

## 10-Demethoxystegane, a New Lignan from *Steganotaenia araliacea*

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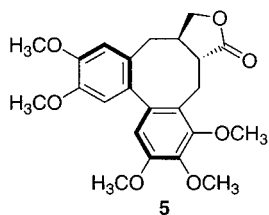
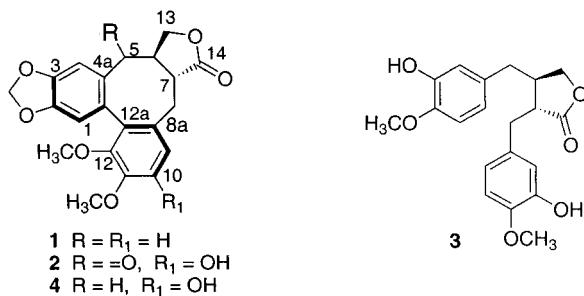
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A new dibenzocyclooctadiene lactone lignan, 10-demethoxystegane (**1**), together with the known compounds steganone (**2**) and prestegane B (**3**), have been isolated from the organic extract of *Steganotaenia araliacea* (Apiaceae). Steganone (**2**) showed antiproliferative activity against an ovarian cancer cell line (OVCAR-3).

*Steganotaenia* Hochst. (Apiaceae) is a genus of trees distributed from Ethiopia to South Africa.<sup>1</sup> Since the first report of antileukemic lactone lignans in *S. araliacea*,<sup>2</sup> several additional lignans<sup>3–11</sup> and saponins<sup>12,13</sup> have been described from this species.

The organic extract of *S. araliacea* showed a lignan-like profile of cytotoxic activity in the NCI 60-cell line screen.<sup>14</sup> The bioassay-guided purification of this extract led to a new dibenzocyclooctadiene lactone lignan, 10-demethoxystegane (**1**), which was isolated along with the known compounds steganone (**2**)<sup>2</sup> and prestegane B (**3**).<sup>3</sup>



The novel compound 10-demethoxystegane (**1**) was isolated as a white solid. Its molecular formula was established as C<sub>21</sub>H<sub>20</sub>O<sub>6</sub> by HRFABMS (*m/z* 368.1257, calcd 368.1260). The IR spectrum of compound **1** showed a strong absorption band at 1773 cm<sup>-1</sup>, indicating the presence of a lactone carbonyl. The <sup>1</sup>H NMR spectrum (Table 1) exhibited signals for four aromatic protons, two of which appeared as singlets and two which formed an *ortho* AB system ( $\delta$  6.53, d,  $J$  = 8.0 Hz and  $\delta$  6.82, d,  $J$  = 8.0 Hz). Additional signals included two doublets at  $\delta$  5.47 ( $J$  = 1.5 Hz) and 5.42 ( $J$  = 1.5 Hz) characteristic of a methylenedioxy group and two singlets assigned as methoxy groups ( $\delta$  3.41 and 3.59). The upfield region ( $\delta$  3.6–1.6) of the spectrum also contained eight signals, which appeared as six doublets of doublets, one doublet of doublets of doublets,

and one multiplet, which integrated for one proton each, and which accounted for all the protons present in the molecular formula.

The <sup>13</sup>C NMR spectrum (Table 1) contained signals for all 21 carbons. The signals included one lactone carbonyl, 12 aromatic carbons, one methylenedioxy, one oxygenated methylene, two methoxy groups, and four aliphatic carbons. Analysis of the proton coupling constants along with the COSY spectrum led to the assignment of all the aliphatic protons. HMBC correlations between some of the aromatic carbons and aliphatic protons (between C-4 and C-4a and H-5 $\alpha$  and H-5 $\beta$ , between C-8a and H-8 $\beta$ , and between C-9 and C-12a and H-8 $\alpha$  and H-8 $\beta$ ) indicated that the four aliphatic carbons linked the aromatic rings. These data suggested that compound **1** was a dibenzocyclooctadiene lactone bearing one methylenedioxy and two methoxy groups.

The position of the protons of the *ortho* AB system was determined to be at C-9 and C-10 by the HMBC correlations between C-8 ( $\delta$  31.9) and H-9 ( $\delta$  6.82). Furthermore, both methoxy groups (OCH<sub>3</sub>-11 and OCH<sub>3</sub>-12) were determined to be substituents of the same aromatic ring by HMBC correlations between C-12 ( $\delta$  147.4) and H-10 ( $\delta$  6.53) and one of the methoxy groups ( $\delta$  3.59), and between C-11 ( $\delta$  152.3) and H-9 ( $\delta$  6.82) and the other methoxy group ( $\delta$  3.41). The presence of two aromatic protons as singlets in the remaining ring indicated that the methylenedioxy group was present between C-2 and C-3. This was further supported by HMBC correlations between the protons of the methylenedioxy group and C-3, between H-1 and C-4a and C-12a, and between H-4 and C-2, C-3, C-5, and C-12b. The coupling constant between H-6 and H-7 ( $J$  = 13.5 Hz) indicated that the ring junction was *trans*.

