

NEOISOSTEGANE, A NEW BISBENZOCYCLOOCTADIENE LIGNAN
LACTONE FROM *STEGANOETAENIA ARALIACEA*¹

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ABSTRACT.—Neoisostegane, the first naturally occurring steganin without a functional group at C-5, was isolated from two different collections of *Steganotaenia araliacea*. The structure of neoisostegane, which contained five aromatic methoxyl groups rather than the more usual three methoxyls and one methylenedioxy moiety, was elucidated by comparison of the pmr coupling constants with those of synthetic steganins and by selective decoupling experiments. The structure was confirmed by cmr data, by preparation of a derivative having five aromatic methoxyl moieties from stegane, which was compared spectroscopically to neoisostegane, and by thermal isomerization experiments.

In the course of separate investigations of two different collections of *Steganotaenia araliacea* Hochst. (Apiaceae), one from the Republic of Guinea and one from Ethiopia, a new bisbenzocyclooctadiene lignan lactone has been isolated (1,2). Unlike the lignans of this type previously isolated by Kupchan and co-workers (3) from *S. araliacea*, neoisostegane (**1**) bears no functionality at C-5.²

Extracts of the two collections of *S. araliacea* were prepared according to previously established protocols (3,4). Preparative tlc of appropriate fractions derived as described in the Experimental section gave, in each case, pure neoisostegane (**1**) as a crystalline solid.³ High resolution ms established the molecular formula of **1** as C₂₃H₂₆O₇ (*m/z* 414.1671 [LSO], 414.1702 [VCU]; calcd 414.1679). The ir spectrum displayed a carbonyl at 1776 cm⁻¹ indicating the presence of a lactone. The pmr spectrum showed three one-proton singlets at δ 6.72, 6.69, and 6.52, three three-proton singlets at δ 3.96, 3.89, and 3.86, and one six-proton singlet at δ 3.94. These data suggested that neoisostegane (**1**) was a bisbenzocyclooctadiene lignan lactone bearing five aromatic methoxyl groups but no methylenedioxy moiety.

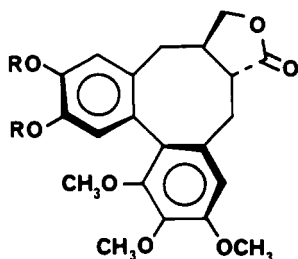
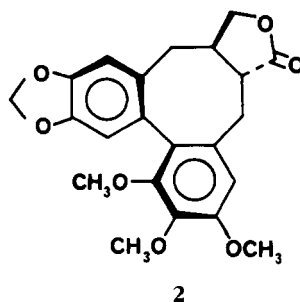
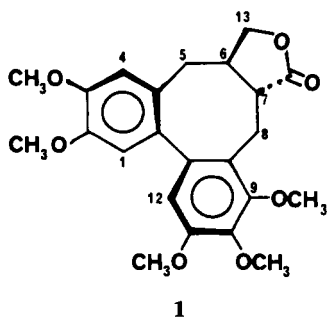
Comparisons of the vicinal ¹H-coupling constants for the aliphatic protons of **1** with those for isostegane (**2**) and stegane (**3**) (5) (Table 1), both of which had been synthesized previously (6,7), clearly indicated the stereochemistry of **1**. The coupling constant of 13.2 Hz between the 6-H and 7-H in the pmr spectrum of **1**, as is also found in the spectra of **2** and **3**, indicated that the lactone was *trans*-fused. A *cis*-lactone would exhibit a coupling constant of 8 Hz (8). In the spectrum of stegane (**3**), which has a "normal" biaryl configuration in combination with a *trans*-lactone, the 5 α ,6 and 7,8 β coupling constants were 7.2 Hz and 8.5 Hz, respectively. In the spectrum of isostegane (**2**), which has the "iso" biaryl configuration and *trans*-lactone, however, these values were both equal to 0.0 Hz (5,9).⁴ The 5 α ,6 and 7,8 β coupling constants in the spec-

¹In memory of Dr. S. Morris Kupchan, 1922-1976.

