Novel Saponins Hainaneosides A and B Isolated from *Marsdenia hainanensis*

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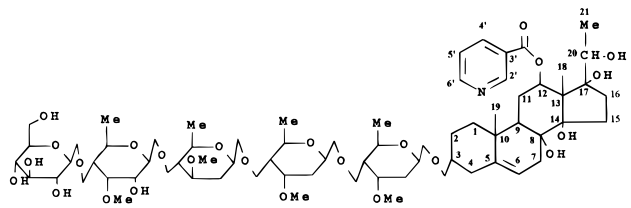
Novel saponins possessing fertility-regulating activity, hainaneosides A (**1**) and B (**2**), have been isolated from the twigs and stems of *Marsdenia hainanensis*, and their structures have been elucidated unambiguously as 12-nicotinoyl sarcostin-3-*O*-*β*-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-*β*-D-allopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside and 12-nicotinoyl-20-cinnamoyl sarcostin-3-*O*-*β*-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-*β*-D-allopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranoside using NMR spectroscopic techniques and chemical transformations.

In the course of evaluating plants for fertility-regulating activity, the MeOH extract of *Marsdenia hainanensis* was found to be most active of eight Asclepiadaceaen species examined. The extract exhibited significant antifertility activity without estrogenicity in rats at the dose of 75 mg/kg po on days 1−10. Bioassay-directed separation finally led to the isolation of two new compounds, hainaneosides A (**1**) and B (**2**). We report here the isolation and structure determination of hainaneosides A and B.[1]



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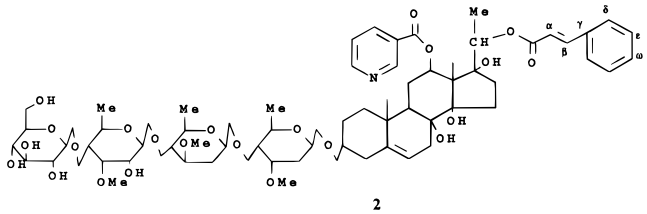

2

Marsdenia hainanensis Tsiang (Asclepiadaceae) is widely distributed in southern China. The dried twigs and stems of *M. hainanensis* were extracted with MeOH. The $CHCl_3$-soluble material of the MeOH extract was repeatedly chromatographed on Si gel columns to give two compounds: hainaneosides A (**1**) and B (**2**).

Hainaneoside A (**1**), an amorphous powder, showed positive Leibermann−Burchard and Kellen−Killiani reactions, indicating the presence of a steroid skeleton and 2-deoxy sugar moieties in the molecule. The positive FABMS exhibits two pseudomolecular ion peaks at *m/z* 1242 $[M + H]^+$ and 1264 $[M + Na]^+$, which together with ^{1}H- and ^{13}C-NMR data indicate the molecular formula $C_{61}H_{95}O_{25}N$ for **1**. The ^{1}H-NMR spectrum of **1** shows well-resolved signals for five anomeric protons: two doublets at δ 4.43 and 4.72 and three doublets of doublets at δ 4.62, 4.79, and 4.86, corresponding to 2-deoxy sugars. The β-linkages of all of the sugars are revealed by the coupling constants of the anomeric protons (7.7−9.7 Hz). In the highfield region, there are four secondary methyl signals at δ 1.15, 1.20, 1.27, and 1.34, suggesting the presence of four 6-deoxy sugar units in the molecule. Because of moderate signal overlap in the 3.2−3.8 ppm region, the scalar coupling network for each sugar unit was then established by the interpretation of the DQF−COSY[2] and TOCSY[3] spectra, using each anomeric proton signal as well as the secondary methyl signal as the starting point. The results, shown in Table 1, indicate that of the five sugar moieties, three are 2,6-dideoxypyranose, one is a 6-deoxypyranose, and the remaining sugar is a normal pyranose residue. The DQF−COSY and TOCSY spectra of **1** showed two parallel sets of proton signals due to two cymarose units, which are characterized by their 2,6-dideoxypyranose skeleton with an equatorial H-3 proton. The latter is indicated by their somewhat lowfield chemical shift values and narrow line width (partly overlapped at δ 3.83, $W_{1/2}$ = 3 Hz). The third 2,6-dideoxypyranose shows quite different chemical-shift values. Its H-4 appears the most highfield (δ 3.19) as a triplet (J = 9.1Hz), indicating H-3, H-4, and H-5 are all in axial orientation. In addition, the anomeric proton shows NOE connectivity with H-3 and H-5 in the ROESY[4] spectrum. Thus it can be identified as oleandropyranose due to its all-axial proton-coupling pattern.

