	~3.53 m	3.32 qd (6,9)	3.39 qd (6,9)
6′	1.35 d (6)	1.30 d (6)	1.17 d (6)
2′-OMe	3.56 s	3.57 s	3.57 s
3′-OMe	3.49 s	3.63 s	3.50 s
OH	4.40 s[b]	4.34 s[b]	4.33 s[b]
OAc	—	—	2.11 s

[a]360 MHz, $CDCl_3$, δ-scale, relative to TMS.
[b]D_2O exchangeable.

with reference to an authenic sample by mmp, ir, ^{1}H nmr, and co-tlc. Mansonin (**2**), the C-4′ epimer of **1**, has only been subjected to preliminary ^{1}H-nmr studies (14). All sugar-moiety protons of **2** were assignable as a result of a ^{1}H-^{1}H nmr COSY experiment (Table 1), including H-2′, -3′, and -4′, which exhibited only minor differences in their chemical shifts. On acetylation, mansonin (**2**) gave a monoacetate (**4**) (12), in which a paramagnetic shift was observed for H-4′ to 4.70 ppm, and the C-3′ methoxy group experienced a slight shielding effect compared to **2** (Table 1), values which were in accord with published observations on a cardenolide with identical substitution in the sugar moiety at C-3′ and -4′ (15). The chemical shift assignments for the equatorial methoxy group affixed to C-3′ in **1**, **2**, and **4** were made by analogy with literature values (15,16). It was apparent, however, that the chemical shift of the C-2′ equatorial methoxy group was relatively unaffected by variations in substitution at C-4′ (Table 1).

^{13}C-nmr data have not thus far been reported for strebloside (**1**) and mansonin (**2**), and assignments made in this investigation are shown in Table 2. The carbons constituting the aglycone portion of **1** and **2**, with the exception of C-3, were assigned according to published values for strophanthidin (**3**) (17), with the use of SFORD and APT (18) experiments. A ^{1}H-^{13}C heteronuclear shift correlated (HETCOR) two-dimensional experiment (19) was used to assign the chemical shifts between 60 and 100 ppm, which represent C-3 and carbons of the sugar moiety of mansonin (**2**). This relatively new nmr technique has already proven to be very useful in the analysis of natural products (20,21), and allows the definitive assignment of ^{13}C-nmr signals if the ^{1}H-nmr resonances of the attached protons are known. Because the previous COSY experiment had led to the determination of all sugar protons in **2**, the corresponding carbon signals could be assigned unambiguously. The HETCOR experiment showed clearly that the single carbon resonance at 60.6

TABLE 2. ^{13}C-nmr Chemical Shifts of Compounds **1-3**

Carbon	**1**[a]	**2**[a]	**3**[a]
1	24.1	24.0	24.8
2	26.8[c]	26.6[c]	27.4[c]
3	72.7	73.2	67.2
4	36.4	36.1	38.1
5	73.1	73.1	75.3
6	34.2	34.3	37.0
7	17.9[d]	17.6[d]	18.1[d]
8	41.6[e]	41.3[e]	42.2[e]
9	39.3[e]	39.1[e]	40.2[e]
10	54.6	54.5	55.8
11	21.9[d]	21.8[d]	22.8[d]
12	39.7	39.4	40.2
13	49.4	49.3	50.1
14	85.1	84.7	85.3
15	31.9	31.7	32.2
16	25.1[c]	25.0[c]	27.5[c]
17	50.4	50.2	51.4
18	15.6	15.5	16.2
19	208.2	208.0	195.7
20	174.4[f]	174.9[f]	177.2[f]
21	73.5	73.5	74.8
22	117.7	117.3	117.8
23	174.3[f]	174.7[f]	176.6[f]
1′	99.8	99.8	—
2′	79.9	83.6	—
3′	83.8	86.1	—
4′	68.3	74.8	—
5′	70.3	71.5	—
6′	16.4	17.5	—
2′-OMe	61.2	60.6	—
3′-OMe	57.6	60.6	—

[a]90.8 MHz, $CDCl_3$, δ-scale, relative to TMS.

[b]25.2 MHz, $CDCl_3$,-CD_3OD (3:2), δ-scale, relative to TMS. Data taken from (17).

[c-f]Chemical shifts denoted by the same letter in each column may be interchanged.

ppm was attributable to both the C-2′ and C-3′ methoxy groups, which were entirely overlapping. The carbon assignments recorded for the sugar-moiety carbons of **2** were in general agreement with those anticipated if methyl quinovoside were methylated at C-2′ and C-3′ (22,23). In the case of strebloside (**1**), ^{13}C-nmr resonances pertaining to the sugar portion of the molecule were assigned by comparison with published data for diginose (24), together with the known effects of methylation on α- and β- substituted carbons (23). The upfield shifts of C-2′, -3′, -4′, and -5′ in the spectrum of **1** in relation to mansonin (**2**) are consistent with predicted carbon chemical shift differences due to the different orientation of OH-4′ (16,25). The signal for C-3′ methoxy group in strebloside (**1**) occurred 3.0 ppm further upfield than that for mansonin (**2**), due to the shielding effect of the adjacent axial C-4′ OH group, as reported in related compounds (16).

Despite the fact that a number of Kedde reagent-positive cardiac glycosides were detected in the orginal CH_2Cl_2 extract of *S. asper* stem bark, it is interesting to note that only two such compounds, strebloside (**1**) and mansonin (**2**), were obtained as a result of

bioactivity-guided fractionation using the KB test system in this investigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points, uv and ir spectral measurements, and preparative tlc were performed as before (1). Optical rotations were carried out using a Perkin-Elmer 241 polarimeter. Nmr spectra were determined with a Nicolet NT-360 instrument. Low resolution mass spectra were obtained with a Finnigan 4510 mass spectrometer operating in the CI(CH_4) mode at 70eV.

