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NEW STEMODANE DITERPENES FROM *STEMODIA MARITIMA*

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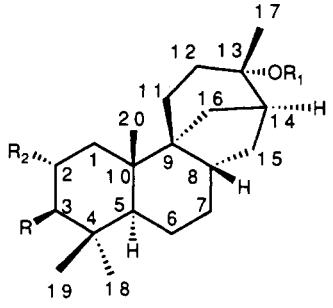
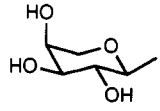
ABSTRACT.—EtOH extraction of *n*-hexane defatted *Stemodia maritima* has yielded four new stemodane diterpenes after solvent partitions and chromatographic separations. These diterpenes have been identified as maristeminol [**2**] and stemodinosides A [**3**], B [**4**], and C [**5**] from ^{13}C-nmr comparisons with stemodin [**1**]. The antiviral activity and cytotoxicity of these derivatives were tested, but they were essentially not active.

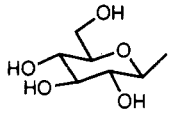
Stemodia maritima L. (Scrophulariaceae) has yielded a number of interesting diterpenes having two different skeletal backbones (1–4). Stemodane-type compounds constitute the major constituents isolated from collection of plant native to Curaçao with stemodin [**1**] being the principal diterpene (2). The diterpenes can be precipitated out by simply concentrating *n*-hexane percolates of *S. maritima*. EtOH extraction of the defatted plant material has now led to the isolation and identification of four new stemodane diterpenes, maristeminol [**2**] and stemodinosides A [**3**], B [**4**], and C [**5**].

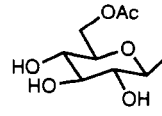
Maristeminol [**2**], mp 180–182°, had molecular formula $C_{20}H_{34}O_3$, as established by lc/ms and ^{13}C nmr. Comparison of the ^{1}H- and ^{13}C-nmr spectral data of **2** with those of stemodin [**5**] (Tables 1 and 2) led to the conclusion that **2** contained the stemodane



1 R=R$_1$=H, R$_2$=OH
2 R=R$_2$=OH, R$_1$=H

3 R=H, R$_1$=  , R$_2$=OH

4 R=H, R$_1$=  , R$_2$=OH

5 R=H, R$_1$=  , R$_2$=OH

6 R=OH, R$_1$=R$_2$=H

TABLE 1. ^{1}H-nmr Data (in δ ppm) of *Stemodia* Diterpenes **1–5**.[a]

Proton	Compound				
	1	**2**	**3**	**4**	**5**
H-1	2.34, ddd (2.7, 2.7, 12.0)	2.34, dd (11.4, 11.4)	2.30, br d (11.7)	2.33, br d (11.7)	2.36, br d (11.5)
	1.56, m	1.7, m	1.6, m	1.7, m	1.6, m
H-2	4.0, m	3.95, br dd (9, 13.8)	3.99, m	3.98, m	4.01, m
H-3	2.00, m		2.0, m	2.0, m	2.0, m
	1.4, m	3.35, d (9.0)	1.4, m	1.4, m	1.4, m
H-5	1.4, m	2.16, m	1.3, m	1.3, m	1.3, m
H-6	1.4, m	1.4, m	1.4, m	1.4, m	1.4, m
	1.2, m		1.2, m	1.2, m	1.2, m
H-7	1.1, m	—	1.2, m	1.8, m	1.8, m
	1.9, m		1.1, m	1.1, m	1.1, m
H-8	1.75, m	—	1.6, m	1.6, m	1.65, m
H-11	1.4, m		1.3, m	1.3, m	2.1, m
	2.0, m	1.95, m	2.0, m	2.1, m	2.2, m
H-12			1.6, m	1.4, m	1.4, m
	1.6, m	1.6, m		1.89, br d (5.7)	1.85, m
H-14	2.16, dd (6.0, 6.0)	—	2.17, dd (6.5, 6.5)	2.27, dd (6.3, 6.3)	2.21, dd (6.0, 16.0)
H-15	1.1, m		1.1, m	1.1, m	1.1, m
	1.5, m	—	1.7, m	1.65, m	1.6, m
H-16	1.75, m		1.6, m	1.7, m	1.6, m
	2.41, d (10.8)	—	2.43, d (11.2)	2.62, br d (11.4)	2.55, d (11.3)
H-17	1.30, s	1.32, s	1.33, s	1.37, s	1.34, s
H-18	0.98, s	1.27, s	0.90, s	0.88, s	0.93, s
H-19	0.94, s	1.20, s	0.93, s	0.91, s	0.91, s
H-20	1.02, s	1.02, s	0.94, s	0.92, s	0.99, s
H-1′			4.83, d (6.6)	4.99, d (7.8)	4.90, d (7.7)
H-2′			4.25, m	4.1, m	3.95, m
H-3′			4.25, m	4.25, m	4.2, m
H-4′			4.25, m	4.25, m	3.9, m
H-5′			4.35, m	3.90, m	3.9, m
			3.77, d (12.0)		
H-6′			—	4.25, m	4.60, dd (6.5, 11.5)
				4.46, br d (11.5)	4.93, br d (11.5)
Ac			—	—	2.04, s
OH			6.91, 6.31, 6.23, 5.61 (D_2O exchangeable)	7.0, 5.85, 5.56 (D_2O exchangeable)	7.13, 5.46 (D_2O exchangeable)

