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NOVEL ANTIMITOTIC DIBENZOCYCLO-OCTADIENE LIGNAN CONSTITUENTS OF THE STEM BARK OF STEGANOTAENIA ARALIACEA

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ABSTRACT.—By means of activity-directed chromatographic fractionation using cultured astrocytoma (ASK) cells, six dibenzocyclo-octadiene lignans were isolated from *Steganotaenia araliacea* stem bark. In addition to the most abundant analogue, steganangin [1], two other known compounds, steganacin [3] and steganolide A [6], and three new compounds, episteganangin [2], steganoate A [4], and steganoate B [5], were obtained. Episteganangin [2] was chemically correlated with the known ketone steganone [7]. All of these compounds demonstrated cytotoxic activity when tested against a panel of eleven human tumor cell lines, with the exception of steganoate A [4]. The magnitude of this activity tended to correlate with antimitotic activity observed with the ASK assay and in vitro inhibition of microtubule assembly. Steganacin [3] was less cytotoxic than colchicine, but more active in these latter two assay systems.

As part of a search for naturally occurring antineoplastic agents, we have investigated an EtOAc-soluble extract of the stem bark of *Steganotaenia araliacea* Hochst. (Umbelliferae), which showed significant astrocyte reversal activity when tested against an astrocytoma (ASK) cell line. This plant has been studied previously by a number of research groups for its biologically active dibenzocyclo-octadiene lactone lignans and their analogues (1–8). Steganangin [1] and steganancin [3] were first isolated by Kupchan and coworkers as constituents of *S. araliacea* stem bark and stem wood, and found to exhibit significant antileukemic activity in the in vivo murine P-388 lymphocytic leukemia test system (1). The absolute stereochemistry of the dibenzocyclo-octadiene lignans of *S. araliacea* was assigned after the synthesis of (−)-steganone [7] was completed, starting from L-glutamic acid via 3R-(3,4,5-trimethoxybenzyl)-4-butanolide (7). Compounds **1** and **3** and their bioactive analogues are known to resemble colchicine and podophyllotoxin in biological action by binding to tubulin and preventing spindle formation and cell division (9–12). In the present study, we report three novel dibenzocyclo-octadiene lignans from *S. araliacea*, namely, episteganangin [2], steganoate A [4], and steganoate B [5]. These compounds, along with several known lignans from the same plant source, have been evaluated against a panel of human cancer cell lines. In addition, their potential to mediate antimitotic activity, as judged by the ASK assay, was assessed, as was their ability to inhibit in vitro microtubule assembly.

RESULTS AND DISCUSSION

In the present study, utilizing astrocytoma cells to monitor chromatographic fractionation, steganangin [1] was isolated as the major bioactive lignan constituent of *S. araliacea* stem bark, and its identity was established by comparison with reported spectral and physical data (1,2). The novel compound **2** showed spectral data similar to those of **1** and was assigned a molecular formula of $C_{27}H_{28}O_9$ on the basis of hreims. The ^{13}C-nmr spectroscopic data of **2** were almost identical to those of **1**, suggesting that the two compounds are based on the same carbon skeleton. All carbon assignments for **2** were assigned unambiguously by performing appropriate ^1H-^{13}C-HETCOR and selective