The 6-deoxypyranose is assigned as 6-deoxyallopyranose on the basis of the following observations. Its H-3

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Table 1. NMR data for the Oligosaccharide Moiety of **1** and **2** in Me_2CO-d_6[a]

	1				2	
position	δ_C	HMBC	δ_H	ROESY	δ_C	δ_H
Cym 1	96.3		4.86 (dd, 9.6, 1.7)	H-3, aglycon	96.5	4.85 (dd, 9.6, 1.7)
2	37.1		1.51, 1.97		37.2	1.49, 1.92
3	78.0	3-Ome	3.83	Cym H-2,4	78.1	3.82
4	83.6		3.22		83.7	3.23
5	69.0		3.78	Cym H-1	69.1	3.79
6	18.0		1.15 (d, 6.2)		18.0	1.16 (d, 6.2)
3-OMe	58.7		3.43 (s)	Cym H-3	58.9	3.43
Cym 1′	100.6	Cym H-4	4.79 (dd, 9.6, 1.7)	Cym H-4		
2′	37.3		1.58, 2.06			
3′	77.9	3-OMe	3.83	Cym H-2′, 4′		
4′	83.5		3.26 (dd, 9.7, 2.6)			
5′	69.0		3.80	Cym H-1′		
6′	18.5		1.20 (d, 6.2)			
3-Ome′	58.6		3.43 (s)	Cym H-3′		
Ole 1	102.0	Cym H-4′	4.62 (dd, 9.7, 1.7)	Cym H-4′	101.5	4.61, (dd, 9.7, 1.7)
2	37.3		1.37, 2.35		37.3	1.35, 2.35
3	79.7	3-OMe	3.38	Ole H-1	79.9	3.36
4	82.5		3.19 (t, 9.1)		82.8	3.17 (t, 9.1)
5	71.5		3.36	Ole H-1	71.6	3.34
6	19.0		1.34 (d, 6.1)		18.5	1.33 (d, 6.1)
3-OMe	56.9		3.40 (s)	Ole H-3	57.2	3.40
Allo 1	101.3	Ole H-4	4.72 (d, 8.2)	Ole H-4	102.2	4.72 (d, 8.2)
2	72.2		3.28		72.3	3.29
3	82.5	3-OMe	3.95 (t, 2.8)	Allo H-2, 4	82.7	3.95
4	83.4		3.32 (dd, 8.4, 2.8)		83.5	3.31
5	69.4		3.75	Allo H-1	69.6	3.76
6	19.2		1.27 (d, 6.2)		18.9	1.28 (d, 6.2)
3-OMe	61.4		3.56 (s)	Allo H-3	61.7	3.55
Glc 1	105.7	Allo H-4	4.43 (d, 7.7)	Allo H-4	105.9	4.43 (d, 7.8)
2	75.0		3.23		75.1	3.20
3	77.7		3.38	Glc H-1	77.8	3.36
4	71.7		3.34		72.0	3.34
5	77.3		3.29	Glc H-1	77.5	3.31
6	62.9		3.68, 3.88		62.9	3.68, 3.88

[a] Measured at 150 MHz for ^{13}C-NMR and at 600 MHz for ^{1}H-NMR; δ_C and δ_H are in ppm; J values in Hz are shown in parentheses.

proton characteristically appears at lowest field (δ 3.95) as a small triplet (J = 2.8Hz), which indicates that is in equatorial orientation. All four 6-deoxypyranose residues are 3-methyl ethers, as indicated by the presence of four methoxy group signals at δ 3.40, 3.43(× 2), and 3.56. Assignment of individual methoxy groups to each sugar residue is determined by NOE cross peaks between them and H-3 proton of the relevant sugar unit in the ROESY spectrum. The remaining sugar moiety is identified as glucopyranose on the basis of its characteristic coupling pattern of all-axial ring protons and H-6 (δ 3.88 and 3.68, respectively, *AB*M spin system). Corroborative evidence for the structure of the sugar moieties is obtained from an HMQC experiment,[5] which leads to the full ^{13}C and ^{1}H assignment of each sugar moiety (Table 1) and acid hydrolysis of **1**, which yields glucose, cymarose, and pachybiose. Thus, the five sugars are two β-cymaroses (Cym, 2,6-dideoxy-3-*O*-methylribohexose), β-oleandrose (Ole, 2,6-dideoxy-3-*O*-methylarabinohexose), β-3-methoxy-6-deoxyallopyranose (Allo), and β-glucose (Glc).