²Steganins without functionality at C-5 have been described previously only as synthetic compounds. For a compilation of references see: M. Mervic, Y. Ben David, and E. Ghera, *Tetrahedron Lett.*, **22**, 5091 (1981) and R.S. Ward, *Chem. Soc. Rev.*, **11**, 75 (1982).

³The Rfs of samples of neoisostegane (**1**) from both sources were identical upon analytical tlc in four different solvent systems, and the 500 MHz pmr spectra of the samples were superimposable.

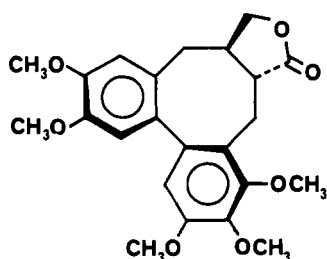
⁴This is in agreement with dihedral angles of 90° in **1** and **2**.



3 R = -CH₂-

4 R = CH₃

5 R = H



trum of **1** were also both equal to 0.0 Hz, thus showing that the stereochemistry of **1** and **2** were identical.

The chemical shifts of the three aromatic singlets in the pmr spectrum of **1** allowed the positions of four of the methoxyls to be assigned unambiguously to C-2, C-3, C-10, and C-11 on the isostegane skeleton. Irradiation of δ 2.68 (5α -H) led to enhancement and sharpening of the singlet at δ 6.69 (4-H, $J_{4,5} = 0.3$ Hz), thus eliminating the possibility of a methoxyl at C-4. Lack of a high field methoxyl [δ 3.57 in isostegane (**2**)] in

TABLE 1. Pmr Chemical Shifts and Coupling Constants for the Aliphatic Protons of **1**, **2**, and **3**^a

Assignment	Chemical Shift (δ)			Assignment	Coupling Constants (Hz)		
	1	2 ^b	3 ^b		1	2 ^b	3 ^b
5 α -H	2.68 d	2.64 d	2.92 dd	5 α -5 β	13.2	12.9	14.2
5 β -H	2.42 dd	2.44 dd	2.32 dd	5 α -6	0	0	7.2
6-H	2.23 m	2.16 m	2.44 m	5 β -6	10.0	9.0	5.9
7-H	2.04 dd	2.13 dd	2.37 m	6-13 α	7.2	6.5	7.2
8 α -H	1.93 dd	2.30 dd	2.84 m	6-13 β	11.4	10.6	10.2
8 β -H	3.69 d	3.12 d	2.96 m	6-7	13.2	13.2	13.2
13 α -H	4.37 dd	4.40 dd	4.30 dd	7-8 α	9.2	9.2	5.5
13 β -H	3.78 dd	3.78 dd	3.76 dd	7-8 β	0	0	8.5
				8 α -8 β	13.4	13.3	14.0
				13 α -13 β	8.4	8.4	8.6

^aSpectra were recorded at 400 MHz (**1**) or 250 MHz (**2**, **3**) in CDCl₃ with TMS as an internal standard.

^bValues for **2** and **3** are from Robin *et al.* (5).

the spectrum of **1** argued against placement of the methoxyl at C-1 or C-12.⁵ Irradiation at δ 3.69 (8 β -H) produced no variation in the aromatic region and indirectly substantiated this argument. Therefore, the remaining methoxyl was assigned to C-9. Examination of Dreiding models also showed that the 8 β -H is sterically compressed between the lactone carbonyl and the substituent at C-9. Therefore, a methoxyl at C-9 would explain the increased low-field shift of the 8 β -H at δ 3.69 in **1** vs. δ 3.12 in **2**.

Examination of the cmr data for **1**, **2**, and **3** (Table 2) resulted in similar conclusions. The resonances for C-6 and C-7 appeared at 40.1 and 43.2 ppm in the spectrum of stegane (**3**), while in the spectrum of isostegane (**2**) these resonances appeared at 47.1 and 50.1 ppm. In the spectrum of neoisostegane (**1**) the C-6 and C-7 resonances were located at 47.0 and 49.8 ppm, thus confirming that **1** had the same stereochemistry as isostegane (**2**). The resonance for C-8 in the spectrum of **1** appeared at 24.3 ppm, a significant upfield shift from the position of C-8 in the spectra of **2** and **3** (ca. 32.4 ppm). This implied that the environment of C-8 in neoisostegane (**1**) was significantly different from **2** and **3**. This was explained by the presence of a methoxyl substituent at C-9 rather than at C-12.