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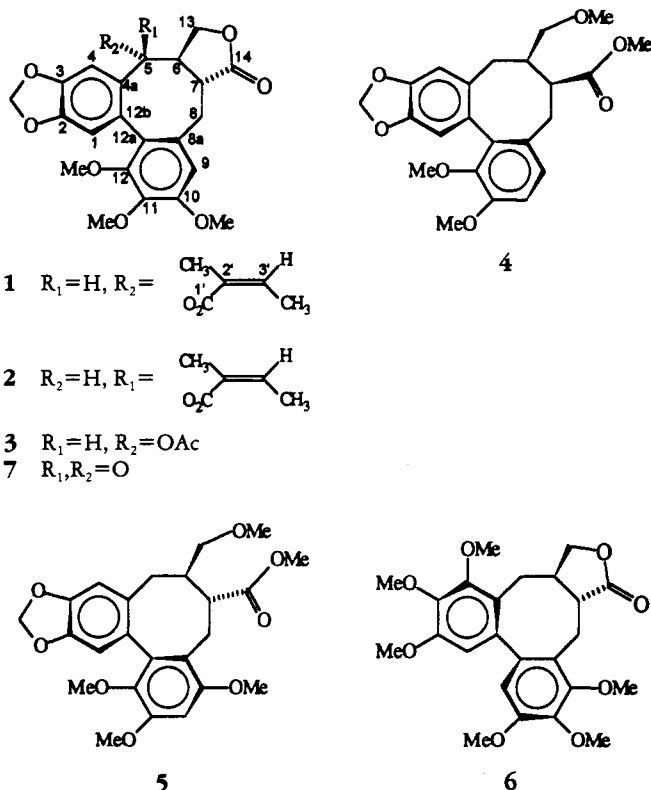
INEPT nmr experiments (13). In the latter context, soft irradiation ($^3J_{CH}=8$ Hz) of H-1 (δ 6.76), H-4 (δ 6.80), and H-9 (δ 6.51) led, in turn, to the enhancement of the C-2, C-3, C-4a, and C-12b; C-2, C-3, and C-5; and C-8, C-10, C-11, and C-12a resonances. Analogous irradiation of H-5 (δ 5.96) and H-7 (δ 2.37) enhanced, respectively, the resonances of C-1', C-4a, C-6, C-12b, and C-13; and C-5, C-8, and C-13.

It is known that lignans of the steganangin type undergo thermal atropisomerization (14). However, steganangin [1], on treatment with MeOH or on heating, was not transformed to compound 2, thus ruling out the possibility of 2 being an atropisomer of 1. The cd spectra of 1 and 2 were almost identical, with both having absorption minima at about 240 nm; this is consistent with 2 possessing an S configuration of the biphenyl group (8). The ^{13}C-nmr data for this compound were closely comparable to those reported for the known 5β-acylated dibenzocyclo-octadiene lignans, araliangin and episteganacin, and the ^{1}H-nmr values of 2 were similar to those of episteganacin (6). Accordingly, it may be proposed that compound 2 is an epimer of steganangin [1]. That the two compounds were epimeric at the C-5 stereocenter was confirmed by analysis of the NOESY nmr spectrum of 2, in which cross peaks were observed between H-5α and H-6α, H-9 and 10-Me, H-5α and 12-Me, and H-8β and H-9. In a 1D nOe nmr experiment performed on 2, irradiation of H-5α gave a 15% enhancement of H-6α, thereby confirming the vicinal cis relationship of those two protons (6). Compounds 1 and 2 were interconverted to a common product, the known compound (−)-steganone [7], using standard reduction and oxidation methods (1). The stereochemistry of episteganangin [2] at its three chiral centers was therefore assigned as 5S, 6R, and 7R.

Two other novel S. araliacea constituents obtained, 4 and 5, showed several close similarities in their overall spectral properties. No ir absorption band was observed for either 4 or 5 at 1775 cm^{-1}, thereby eliminating the presence of a five-membered lactone