The sequence and linkage sites of the sugar units were established as Glc(1−4)-Allo(1−4)-Ole(1−4)-Cym-(1−4)-Cym(1−3)-aglycon on the basis of ROESY and HMBC[6] data, which are summarized in Figure 1. This assignment is consistent with the FABMS data, which show main fragment ions at m/z 1079 $[M + H - Glc]^+$, 901 $[M + H - Glc - Allo]^+$, 775 $[M + H - Glc - Allo - Ole]^+$, 631 $[M + H - Glc - Allo - Ole - Cym]^+$ and 487, which corresponds to the aglycon moiety. The oligosacchride portion of **1** counts for the $C_{34}H_{59}O_{18}$ constituent. The remaining entity of the molecule

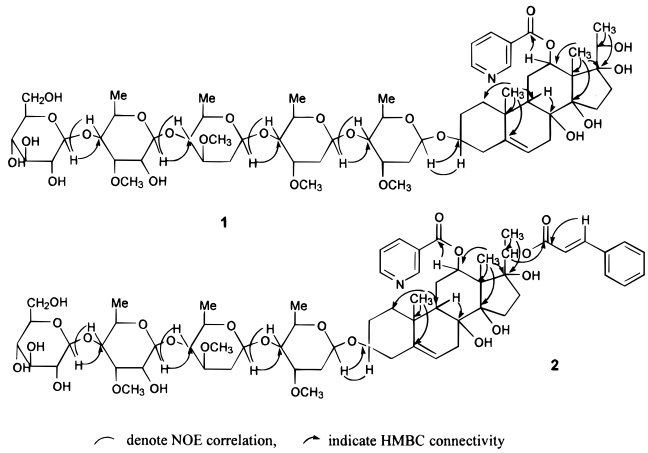

Figure 1. The NOE correlations and ^{13}C−^{1}H connectivities of **1** and **2**.

consisting of $C_{27}H_{36}O_7N$ is the aglycon and its appended group. The nmr data for this remaining portion of the molecule shows two singlet methyl signals at δ 1.17, 1.70 ppm (18- and 19-Me), a doublet methyl signal at δ 1.06 assignable to 20-methyl of the aglycon, and an olefinic proton signal at δ 5.34, which indicates the presence of a trisubstituted double bond. The interpretation of the DQF−COSY, TOCSY, and HMQC spectra for the aglycon of the molecule results in the following fragments: $-CH_2CH_2CH(OR)CH_2-$, $-C{=}CHCH_2-$, $-CHCH_2CH(OR)-$, and $-CH_2CH_2-$ bearing quaternary carbons at both ends, as well as a $-CH(OH)CH_3$ group corresponding to the partial structures of C-1 to C-4, C-5 to C-7, C-9 to C-12, C-15 to C-16, and C-20 to C-21

Table 2. NMR Data for the Aglycon Moieties of Hainaneoside A (**1**) and B (**2**) in Comparison with the ^{13}C-NMR Data of an Authentic Sample of Sarcostin[a]