Support for the "iso" conformation around the biaryl bond in **1** was obtained by examination of the cd spectra of **1**, **2**, and **3**. It has been shown that the sign and inten-

TABLE 2. Cmr Data for Steganes **1**, **2**, **3**, and **4**

Assignment	Chemical Shift, ppm ^a			
	1	2	3	4
1	112.7 d ^b	108.8 d ^b	110.3 d ^b	113.9 d ^b
2	147.7 s ^c	obs. ^c	146.3 s ^c	obs.
3	150.7 s ^c	145.9 s ^c	147.0 s ^c	obs.
4	114.5 d ^b	111.9 d ^b	111.7 d ^b	115.4 d ^b
4a	131.2 s ^d	132.5 s ^d	130.7 s ^d	obs.
5	34.4 t	34.2 t ^e	32.5 t ^e	32.2 t ^e
6	47.0 d	47.1 d	40.1 d	39.6 d
7	49.8 d	50.1 d	43.2 d	44.0 d
8	24.3 t	32.3 t ^e	33.9 t ^e	34.7 t ^e
8a	126.8 s ^d	136.0 s ^{c,d}	133.6 s ^d	obs.
9	151.8 s ^c	107.5 d ^b	109.3 d ^b	obs.
10	132.8 s ^d	147.7 s ^c	151.6 s ^c	obs.
11	149.2 s ^c	128.4 s ^d	130.0 s ^d	obs.
12	110.1 d ^b	153.4 s ^c	152.8 s ^c	108.9 d ^b
12a	141.9 s ^c	126.5 s ^d	127.7 s ^d	obs.
12b	136.3 s ^c	obs. ^c	141.1 s ^c	obs.
13	70.0 t	70.1 t	71.0 t	71.5 t
OCH ₃	56.3 q ^f	56.1 q	56.1 q	56.2 q ^f
	60.8 q	60.9 q	60.7 q	60.9 q
	61.1 q	61.1 q	61.0 q	61.1 q
OCH ₂ O	—	101.2 t	101.2 t	—
C=O	176.0 s	176.5 s	178.1 s	obs.

^aSpectra were recorded at 22.5 MHz in CDCl₃ with TMS as an internal standard. Chemical shift assignments were based upon calculated values (17) and literature data for related lignans (18).

^{b-e}Assignments may be interchanged within the same column.

^fResonance represents more than one OCH₃ group.

obs. = Resonance was obscured by baseline noise.

⁵The high field methoxyl is usually attributed to the anisotropic shielding of the hydrogen situated above the plane of the other phenyl ring. See: A. Brossi, J.C. Brien, and S. Teitel, *Helv. Chim. Acta*, **52**, 678 (1969).

sity of the Cotton effect recorded in cd spectra of bridged biphenyls can be related to the conformation about the biaryl bond (10). Bridged biphenyls with the same conformation would be expected to exhibit Cotton effects of the same sign. The cd spectra of **1**, **2**, and **3** are illustrated in Figure 1. The cd spectrum of stegane (**3**) exhibited a negative first Cotton effect, relative to D-(+)-10-camphorsulfonic acid, at 248 nm. The positive second Cotton effect below 225 nm could not be resolved on our instrument. The cd spectrum of isostegane (**2**), on the other hand, exhibited an equal but positive first Cotton effect at 248 nm. The cd spectrum of neoisostegane (**1**) also exhibited a positive first Cotton effect but centered at 238 nm. This confirmed that **1** is also in the "iso" conformation. The blue shift in the λ max is most likely due to the different substitution pattern of the aromatic rings.