ring in each case. In the ^{1}H-nmr spectrum of **5**, the proton resonances in the aliphatic region were more highly resolved than was the case for compound **4**, although both compounds possessed the same total number of aliphatic protons. The cd spectra of compounds **4** and **5** exhibited a negative absorption band at about 237 nm, which, by comparison with literature values, indicated that the biphenyl ring configurations were S (8,14). Interpretation of the spectral data of **4** and **5** showed in both cases that -CH$_2$OMe and -COOMe groups were attached, in turn, to C-6 and C-7. Unambiguous assignments of the ^{13}C-nmr spectral data for **4** and **5** were achieved by analysis of their ^{1}H-^{13}C-HETCOR and selective INEPT nmr spectra. In selective INEPT nmr experiments on **4**, soft irradiation ($^3J_{CH}$=8 Hz) of H-1 (δ 6.71), H-4 (δ 6.80), H-9 (δ 6.89), and H-10 (δ 6.83) led to the enhancement, in turn, of the C-3, C-4a, and C-12b; C-2 and C-5; C-8, C-8a, C-10, C-11, and C-12a; and C-8a, C-9, C-11, and C-12 resonances. In addition, irradiation ($^3J_{CH}$=6 Hz) of H-6 (δ 2.54) and H-7 (δ 2.97) enhanced the C-4a, C-5, C-8, and C-13; and C-5, C-8, C-8a, C-13, and C-14 resonances, respectively. Irradiation of H-1 (δ 6.75), H-4 (δ 6.83), and H-10 (δ 6.45) was carried out in a similar manner for compound **5** and led to carbon enhancements analogous to those observed for compound **4**. ^{1}H-Nmr coupling constants for **4** and **5** were measured by performing 1D double-irradiation experiments for each proton. Thus, in the ^{1}H-nmr spectrum of compound **5**, the H-5α and H-5β signals were well resolved and individual multiplicities were observed as a double doublet (δ 2.70) and a doublet (δ 3.05), respectively, whereas in compound **4**, the H-5α and H-5β signals overlapped (m, δ 2.77). The H-8β proton of both isolates appeared as a doublet of doublets, but the H-8α proton of compound **5** was a double doublet (δ 2.19) while that of **4** was a broad doublet (δ 2.96), suggesting a difference in stereochemistry at the C-7 position. The stereochemistry at the C-6 and C-7 positions was determined by performing NOESY experiments for both compounds. Compound **5**, which has been named steganoate B, showed in its NOESY nmr spectrum correlations between H-5β and H-4, H-13 and H-4, and H-13 and H-8β, thereby confirming the β-axial orientation of the -CH$_2$OMe group and the twist-boat-chair conformation of the cyclo-octadiene ring (14). A further nOe correlation between H-5β and H-7 confirmed that the -COOMe group was α-equatorial and gave additional further evidence for the structure. Given the aforementioned data, the configurations at these chiral centers were assigned at $6R$ and $7R$ for steganoate B [**5**]. In the case of steganoate A [**4**], NOESY nmr correlations were observed between protons H-5α and H-7, H-5β and H-4, and H-8β and H-9, thus confirming the β orientation of the -COOMe group, as well as the $6R$ and $7S$ configurations of this isolate. It may be pointed out that compounds **4** and **5** are not artifacts of extraction with MeOH, since both compounds were extracted from *S. araliacea* stem bark using CHCl$_3$ in place of MeOH.

Spectroscopic investigation of the two remaining lignans that were isolated in this investigation permitted their identification as the known compounds steganacin [**3**] and steganolide A [**6**]. Steganacin [**3**] is a known constituent of *S. araliacea* stems (1) and has a well-documented profile of biological activity (1,10,11). Unlike compounds **1–5**, steganolide A [**6**] was assigned the R configuration in relation to its biphenyl group, on the basis of ^{1}H-nmr data (5). For our isolate, the cd spectrum showed a positive absorption maximum at about 240 nm (15), thereby offering additional support for an R biaryl configuration of steganolide A [**6**].

As summarized in Table 1, compounds **1**, **3**, and **7** demonstrated, in the ASK assay system, dose-dependent activity that was more potent than that of colchicine. Less active were compounds **2** and **4–6**. In order to confirm the mechanism of this activity, in vitro studies were performed to investigate the potential of these compounds to inhibit

TABLE 1. Reversal of ASK Cell Morphology and Depolymerization of Tubulin Mediated by Isolates Obtained from *Steganotaenia araliacea* and Colchicine.

Compound	ASK Activity[a] (µg/ml)								ED_{50}[b]	(r^2)[c]
	100.0	20.0	4.0	0.8	0.16	0.032	0.0064	0.00128		
1	100	100	100	100	50	0	0	NT	38.1	0.996
2	100	0	0	0	NT[d]	NT	NT	NT	78.1	1.000
3	100	100	100	100	100	0	NT	NT	1.7	0.979
4	40	0	0	0	NT	NT	NT	NT	76.9	0.944
5	100	100	0	NT	NT	NT	NT	NT	59.3	1.00
6	100	100	0	0	0	0	NT	NT	NT	NT
7	100	100	100	100	0	0	0	NT	68.2	0.983
Colchicine	100	100	100	70	0	0	0	0	7.4	0.997

[a]The values given corresponding to the % reversal in the ASK assay.
[b]ED_{50}=concentration required to inhibit the tubulin polymerization reaction by 50% (µg/ml).
[c]Regression coefficient obtained when data from the tubulin polymerization assay were analyzed by linear regression.
[d]NT=not tested.

microtubule assembly. Typical data are illustrated in Figure 1, in which inhibition of the polymerization reaction is demonstrated with compound **3**. The inhibition reaction is dose-dependent and can be analyzed by linear regression to yield ED_{50} values. These are summarized in Table 1 for compounds **1–5, 7**, and colchicine. There is a general, but imprecise, correlation with activity in the ASK test system: e.g., compound **3** is the most active in both systems and compounds **2** and **4** are the least active in both systems.