The biaryl configuration was determined by analysis of the coupling constants H-5 $\alpha$ , H-6 and H-7, H-8 $\beta$  and comparison with literature.<sup>4–10</sup> In compounds with a "normal" biaryl configuration<sup>15</sup> in combination with a *trans*-lactone, such as stegane (**4**),<sup>4</sup> the H-5 $\alpha$ , H-6 and H-7, H-8 $\beta$  coupling constants range from 7.0 to 11.0 Hz.<sup>4–7</sup> However, in compounds with "iso" biaryl configuration<sup>15</sup> and *trans*-lactone, such as neoisostegane (**5**),<sup>8,9</sup> these values are equal to 0.0 Hz.<sup>4,8–10</sup> The coupling constants for H-5 $\alpha$ , H-6 and H-7, H-8 $\beta$  in the <sup>1</sup>H NMR spectrum of 10-demethoxystegane (**1**) were 7.5 and 9.5 Hz, respectively, indicating a "normal" biaryl configuration. NOE experiments (Table 1) and the very closely similarity of the <sup>13</sup>C NMR data of compound **1** with those published for the known lignan stegane (**4**)<sup>4</sup> suggested that the stereochemistry at C-6 and C-7 was 6*R*, 7*R* in **1**, as in stegane (**4**). Since the only difference between these two compounds is the absence of

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**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Assignments of 10-Demethoxystegane (**1**) at 500 and 125 MHz, Respectively, in Benzene- $d_6$ 

position	$^{13}\text{C}$ $\delta$ mult	$^1\text{H}$ $\delta$ mult ( $J$ in Hz)	HMBC	NOE
1	111.6 d	6.70 s		
2	147.5 s <sup>a,b</sup>		H-4	
3	146.6 s <sup>a</sup>		H-1, H-4, OCH <sub>2</sub> O	
4	110.7 d	6.41 s	H-5 $\alpha$ , H-5 $\beta$	H-5 $\beta$
4a	131.0 s		H-1, H-5 $\alpha$ , H-5 $\beta$	
5 $\alpha$	33.9 t	2.47 dd (15.0,7.5)	H-4	H-5 $\beta$ , H-6, H-13 $\alpha$
$\beta$		1.61 dd (15.0,7.0)		H-4, H-5 $\alpha$ , H-6, H-13 $\beta$
6	39.7 d	2.04 m	H-5 $\alpha$ , H-5 $\beta$ , H-8 $\alpha$	H-5 $\alpha$ , H-5 $\beta$ , H-13 $\alpha$
7	43.6 d	1.94 ddd (13.5,9.5,5.0)	H-5 $\alpha$ , H-8 $\alpha$ , H-8 $\beta$ , H-13 $\alpha$	H-8 $\beta$
8 $\alpha$	31.9 t	3.13 dd (14.5,5.0)	H-9	
$\beta$		2.90 dd (14.5,9.5)		
8a	131.8 s		H-8 $\beta$ , H-10	
9	125.0 d	6.82 d (8.0)	H-8 $\alpha$ , H-8 $\beta$	H-8 $\alpha$
10	112.1 d	6.53 d (8.0)		
11	152.3 s		H-9, OCH <sub>3</sub> -11	
12	147.4 s <sup>b</sup>		H-10, OCH <sub>3</sub> -12	
12a	136.3 s		H-1, H-8 $\alpha$ , H-8 $\beta$ , H-9	
12b	130.7 s		H-4	
13 $\alpha$	70.5 t	3.51 dd (15.5,9.0)	H-5 $\alpha$ , H-5 $\beta$	
$\beta$		3.00 dd (10.5,9.0)		
14	177.6 s		H-8 $\alpha$ , H-8 $\beta$ , H-13 $\alpha$	
OCH <sub>2</sub> O	101.1 t	5.47 d (1.5) 5.42 d (1.5)		
11-OCH <sub>3</sub>	55.3 q	3.41 s		
12-OCH <sub>3</sub>	60.1 q	3.59 s		

<sup>a,b</sup> Assignments bearing the same superscript may be interchanged.

the methoxy group at C-10 in compound **1**, we have named it 10-demethoxystegane.

All the isolated compounds were tested in triplicate against ovarian (OVCAR-3) and colon (COLO-205) cancer cells.<sup>16</sup> Only steganone (**2**) showed antiproliferative activity against OVCAR-3 ( $\text{IC}_{50} = 2.3 \pm 0.5 \mu\text{g/mL}$ ) and moderate growth-inhibition against COLO-205 ( $\text{IC}_{50} = 9.2 \pm 1.7 \mu\text{g/mL}$ ).