[a]Data recorded in pyridine-d_5. Figures in parentheses are J in Hz. The assignments were made by a combination of COSY and HETCOR. The chemical shifts for multiplets were obtained from HETCOR plots and are reported ±0.05 ppm.

TABLE 2. ^{13}C-nmr Chemical Shift Values of *Stemodia* Diterpenes **1–5**.[a]

Carbon	Compound						
	1[b]	**2**	**3**	**4**	**5**	Methyl-α-L-ara[c]	Methyl-β-D-glu[c]
C-1	46.3	43.6	46.4	46.4	47.0		
C-2	64.1	68.3	64.1	64.1	64.1		
C-3	51.7	83.6	51.9	51.8	52.0		
C-4	34.6	39.7	34.8	34.8	34.8		
C-5	46.9	46.9	47.0	46.9	47.0		
C-6	22.3	22.5	22.4	22.5	22.5		
C-7	36.7	37.0	36.8	36.8	36.8		
C-8	37.5	37.4	37.7	37.8	37.9		
C-9	50.6	50.6	50.1	50.2	50.1		
C-10	40.3	39.7	40.3	40.3	40.4		
C-11	28.3	28.3	28.3	28.2	28.2		
C-12	33.4	33.4	31.3	31.9	31.0		
C-13	71.1	71.0	79.2	79.2	79.4		
C-14	46.9	47.4	44.8	44.5	45.1		
C-15	38.3	38.2	38.1	38.1	38.0		
C-16	30.3	30.5	30.0	29.9	29.9		
C-17	28.4	28.7	25.1	25.3	25.1		
C-18	34.6	30.4	34.9	34.8	34.8		
C-19	23.7	19.9	23.9	23.9	23.9		
C-20	19.5	18.3	19.9	19.9	19.9		
C-1′			99.4	99.0	99.9	105.1	104.3
C-2′			72.7	75.4	74.8	71.8	74.2
C-3′			74.8	79.0	78.7	73.4	76.9
C-4′			69.3	72.2	71.8	69.4	70.8
C-5′			66.5	77.9	75.2	67.3	76.9
C-6′				63.3	64.8		61.9
COMe					20.9		
*CO*Me					170.8		

[a]Data were recorded in pyridine-d_5. The multiplicities were verified by DEPTGL and APT expeirments.

[b]Assignments have been rigorously established (5) and are listed here for comparison with other diterpenes.

[c]Data were reported previously (7) and are listed here for comparison.

ring system with one additional hydroxyl group. (Stemarin has been isolated from *Stemodia* and has a different ring system (3). The complete ^{13}C-nmr assignments have not been established for stemarin, but the ^{13}C-nmr pattern for stemarin is quite different from that of stemodin. The data are listed here for comparison: methyl [29.6, 18.2, 17.2], methylene [71.8, 39.5, 36.3, 32.3, 30.9, 29.9, 27.6, 26.9, 23.0, 18.7], methine [49.2, 42.7, 40.0], quaternary [72.2, 52.0, 38.9, 38.2]. Since all of the ^{1}H- and ^{13}C-nmr assignments for stemodin [**1**] and maritimol [**6**] have been rigorously established (5), the location of the hydroxyl group at C-3 was straightforward (Table 2). The hydroxyl group was oriented in the equatorial configuration by noting that the signal for H-3 appeared as a doublet ($J = 9.0$ Hz), requiring the proton to be axial.