In addition, lignans **1–7** were evaluated against a panel of eleven human cell lines. As summarized in Table 2, steganangin [**1**], steganacin [**3**], and steganone [**7**] were
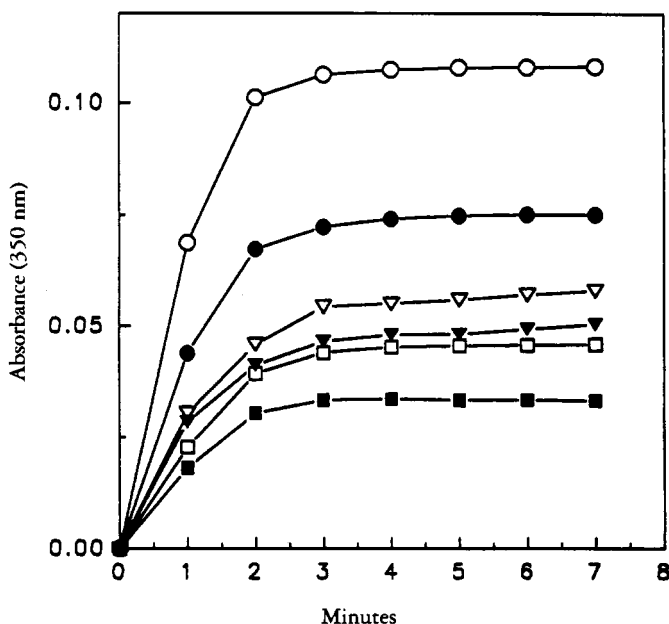

FIGURE 1. Evaluation of the effect of steganacin [**3**] on in vitro microtubule assembly by turbidimetric analysis at 37°. Each reaction mixture contained the following concentrations of **3** (µg/ml): 0 (○); 1.25 (●); 1.50 (▽); 1.75 (▼); 2.0 (□); 2.50 (■). For additional details, see the Experimental section.

TABLE 2.   Cytotoxic Activity of Isolates Obtained from *Steganotaenia araliacea* and Colchicine.[a]

Compound	Cell line[b]										
	A431	BC1	Col2	HT	KB	KB-V1	LNCaP	Lu1	Mel2	U373	ZR75-1
1	>20	0.7	0.6	0.6	0.4	0.7	0.9	0.4	0.7	>20	1.0
2	>20	1.1	>20	7.4	7.4	0.9	>20	6.9	20	>20	13.9
3	0.2	0.6	0.4	0.7	0.1	0.2	0.9	0.4	>20	0.1	5.0
4	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20
5	6.7	17.3	9.9	11.5	9.2	0.8	>20	5.5	14.9	7.8	4.7
6	5.5	8.0	5.1	6.5	4.9	0.8	9.0	5.5	9.2	3.1	8.9
7	>20	1.8	2.1	0.7	1.5	7.1	2.0	1.0	>20	>20	>20
Colchicine ...	0.002	0.008	0.001	0.004	0.02	3.5	0.06	0.02	0.007	0.005	0.1

[a]Results are expressed as ED_{50} values (μg/ml).
[b]Key: A431=human epidermoid carcinoma; BC1=human breast cancer; Col2=human colon cancer; HT=human fibrosarcoma; KB=human oral epidermoid carcinoma; KB-V1=drug-resistant KB; LNCaP=human prostate cancer; Lu1=human lung cancer; Mel2=human melanoma; U373=human glioma; ZR75-1=hormone-dependent human breast cancer.