### Experimental Section

**General Experimental Procedures.** Optical rotations were measured using a Perkin-Elmer 241 polarimeter. NMR spectra were performed on a Varian Inova I Unity 500 spectrometer in  $\text{CDCl}_3$  and benzene- $d_6$ . Mass spectra were obtained with a JEOL SX102 mass spectrometer. UV spectra were obtained on a Beckman DU 640 spectrophotometer, and IR spectra on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. HPLC separations were performed on a Rainin System using a C-18 column (Dynamax, 1 cm  $\times$  25 cm, 50:50 or gradient 55:45 to 70:30 MeOH– $\text{H}_2\text{O}$ , flow rate 1.0 mL/min, UV detection at 225 nm).

**Material.** *S. araliacea* Hochst. was collected in the Tanga Region, Handeni District, Kwachaga, Tanzania, in September 1989, by Peter Mambo under contract with the National Cancer Institute. A voucher specimen has been deposited at the Missouri Botanical Garden. The plant taxonomy was identified as *Steganotaenia araliacea* by Rogasian Mahunnah (Institute of Traditional Medicine, Dar es Salaam) and Boniface Muhoro (Dar es Salaam University).

**Extraction and Isolation.** A portion (1.03 g) of organic extract of *S. araliacea* was subjected to solvent–solvent partitioning.<sup>17</sup> The cytotoxic activity was present in the MeOtBu fraction. It was subjected to two successive gel permeation columns of Sephadex LH-20 ( $\text{CH}_2\text{Cl}_2$ –MeOH, 1:1, followed by hexane– $\text{CH}_2\text{Cl}_2$ –MeOH, 2:5:1) to give nine fractions (A–I). Fraction A was purified by HPLC [Dynamax C-18, MeOH– $\text{H}_2\text{O}$  (gradient 55:45 to 70:30)] to afford 10-demethoxystegane (**1**) (0.8 mg, 0.08% of extract) and steganone (**2**) (4.0 mg, 0.40% of extract). Fraction E was purified by the reversed-phase HPLC system using MeOH– $\text{H}_2\text{O}$  (1:1) as the solvent system to afford prestegane B (**3**)<sup>3</sup> (2.2 mg, 0.22% of extract).

**10-Demethoxystegane (1):** white solid;  $[\alpha]_{\text{D}}^{25} -67.5^\circ$  ( $c$  0.04, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 251.5 (3.50), 286.5 (3.58)

nm; IR (NaCl)  $\nu_{\text{max}}$  2923, 2853, 1773, 1632, 1612, 1561, 1485, 1458, 1221  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR in benzene- $d_6$ , see Table 1; HRFABMS (magic-bullet matrix)  $m/z$  368.1257, calcd for  $\text{C}_{21}\text{H}_{20}\text{O}_6$ , 368.1260; FABMS (magic-bullet matrix)  $m/z$  369  $[\text{M} + \text{H}]^+$  (17), 368  $[\text{M}]^+$  (10), 309 (62), 251 (45).

**Steganone (2):**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  195.2 (s, C-5), 175.9 (s, C-14), 154.0 (s, C-10), 151.8 (s, C-12), 151.4 (s, C-3)\*, 147.9 (s, C-2)\*, 141.3 (s, C-11), 133.4 (s, C-12b), 132.1 (s, C-8a), 131.5 (s, C-4a), 126.7 (s, C-12a), 112.6 (d, C-1), 108.6 (d, C-4), 107.9 (d, C-9), 102.2 (t,  $-\text{OCH}_2\text{O}-$ ), 66.9 (t, C-13), 61.1 (q, OCH<sub>3</sub>-12), 61.0 (q, OCH<sub>3</sub>-11), 56.1 (q, OCH<sub>3</sub>-10), 49.8 (d, C-6), 44.7 (d, C-7), 30.2 (t, C-8) (\* assignments may be interchanged). Other spectral data consistent with published values.<sup>2</sup>

**Prestegane B (3):**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  178.6 (s, C-9'), 145.7 (s, C-3 and C-3'), 145.6 (s, C-4'), 145.4 (s, C-4), 131.3 (s, C-1), 130.9 (s, C-1'), 120.7 (d, C-6'), 120.1 (d, C-6), 115.4 (d, C-2'), 114.7 (d, C-2), 110.8 (d, C-5')\*, 110.7 (d, C-5)\*, 71.2 (t, C-9), 56.0 (q, OCH<sub>3</sub>-4')<sup>†</sup>, 55.9 (q, OCH<sub>3</sub>-4)<sup>†</sup>, 46.4 (d, C-8'), 41.4 (d, C-8), 38.0 (t, C-7), 34.6 (t, C-7) (\*,† assignments bearing the same superscript may be interchanged). Other spectral data consistent with published values.<sup>3</sup>

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