	1		**2**	sarcostin	**1**	**2**
position	δ_C	HMBC	δ_C	δ_C	δ_H	δ_H
1	39.5	H-19	39.5	39.5	1.14, 1.84	1.11, 1.78
2	29.8		29.8	31.7	1.56, 1.81	1.55, 1.83
3	77.6	Cym H-1	77.9	73.0	3.54	3.52
4	39.2	H-6	39.3	42.9	2.20, 2.37	2.20, 2.37
5	139.5	H-19	138.6	140.4		
6	119.4	H-4	119.4	119.0	5.34	5.34
7	34.1		34.0	34.5	2.19, 2.12	2.20, 2.18
8	74.2	H-6, H-9	74.2	74.2		
9	44.0	H-19	44.1	44.5	1.64	1.61
10	37.5	H-6, H-19	37.7	37.5		
11	25.4		25.6	28.9	2.16, 1.90	2.00, 1.72
12	75.7	H-18	74.5	71.9	4.93	4.86
13	57.1	H-18	57.3	58.5		
14	88.4	H-18, H-9	88.9	89.0		
15	32.5		33.7	33.7	1.79, 1.86	1.97, 2.11
16	35.0		35.1	35.3	1.91, 1.92	2.16, 2.17
17	88.5	H-18, H-21	88.0	88.9		
18	11.7		11.3	10.7	1.70	1.68
19	18.3		18.7	18.5	1.17	1.11
20	70.8	H-21	76.3	70.0	3.62	4.88
21	18.5		15.2	17.1	1.06	1.33
C=O	165.3	H-12	164.7			
2′	151.7		151.6		9.24	9.21
3′	127.7		128.0			
4′	139.1		139.7		8.46 (d, 7.9)	8.24 (d, 7.8)
5′	124.4		124.4		7.54 (dd, 7.9, 4.9)	7.40 (dd, 7.8, 4.8)
6′	154.3		154.2		8.79 (d, 4.9)	8.76 (d, 4.8)
C=O			166.6			
α			120.0			6.14 (d, 16.2)
β			144.3			7.39 (d, 16.2)
γ			131.0			
δ			128.8			7.39
ϵ			129.7			7.41
ω			135.2			7.40

[a] Measured at 150 MHz for ^{13}C-NMR and at 600 MHz for ^{1}H-NMR in Me_2CO-d_6; δ_C and δ_H are in ppm; *J* values (in Hz) are shown in parentheses.

of the aglycon, respectively. The HMBC experiment was used to clarify the correlations between the partial structures. The long-range connectivities obtained by the HMBC experiment (see Figure 1) indicate that the aglycon of **1** has a highly oxidized pregnane skeleton with oxygenated quaternary carbons at 8, 14, and 17 and oxygenated tertiary carbons at the 3, 12, and 20 positions (i.e., the sarcostin skeleton). Comparison of ^{13}C-NMR data between the aglycon portion of **1** and an authentic sample of sarcostin reveals close similarity except for C-2, C-3, C-4, C-12, and C-13 due to the substitution (Table 2). Glycosylation shifts are observed at C-3 (+6.7 ppm) and C-4 (−3.7 ppm), indicating that the oligosaccharide chain is attached to C-3 through an ether linkage. Furthermore, the cymarose H-1 shows an NOE cross peak with H-3 of the aglycon in the ROESY spectrum and the long-range correlation with C-3 of the aglycon in the HMBC spectrum. In order to confirm the structure, **1** was submitted to acid hydrolysis and subsequently alkaline hydrolysis to afford sarcostin, which was identified by comparison on TLC with the authentic sample.

The remaining portion of the molecule, C_6H_4ON, was assigned to a nicotinoyl group, which is characterized by the UV absorption bands at 226, 362, and 370 nm; the aromatic ^{1}H-NMR proton signals at δ 9.24 (br s), 8.79 (br d, J = 4.9Hz), 8.46 (d, J = 7.9Hz), and 7.54 (dd, J = 7.9, 4.9 Hz); and the ^{13}C-NMR spectrum, which exhibits signals for the nicotinoyl group at δ 124.0 (C′-5), 127.4 (C′-3), 137.7 (C′-4), 151.6 (C′-2), 154.0 (C′-6), and 165.3 (C=O). The carbonyl carbon shows a long-range correlation with H-12 of the aglycon in the HMBC spectrum, which clearly indicates the substitution site at C-12. Therefore, the structure of **1** can be established as 12-nicotinoyl sarcostin-3-*O*-*β*-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-*β*-D-allopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside.

Hainaneoside B (**2**) was obtained as an amorphous powder. The molecular formula was determined as $C_{63}H_{89}O_{23}N$ on the basis of FABMS and NMR data. The 1D and 2D ^{1}H-NMR spectra of **2** are closely related to those of **1**. Compound **2** shows signals for four anomeric protons, three methoxy groups, and three terminal methyl groups for the oligosaccharide moiety. Careful comparison of ^{13}C-NMR, DQF−COSY, TOCSY, ROESY, and HMQC spectra for **1** and **2** (Tables 1 and 2) reveals that, except for the absence of a cymarose moiety in **2**, the remaining four sugars are identical to those of **1**. On the other hand, besides the signals for the nicotinoyl moiety, additional peaks at δ 6.14 (d, J = 16 Hz), 7.38 (d, J = 16 Hz), 7.39−7.41 (m, 5H) in the lowfield region of the ^{1}H-NMR spectrum indicate the presence of a *trans*-cinnamoyl group in the molecule, which is confirmed by the carbon signals at δ 120.0 (α-C), 128.8 (C × 2, δ-C), 129.7 (C × 2, ϵ-C), 131.0 (γ-C), 135.2 (ω-C), 144.3 (β-C), and 166.6 (C=O). Meanwhile, the signal for H-20 of the aglycon of **2**, which is easily recognized by the cross peak with 20-Me in the DQF−COSY spectrum, is significantly shifted downfield to δ 4.86. These data suggest that the second substitution should be at C-20. Because there are two appended groups in