generally cytotoxic, yielding ED_{50} values of <4 µg/ml in several culture systems. The relative intensities of these activites correspond with those in Table 1. The new compound, episteganangin [2], was markedly less active in these assays when compared with the parent compound 1 or compound 3. The two additional new compounds, steganoate A [4] and steganoate B [5], showed clearly that biological activity for the *S. araliacea* lignans is dependent on the presence of a 7*R*-substituted functionality, since 4 was completely inactive in all systems tested. Compound 5 was significantly less active, however, than compounds 1–3 and 7, all of which have an intact butanolide lactone ring. Finally, steganolide A [6], with an *R* biaryl configuration, was less active than steganangin [1] and steganacin [3], which both possess the *S* biaryl configuration.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Cd spectra were recorded using a Jasco-600 CD polarimeter. Uv spectra were obtained on a Beckman DU-7 spectrometer. Ir spectra were taken with a Midac Collegian FT-IR spectrophotometer. ^{1}H-Nmr and ^{13}C-nmr spectra were measured with TMS as internal standard, employing a Varian XL-300 instrument operating at 300 MHz and 75.6 MHz, respectively. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR nmr experiments were also performed on the Varian XL-300, using standard Varian pulse sequences. Selective INEPT nmr experiments were conducted on a Nicolet NT-360 spectrometer, operating at 90.8 MHz. Eims, cims, and hreims were obtained using a Finnigan MAT 90 instrument. Preparative tlc was performed on Merck Si gel G plates (0.5-mm layer thickness).

PLANT MATERIAL.—The stem bark of *S. araliacea* was collected in Mazowe, Zimbabwe, in October 1991, and identified by one of us (T.E.C.). A voucher specimen (V00174) has been deposited at the Field Museum of Natural History, Chicago, Illinois.

EXTRACTION PROCEDURE.—The air-dried, milled stem bark (1.7 kg) was extracted twice overnight with MeOH (10×2 liters). The resultant extracts were combined, concentrated, and diluted with H_2O to afford an aqueous MeOH solution (1:9) (1.5 liters), which was washed with petroleum ether (3×1 liter). The MeOH layer was concentrated to dryness and partitioned three times between EtOAc (3×1 liter) and H_2O (3×1 liter). The combined EtOAc layers were dried ($MgSO_4$), filtered, and concentrated to give an extract (28 g), which exhibited 100% conversion of ASK cells (16,17) at 4 µg/ml.

ISOLATION AND CHARACTERIZATION OF ISOLATES.—A portion (27 g) of the EtOAc extract was adsorbed onto Si gel (80 g) and separated over additional Si gel (120 g) by vlc, using toluene/EtOAc gradient mixtures. Nine fractions were collected and tested against the ASK cell line (astrocyte reversal). The second fraction (1.2 g), eluted with toluene-EtOAc (3:2), showed the highest activity and was further purified by Si gel cc, eluting with toluene-EtOAc (9:1), and then by flash chromatography using hexane-Me_2CO (7:3) as eluent, to afford steganangin [1] (560 mg, 0.03% w/w) and episteganangin [2] (280 mg, 0.012% w/w). The third fraction (980 mg), obtained through vlc by elution with toluene-EtOAc (3:2), was purified with Si gel chromatography using this same solvent mixture, followed by Al_2O_3 chromatography with toluene-Et_2O (9:1) as solvent, and then separated on a column containing Sephadex LH-20 using MeOH-$CHCl_3$ (1:1), to afford steganoate A [4] (160 mg, 0.0085% w/w). A fraction obtained from vlc by elution with toluene-EtOAc (1:1) showed the presence of two more lignans, which were purified by sequential separation over Al_2O_3, with toluene-Et_2O (9:1) as solvent, then Sephadex LH-20 using MeOH-$CHCl_3$ (1:1) for elution, followed by preparative tlc on Si gel in toluene-EtOAc (4:1), and finally preparative tlc on Al_2O_3 in pretroleum ether-EtOAc (4:1). The two compounds purified in this manner were identified as steganacin [3] (15 mg, 0.0008% w/w) and steganolide A [6] (12 mg, 0.0007% w/w). A final lignan, steganoate B [5] (65 mg, 0.003% w/w), was isolated from a vlc fraction eluted with toluene-EtOAc (2:3), using preparative tlc with toluene-EtOAc (3:2) as solvent (eluted three times).

Steganangin [1].—Colorless needles from Et_2O/petroleum ether: mp 138°; $[\alpha]_D$ −122.6° (*c*=1.02, $CHCl_3$) [lit. (1), mp 142°, $[\alpha]_D$ −113°; lit. (2), mp 156–158°, $[\alpha]_D$ −103°]; cd Δε (MeCN) (nm) +42.9 (208), −31.9 (239). This isolate was identified as steganangin, a known constituent of *S. araliacea* stems, on the basis of spectral data comparison (uv, ir, ^{1}H nmr, ^{13}C nmr and ms) with reported values (1,2,6).