Stemodinosides A, B, and C were established as glycosides of stemodin by examining the ir and ^{13}C-nmr spectral data (Table 2) and by acid hydrolysis. Stemodinoside A [**3**] has stemodin as its aglycone and a pentose sugar identified as arabinose (^{13}C nmr, tlc). The sugar was attached to stemodin through the tertiary alcohol function at C-13 (^{13}C nmr, Table 2).

Stemodinoside B [**4**] has stemodin as its aglycone and glucose as its sugar (^{13}C-nmr,

hydrolysis), with the glucose attached through the oxygen at C-13, as was the case for **3** (^{13}C nmr, Table 2).

Stemodinoside C [**5**] is also composed of glucose and stemodin, but possesses in addition an acetate group (δ_H 2.03 CH_3CO; δ_C 20.9 and 170.8; ν max 1760 cm^{-1}). Controlled basic hydrolysis produced **4**, identical with the natural sample. The acetate was located at C-6′ based on the ^{1}H- and ^{13}C-nmr shifts of H-6′ as compared with those of **4**. Thus, stemodinoside C [**5**] is a glucose-6′ acetate glycoside of stemodin.

Because optical rotations were not obtained on the sugars, the absolute configurations of arabinose and glucose are not known. However, it is known that the naturally occurring forms in most plants are L-arabinose and D-glucose. The anomeric proton in both glycosides resonates as a doublet (J = ca. 7 Hz), suggesting an α-L-arabinoside and a β-D-glucoside.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H-nmr spectra were run at 300 MHz and ^{13}C-nmr spectra at 75 MHz in pyridine-d_5 unless otherwise stated, using a VXR 300 MHz spectrometer. Multiplicity (APT, DEPTGL) and all 2D nmr experiments (COSY, HETCOR) were run using standard varian pulse sequences. Tlc was performed on Si gel GF_{254} and cc on Si gel. Compounds were visualized by spraying with 1% vanillin/H_2SO_4. Fab mass spectra were obtained from the mass spectrometer laboratory at the University of Kansas. Optical rotations were recorded on a Perkin-Elmer polarimeter in solution in a 1 dm cell. The plant material used in this study was obtained from Curaçao by Dr. R.O. Guerrero of the University of Puerto Rico. A voucher specimen is on deposit at the Herbarium of the Department of Pharmacognosy, University of Mississippi and was verified by Dr. Julia Morton, Morton Collectanea, University of Miami.

EXTRACTION AND ISOLATION OF TERPENOIDS AND DITERPENE GLYCOSIDES.—The dried ground whole plant (3.6 kg) was percolated successively with *n*-hexane and EtOH. The EtOH extract (325.9 g) was subjected to solvent partitioning between *n*-hexane and EtOAc to afford, after evaporation, an EtOAc extract (225 g) and an *n*-hexane extract (95.5 g). A portion of the EtOAc fraction (15 g) was subjected to cc over Si gel (900 g), eluting initially with EtOAc and then with increasing amounts of MeOH in EtOAc. Elution with EtOAc yielded a large amount of the known triterpene betulinic acid (670 mg), and elution with 1% MeOH in EtOAc gave a mixture of components. This mixture was further subjected to cc over Si gel [with *n*-hexane–EtOAc (8:2) as eluent] to afford maristeminol [**2**] (20 mg).

Further elution of the column with 3% MeOH in EtOAc revealed a mixture that was subjected to further Si gel cc. Elution with 2% MeOH in EtOAc gave stemodinosides C [**5**] (140 mg) and A [**3**] (150 mg). Finally, elution with 4% MeOH in EtOAc yielded stemodinoside B [**4**] (200 mg).

Stemodin [**1**] could be obtained easily by concentrating the *n*-hexane percolates to about half volume, keeping the percolate at 4° and filtering the crude greenish precipitate (0.5%). Chromatography (Si gel) of the evaporated filtrate yielded additional quantities of stemodin [**1**] (21.5%) as well as stemarin (0.6%), maritimol [**6**] (0.5%), and stemodinone (0.2%) (2). Rechromatography of the very polar fractions from these columns (MeOH wash) yielded additional quantities of **2**–**5**. The *n*-hexane filtrate (91 g) (representing 9.9 kg of *S. maritima*) was processed to yield 247 mg of **2** (0.0005%), 217 mg of **3** (0.0041%), 225 mg of **4** (0.005%), and 205 mg of **5** (0.0038%).