the aglycon, the remaining problem is to determine the sites of these two substituents (nicotinoyl and cinnamoyl), respectively. In the HMBC spectrum, H-20 (4.86 ppm) shows a long-range connectivity with the carbonyl carbon atom (167.0 ppm), which in turn couples with the β-proton (6.14 ppm) of the cinnamoyl moiety. Thus, the cinnamoyl group is attached to C-20, and the nicotinoyl moiety is linked to C-12 of the aglycon by ester linkages. Therefore, hainaneoside B (**2**) is identified as 12-nicotinoyl-20-cinnamoyl sarcostin-3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside.

To date, a number of novel glycosides with a tri-, tetra-, or pentaglycosidic chain have been isolated from the family Asclepiadaceae. The commonly encountered aglycons of the glycosides are ester-type derivatives of polyoxypregnane such as sarcostin, deacylmetaplexigenin, pergularin, utedin, and tomentogenin.[7] Various appended groups including cinnamoyl, benzoyl, tigloyl, ikemaoyl, acetyl, and nicotinoyl, which usually substituted at 12- and/or 20-position of the aglycon, were reported in the literature.[8] Hainaneosides A and B are believed to be the first examples to contain a sarcostin aglycon with a nicotinoyl substitution group at 12-position. Althouph a similar aglycon, gagaminin (12-cinnamoyl-20-nicotinylsarcostin), was previously reported for wilforside G1G, the aglycon of **2** is distinct from gagaminin in the sites of the two appended groups. In addition, the sugar chain of **1** appears to be identical to that of dregeoside A_{01}.[9] However, the tetraglycosidic chain of **2** is unique in this family of compounds.

Upon the test of terminating early pregnancy, hainaneoside A was active at a dose of 5 mg/kg po in rats.

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert hot stage apparatus and are uncorrected. Optical rotations were measured in Me_2CO with a Perkin-Elmer 241 MC digital polarimeter. UV (MeOH) and IR (KBr) spectra were recorded on an HP 8451 A Diode Array spectrophotometer and a Digilab FTS-20E, respectively. The positive ion FABMS was obtained with a VG Quattro spectrometer, using glycerol as matrix. The ^{1}H-NMR (1D and 2D) and ^{13}C-NMR (BB) spectra were taken in Me_2CO-d_6 on a Bruker Aspect AMX-600 spectrometer operating at 600 MHz. The chemical shifts are expressed as ppm (δ) with TMS as an internal standard and coupling constants (J) are in Hz . The proton−proton connectivities were established using DQF−COSY and TOCSY spectra. The carbon−proton connectivities were established using a proton-detected heteronuclear multiple quantum correlation experiment (HMQC). Nuclear Overhauser connectivities were obtained using ROESY spectrum. The proton−carbon long-range connectivities through two or three bonds were determined by heteronuclear multiple bonds connection (HMBC) experiments. Typical experiment parameters for 2D spectra included 512 fid's (for DQFCOSY, TOCSY, ROESY), 280 fid's (for HMBC), and 256 fid's (for HMQC) acquired with 1K data points zero-filled to 1K × 1K (for DQFCOSY, TOCSY, ROESY, and HMBC) and 1K × 512 (for HMQC), using sine bell apodization. The typical ^{1}H 90° pulse width was 9.5 μs, and ^{13}C indirect detection 90° pulse width was 12 μs.

Plant Materials. The twig and stem parts of *M. hainanensis* Tsiang used in this investigation were collected in Hainan Province, China. The sample was identified by Mr. Zi-en Zhao of the Wuhan Institute of Botany, Chinese Academy of Sciences. The herbarium specimens representing the collection were deposited in the herbarium of Wuhan Institute of Botany, China, and sent to The Field Museum of Natural History, Chicago, IL, and to The Kew Herbarium in London, England, for confirmation and preservation.