Episteganangin [2].—Amorphous solid: $[\alpha]_D$ −143.6° (*c*=0.66, $CHCl_3$); uv λ max (MeOH) (log ε) 215 (4.23), 255 (3.51), 291 (3.28) nm; ir ν max (KBr) 2941, 1774, 1722, 1595, 1485, 800 cm^{-1}; ^{1}H nmr (CDCl$_3$) δ 6.80 (1H, s, H-4), 6.76 (1H, s, H-1), 6.51 (1H, s, H-9), 6.16 (1H, q, *J*=6.1 Hz, H-3'), 6.05 (1H, d, *J*=1.3 Hz, -OCH$_2$O-), 6.03 (1H, d, *J*=1.3 Hz, -OCH$_2$O-), 5.96 (1H, d, *J*=8.3 Hz, H-5), 4.24 (1H, dd, *J*=9.0 and 7.8 Hz, H-13α), 4.05 (1H, dd, *J*=11.3 and 9.0 Hz, H-13β), 3.90 (3H, s, 11-OMe), 3.88 (3H, s, 10-

OMe), 3.59 (3H, s, 12-OMe), 3.28 (1H, dd, *J*=16.3 and 7.5 Hz, H-8α), 3.09 (1H, dddd, *J*=13.3, 11.3, 8.3, and 7.8 Hz, H-6), 2.70 (1H, dd, *J*=16.3 and 11.0 Hz, H-8β), 2.37 (1H, dd, *J*=13.3, 11.0 and 7.4 Hz, H-7), 2.00 (6H, m, 2′-Me and 3′-Me); ^{13}C nmr (CDCl$_3$) δ 109.8 (C-1), 147.7 (C-2), 146.9 (C-3), 112.0 (C-4), 129.4 (C-4a), 70.6 (C-5), 45.2 (C-6), 43.1 (C-7), 33.9 (C-8), 133.0 (C-8a), 104.2 (C-9), 152.8 (C-10), 141.5 (C-11), 151.6 (C-12), 125.3 (C-12a), 127.9 (C-12b) 65.7 (C-13), 177.5 (C-14), 168.8 (C-1′), 126.7 (C-2′), 140.5 (C-3′), 15.9 (C-4′), 20.6 (C-5′), 101.4 (O-CH$_2$-O), 55.9 (12-OMe), 60.9 (11-OMe), 60.8 (10-OMe); eims *m/z* (rel. int.) [M]$^-$ 496 (100), 396 (11), 312 (14), 351 (3), 300 (6), 83 (30); hreims *m/z* [M]$^+$ 496.1727 (calcd for C$_{27}$H$_{28}$O$_9$, 496.1733); cd Δε (MeCN) (nm) +10.8 (211), −20.1 (239), −5.98 (293).

Steganacin [**3**].—Amorphous solid: [α]D −120° (*c*=0.66, CHCl$_3$); [lit. (1) [α]D −114°; lit. (2) [α]D −127°]. The compound was identified as steganacin, a known constituent of *S. araliacea* stems, on the basis of spectral data comparison (uv, ir, ^{1}H nmr, ^{13}C nmr, and ms) with reported values (1,2,6).