PLANT MATERIAL.—The stem bark of *S. asper* was collected in Thailand in 1978 by staff members of the Economic Botany Laboratory, Agricultural Research Service, BARC-East, USDA, Beltsville, Maryland. A representative voucher specimen has been deposited in the Herbarium of the National Arboretum, Washington, DC.

EXTRACTION AND FRACTIONATION.—The dried, milled stem bark of *S. asper* (20 kg) was extracted in turn with petroleum ether (bp. 60-80°) and MeOH. The MeOH extract was dried, and extracted sequentially with petroleum ether, cyclohexane, CH_2Cl_2, EtOAc, MeOH, and H_2O. Cytotoxic activity against the KB system was found to be concentrated in the CH_2Cl_2 extract (177g, ED_{50}, < 1.0 μg/ml), which was fractionated by gravity column chromatography over silica gel 60 (Merck, Darmstadt, W. Germany) by elution with $CHCl_3$-MeOH mixtures of increasing polarity. A total of 63 fractions (2 liters each) were collected. Fractions 31-36, eluted with $CHCl_3$-MeOH (19:1), were active against KB cells (ED_{50} 0.049-0.1 μg/ml). Fractions 33 and 34, the two most cytotoxic fractions obtained, were combined (8.2 g). A 3.3 g portion of this mixture was crystallized in the lower phase of the solvent system hexane-Et_2O-PrOH-EtOH-H_2O (7:16:6:10:8). The resultant crystalline mixture of cardiac glycosides (335 mg) was purified by repetitve preparative tlc in the upper phase of this solvent system, to afford 50 mg of strebloside (**1**, Rf 0.60) and 187 mg of mansonin (**2**, Rf 0.74).

STREBLOSIDE (**1**).—Strebloside (**1**, 0.0003% w/w) exhibited the following data: mp 151°, $[\alpha]^{20}D$ + 11.1° (c 0.18 $CHCl_3$) [lit. mp 153-158°; $[\alpha]^{29}D$ + 25.3° ± 3° (c 0.88 MeOH) (3)]; uv λmax (MeOH)(log ε) 217 nm (4.36);ir νmax (AgCl) 3517, 2939, 1779, 1743, 1620, 1450, 1097, 1069, 753 cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; ci ms (CH_4) *m/z* 579 (MH^+, 1%), 405 (23), 341 (21), 175 (67), 143 (60), 101(100); fab ms ($CHCl_3$/nitrophenyl octyl ether) *m/z* 579 (MH^+). This isolate was identical with an authenic sample of strebloside (**1**) by mmp, ir, ^1H nmr, and co-tlc.

MANSONIN (**2**).—Mansonin (**2**, 0.001% w/w) exhibited the following data: mp 160°, $[\alpha]^{20}D$ +2.3° (c 1.0 $CHCl_3$), {lit. mp 170-175°, $[\alpha]^{23}D$ +6.7° ±2° (c 1.0 MeOH) (13)}; uv λ max (MeOH) (log ε) 217 nm (4.20); ir νmax (KBr) 3489, 2939, 1778, 1742, 1620, 1450, 1154, 1072, 1031, 991, 953, 887, 860, 833, 732, cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; ci ms (CH_4) *m/z* 579 (MH^+, 1%), 405 (6), 341 (38), 175 (47), 143 (89), 101 (100); fab, ms ($CHCl_3$/nitrophenyl octyl ether) 579 (MH^+). This isolate was identical with an authenic sample of mansonin (**2**) when compared by mmp, ir, ^1H-nmr, and co-tlc.

HYDROLYSIS OF **2** TO STROPHANTHIDIN (**3**).—Mansonin (**2**, 3 mg) was dissolved in 2N CF_3COOH (3 ml), and refluxed for 1 h. The acid was removed under reduced pressure by adding 5 × 5 ml MeOH at intervals. Analysis of the residue by tlc on silica gel 60 F-254 (Merck, Darmstadt, W. Germany) in EtOAc-MeOH-H_2O (50:7:5) indicated the presence of strophanthidin (**3**), Rf 0.70, when compared with an authentic sample.

ACETYLATION OF **2** TO MONO-*O*-ACETYL-MANSONIN (**4**).—Mansonin (**2**, 44 mg) was acetylated with pyridine-Ac_2O (1:1, 2 ml). The reaction was stopped after 3 days by the addition of iced H_2O, and the product extracted into $CHCl_3$. After recrystallization with MeOH-H_2O, **4** (33 mg) exhibited the following data: mp 229°, $[\alpha]^{20}D$ +5.6° (c 0.61 $CHCl_3$) [lit. mp 229-233°, $[\alpha]D$ +7.8° ±2° (c 1.0 MeOH) (13)];uv λmax (MeOH) (log ε) 217 nm (4.36); ir νmax (KBr) 3517, 2939, 1779, 1745, 1621, 1450, 1376, 1235, 1172, 1159, 1070, 993, 956, 892, 862, 833, 738, 697 cm^{-1}; ^1H nmr, see Table 1; ci ms (CH_4) *m/z* 621 (MH^+, 2%), 217 (100), 143 (12), 101 (10).

BIOLOGICAL ACTIVITY OF THE ISOLATES.—Strebloside (**1**) and mansonin (**2**) displayed significant activity in the KB cell culture system (8,9), with ED_{50} values of 0.035 μg/ml and 0.042 μg/ml, respectively. An isolate is considered active in this system if it shows an ED_{50} of ≤4 μg/ml.

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