Extraction and Isolation. The dried and powdered twig and stem of *M. hainanensis* Tsiang (3.1 kg) were repeatedly extracted with MeOH at room temperature. The solvent was evaporated under reduced pressure to give a crude extract (89 g), which was partitioned between petroleum ether and H_2O, followed by $CHCl_3$ and H_2O. The $CHCl_3$ solubles (19 g), which showed antifertility activity, were repeatedly chromatographed on a Si gel column, eluting with a $CHCl_3$−MeOH gradient to give hainaneoside A (100 mg) and hainaneoside B (120 mg). The partition and fractionation were guided by the postcoital antifertility tests using Sprague−Dawley rats as described by Che *et al.*[10]

Hainaneoside A (**1**): white powder (MeOH); mp 165−166 °C; $[\alpha]^{23}_D$ +17.6 (*c* 0.6, Me_2CO); IR (KBr) ν max 3440 (OH), 1720 (C=O), 1640 and 1600 cm^{-1} (C_5H_5N); UV (MeOH) λ max 266, 362, 370 nm; ^{1}H-NMR (-d_6, 600 MHz) and ^{13}C-NMR (Me_2CO-d_6, 150 MHz) see Tables 1 and 2; positive ion FABMS *m/z* 1264 $[M + Na]^+$, 1242 $[M + H]^+$, 1079 $[M + H - Glc]^+$, 901 $[M + H - Glc - Allo]^+$, 775 $[M + H - Glc - Allo - Ole]^+$, 631 $[M + H - Glc - Allo - Ole - Cym]^+$ and 487[aglycon moiety]$^+$.

Hainaneoside B (**2**): white powder (MeOH): mp 174−175 °C; $[\alpha]^{23}_D$ +86.2 (*c* 2.7, Me_2CO); IR (KBr) ν max 3430 (OH), 1720 (C=O), 1640, and 1600 cm^{-1} (C_5H_5N, C_6H_5); UV (MeOH) λ max 274, 226, 214 nm; ^{1}H-NMR (Me_2CO-d_6, 600 MHz) and ^{13}C-NMR (-d_6, 150 MHz) see Tables 1 and 2; positive ion FABMS *m/z* 1228 $[M + H]^+$, 1250 $[M + Na]^+$, and 1360 $[M + Cs]^+$.

Hydrolysis of 1. A solution of **1** (50 mg) in MeOH (25 mL) and 0.1 M H_2SO_4 (25 mL) was heated at 50 °C for 1 h, then the MeOH was evaporated, and H_2O (25 mL) was added. The aqueous layer was extracted with Et_2O. After of evaporation of Et_2O, the residue was dissolved in a solution of MeOH (10 mL) and 5% K_2CO_3 (10 mL), the mixture was stirred at 50 °C for 48 h and then evaporated to dryness. The residue was examined by TLC in direct comparison with an authentic sample to confirm the presence of sarcostin. The aqueous layer of acidic hydrolysis was neutralized with $Ba(OH)_2$ and filtered. The solution was evaporated to dryness. The residue was examined by paper chromatography and TLC and showed the presence of glucose, cymarose, and pachybiose by direct comparison with the authentic samples.

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References and Notes

(1) The preliminary report was published in *Chin. Chem. Lett.* **1994**, *5*, 209−210.
(2) Rance, M.; Sorensen, O. W.; Bodenhausen, Wagner, G.; Ernst, R. R.; Wuthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479−481.
(3) Davis, D. G.; Bax, A. *J. Am. Chem. Soc.* **1985**, *107*, 2820−2821.
(4) Bothner-By, A. A.; Stephens, R. L.; Lee, J. T.; Waren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, 811−813.
(5) Bax, A.; Griffey, R. G.; Haukins, B. L. *J. Am. Chem. Soc.* **1983**, *105*, 7188−7190.
(6) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.
(7) Seto, H.; Hayashi, K.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1977**, *25*, 611−616.
(8) Tsukamoto, S.; Hayashi, K.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1985**, *33*, 2294−2304.
(9) Yoshimura, S.; Narita, H.; Hayashi, K.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1983**, *31*, 3971−3983.
(10) Che, C.-T.; Ahmed, M. S.; Kang, S. S. *et al*, *J. Nat. Prod.* **1984**, *47*, 331−341.

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