Steganoate A [**4**].—Colorless cubes from Et$_2$O: mp 157–158°; [α]D −63.5° (*c*=0.90, CHCl$_3$); uv λ max (MeOH) (log ε) 218 (4.37), 256 (3.86), 286 (3.56) nm; ir ν max (KBr) 2940, 1721, 1595, 1485, 1039, 800 cm^{-1}; ^{1}H nmr (CDCl$_3$) δ 6.89 (1H, d, *J*=10.2 Hz, H-9), 6.83 (1H, d, *J*=10.2 Hz, H-10), 6.80 (1H, s, H-4), 6.71 (1H, s, H-1), 6.01 (2H, dd, *J*=6.3 Hz, -OCH$_2$O-), 3.89 (3H, s, 11-OMe), 3.69 (3H, s, 14-OMe), 3.43 (3H, s, 12-OMe), 3.37 (3H, s, 13-OMe), 3.45 (1H, dd, *J*=9.2 and 7.2 Hz, H-13), 3.03 (1H, dd, *J*=9.2 and 8.7 Hz, H-13), 2.97 (1H, d, *J*=9.5 Hz, H-7), 2.96 (1H, bd, *J*=10.4 Hz, H-8α), 2.77 (2H, m, H-5α and -5β), 2.54 (1H, m, H-6), 2.46 (1H, dd, *J*=10.4 and 9.5 Hz, H-8β); ^{13}C nmr (CDCl$_3$) δ 110.0 (C-1), 145.2 (C-2), 145.3 (C-3), 110.3 (C-4), 130.7 (C-4a), 29.3 (C-5), 34.9 (C-6), 43.1 (C-7), 30.8 (C-8), 129.2 (C-8a), 124.7 (C-9), 110.7 (C-10), 151.3 (C-11), 146.6 (C-12), 130.7 (C-12a), 135.0 (C-12b), 73.8 (C-13), 174.7 (C-14), 100.8 (-OCH$_2$O-), 55.6 (11-OMe), 60.0 (12-OMe), 59.1 (13-OMe), 50.9 (14-OMe); eims *m/z* (rel. int.) [M]$^+$ 414 (100), 383 (7), 291 (4), 270 (7), 239 (8), 165 (3); hreims *m/z* [M]$^-$ 414.1670 (calcd for C$_{23}$H$_{26}$O$_7$, 414.1679); cd Δε (MeCN) (nm) +36.7 (216), −11.3 (236), −18.8 (257).

Steganoate B [**5**].—Colorless needles from Et$_2$O/petroleum ether: mp 122–123°, [α]D −41.6° (*c*=0.16, CHCl$_3$); uv λ max (MeOH) (log ε) 222 (4.41), 259 (3.76), 290 (3.91) nm; ir ν max (KBr) 2940, 1721, 1595, 1485, 1039, 800 cm^{-1}; ^{1}H nmr (CDCl$_3$) δ 6.83 (1H, s, H-4), 6.75 (1H, s H-1), 6.45 (1H, s, H-10), 6.01 (2H, *J*=6.3 Hz, -OCH$_2$O-), 3.90 (3H, s, 11-OMe), 3.78 (3H, s, 9-OMe), 3.61 (3H, s, 14-OMe), 3.35 (3H, s, 12-OMe), 3.34 (3H, s, 13-OMe), 3.43 (1H, dd, *J*=9.0 and 7.6 Hz, *J*=9.0 and 7.6 Hz, H-13), 3.17 (1H, dd, *J*=14.0 and 6.5 Hz, H-8β), 3.05 (1H, d, *J*=14.2 Hz, H-5β), 3.01 (1H, dd, *J*=9.0 and 8.1 Hz, H-13), 2.79 (1H, bd, *J*=6.5 Hz, H-7), 2.70 (1H, dd, *J*=14.2 and 6.1 Hz, H-5α), 2.50 (1H, m, H-6), 2.19 (1H, dd, *J*=14.0 and 2.0 Hz, H-8α); ^{13}C nmr (CDCl$_3$) δ 110.6 (C-1), 145.2 (C-2), 146.6 (C-3), 110.1 (C-4), 129.4 (C-4a), 23.2 (C-5), 36.8 (C-6), 41.5 (C-7), 30.5 (C-8), 118.5 (C-8a), 153.8 (C-9), 95.1 (C-10), 151.4 (C-11), 139.5 (C-12), 135.3 (C-12a), 131.2 (C-12b), 74.2 (C-13), 175.7 (C-14), 100.8 (-OCH$_2$O-), 55.4 (9-OMe), 55.6 (11-OMe), 58.5 (12-OMe), 60.2 (13-OMe) 51.1 (14-OMe); eims *m/z* (rel. int.) [M]$^-$ 444 (100), 397 (3), 337 (7), 299 (20), 199 (4), 165 (4); hreims *m/z* [M]$^+$ 444.1777 (calcd for C$_{24}$H$_{28}$O$_8$, 444.1784); cd Δε (MeCN) (nm) +60.8 (205), −32.4 (227), −1.83 (239), −14.9 (257).