MARISTEMINOL [**2**].—Crystallized from MeOH/EtOAc as colorless needles: mp 180–182°; $[\alpha]^{25}D$ +17.5° (MeOH, c = 1.0); ir (KBr) ν max, cm^{-1} 3540 (OH), 3010, 1550, 1265; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; hr fabms 345.2404 ($C_{20}H_{34}O_3Na^+$ requires 345.2422).

STEMODINOSIDE A [**3**].—Obtained as colorless needles from EtOAc/MeOH: mp 130–132°; $[\alpha]^{25}D$ +11.1° (MeOH, c = 2.0); ir (KBr) ν max, cm^{-1} 3550 (OH), 2990, 1545, 1165; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; hr fabms 461.2870 ($C_{25}H_{42}O_6Na^+$ requires 461.2876).

Stemodinoside A [**3**] (60 mg) was dissolved in absolute EtOH (5 ml), 3 N HCl was added (5 ml), and the reaction mixture was refluxed at 80–100° for 1 h. The reaction mixture was cooled, diluted with H_2O (25 ml), and neutralized with 10% KOH. The neutralized reaction mixture was then extracted with EtOAc (3 × 25 ml), and the combined EtOAc extract was dried over anhydrous Na_2SO_4, filtered, and the filtrate evaporated in vacuo. The residue was redissolved in EtOAc and on cooling to 4° afforded colorless plates that were washed and dried under vacuo (30 mg). The neutralized aqueous layer was concentrated, and tlc analysis [Si gel, MeOH-$CHCl_3$-H_2O (4:6:0.5)] showed a single sugar, identical to that of arabinose (R_f ara = 0.59, xyl = 0.67, rib = 0.75). The white crystalline aglycone (mp 194–195°) was confirmed as

stemodin [**1**] by spectral data (^{1}H and ^{13}C nmr) and also by direct comparison (tlc, ^{13}C nmr, mmp) with an authentic sample.

STEMODINOSIDE B [**4**].—Obtained as colorless ovoid-shaped crystals from MeOH/EtOAc: mp 223–225°; $[\alpha]^{25}D$ +12.8° (MeOH, c = 2.5); ir (KBr) ν max cm^{-1} 3560 (OH), 3010, 1560, 1160; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Using the same method as described for **3**, a sample of **4** (50 mg) was hydrolyzed, and the aglycone crystallized from EtOAc as plates (38 mg, mp 194–195°). The aglycone was identified as stemodin by its spectral data (^{1}H and ^{13}C nmr) and also by direct comparison with an authentic sample. Tlc analysis of the concentrated neutralized aqueous layer showed the presence of a single sugar, identical to that of glucose (R_f 0.36, same system as for hydrolysis of **3**).

STEMODINOSIDE C [**5**].—Obtained as colorless needles from MeOH/EtOAc: mp 187–189°; $[\alpha]^{25}D$ +3.75° (MeOH, c = 3.2); ir (KBr) ν max, cm^{-1} 3555 (OH), 3010, 1760 (OAc), 1535, 1170; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; hr fabms 533.3083 ($C_{28}H_{46}O_8Na^+$ requires 533.3090).

Alkaline hydrolysis of **5** was achieved by using the following procedure. KOH (8 mg) was added to a sample of **5** (51 mg) dissolved in absolute EtOH (5 ml), and the mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with H_2O (25 ml) and extracted with EtOAc (3 × 25 ml). After repeated washing with a saturated solution of KCl, the EtOAc extract was dried over anhydrous Na_2SO_4. The residue was dissolved in MeOH-EtOAc (1:10) and cooled to 4° to afford white round crystals, mp 222–224° (20 mg). The product was found to be identical to **4**, by mmp and spectral data (^{1}H and ^{13}C nmr).

ANTIVIRAL AND CYTOTOXICITY TESTING.—Because of the structural similarity to aphidicolin, which is a potent antiviral agent, the diterpenes were tested for HSV-1 activity according to the protocol previously published (6,8). The antiviral activities range from 30–200 mg/ml with a maximum of 77% plaque reduction for **4**.

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