Steganolide A [**6**].—Amorphous solid [α]D +57.7° (*c*=0.17, CHCl$_3$), [lit. (5) [α]D +67.9°]; cd Δε (MeCN) (nm) −14.2 (205), +10.5 (233). The compound was identified as steganolide A, a known constituent of *S. araliacea*, by comparison with spectral data (uv, ir, ^{1}H nmr, and ms) reported previously (5,14).

REDUCTION OF STEGANANGIN [**1**] AND EPISTEGANANGIN [**2**] TO STEGANONE [**7**].—Compounds **1** and **2** (20 mg) were separately treated with LiAlH$_4$ (20 mg) in dry Et$_2$O (10 ml) for 5 h at 25°. At the completion of each reaction, the mixtures were filtered through a bed containing Celite and Na$_2$SO$_4$·8H$_2$O (1:1), to obtain the crude reduction products. These products were then treated with excess PCC in CH$_2$Cl$_2$ for 6 h. After workup and purification by preparative tlc [EtOAc-hexane (2:3)] the product **7** from each reaction (5 mg from **1**, 3 mg from **2**) showed identical physical (mp, [α]D) and spectral (uv, ir, ^{1}H nmr, ^{13}C nmr, and ms) properties, and was identified as (−)-steganone [**7**], which was crystallized from MeOH: mp 149–151°, [α]D −143.6° (*c*=0.66, CHCl$_3$), [lit. (1) mp 155–156°, [α]D −202° (*c*=0.76), lit. (8), mp 154–156°, [α]D −140°]. (−)-Steganone was previously isolated from *S. araliacea* stems (1).

CELL-BASED BIOLOGICAL EVALUATION PROCEDURES.—Test materials were evaluated for cell reversal against ASK cells (16), according to a published method (17). In additon, pure isolates **1–7** were tested against a panel of human cancer cell lines and murine P-388 cells, using established protocols (18).

TUBULIN ISOLATION AND POLYMERIZATION ASSAY.—Microtubules were purified by the method of Weingarten *et al.* (19). The superficial blood vessels and meninges were removed from fresh calf brain (Brown Packing Company, Chicago, IL), and the specimen was homogenized in purification buffer (0.1 mM MES buffer, pH 6.4; 1 mM EGTA; 0.5 mM MgCl$_2$; 1 mM β-mercaptoethanol; 0.1 mM EDTA; and 1 mM GTP; 0.75 ml/g of tissue) using a Polytron (0–4°). The homogenate was centrifuged (100,000×*g*, 1 h, 4°), and

the supernatant was removed, diluted with purification buffer containing 8 M glycerol (1:1), and warmed to 37° for 20 min to allow microtubule polymerization. This suspension was centrifuged (20 min, 30°) and the supernatant fraction was discarded. The pellets were resuspended in purification buffer (0.2 ml/g of brain), chilled on ice (60 min), and stored at −85°. This preparation was centrifuged (10 min, 4°) to remove debris and unwanted polymers of tubulin. The supernatant was diluted with purification buffer containing 8 M glycerol (1:1) and warmed to 37° for 20 min. This microtubule suspension was centrifuged (30 min, 30°), and the pellets (containing the purified microtubule protein) were suspended in cold purification buffer (supplemented with 8 M glycerol) and stored at −85°. Prior to each experiment, an aliquot of this sample was removed, diluted with reassembly buffer (0.1 mM MES buffer pH 6.4, 0.1 mM EGTA, 0.5 mM MgCl$_2$, and 1 mM GTP 1:1), warmed to 37° for 20 min, and centrifuged (45 min, 30°) for the recovery of microtubules. The effect of test compounds on the polymerization reaction was then determined by a turbidimetric procedure (20,21). Briefly, the purification buffer (without GTP) was warmed to 37°, 0.1 M GTP was added to a concentration of 1 mM, and tubulin suspension was added to yield a final protein concentration of 1 mg/ml. Part of the solution (1 ml) was then transferred to a cuvette and monitored at 350 nm; DMSO (12.5 μl) in reassembly buffer was used as a reference. The assembly of the microtubules was indicated by an increase in absorption. For the evaluation of test compounds, samples (dissolved in 12.5 μl DMSO) were added to both cuvettes prior to initiation of the polymerization reaction. Colchicine (Sigma Chemical Co., St. Louis, MO) was used as a control.

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