To confirm further the structure of neoisostegane (**1**), stegane (**3**) was converted

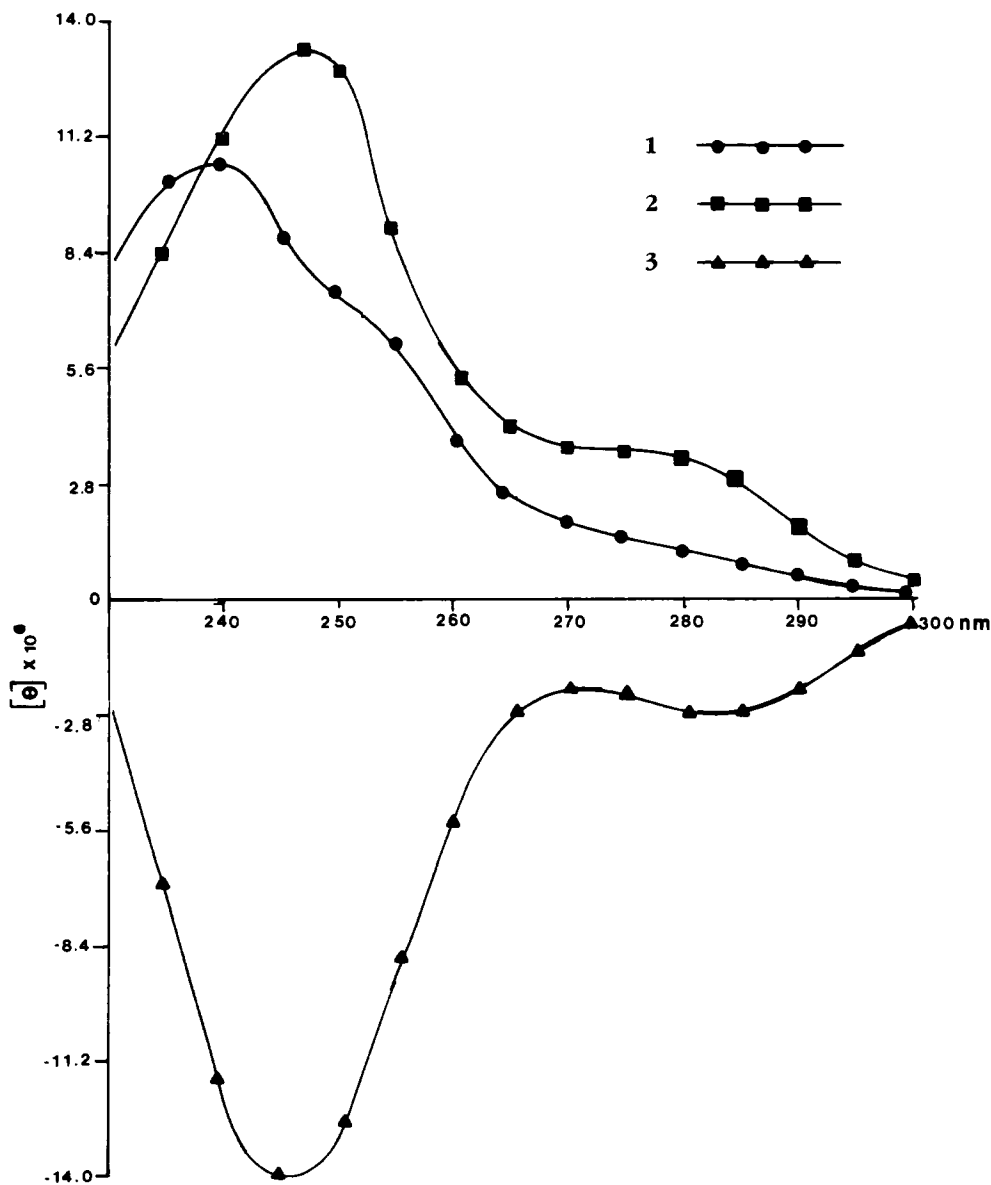


FIGURE 1. Circular dichroism spectra of neoisostegane (**1**), isostegane (**2**), and stegane (**3**).

into **4**. Stegane (**3**) prepared from steganacin by hydrogenolysis (7), was treated with a mixture of phenol, phosphoric acid, and HOAc to remove the methylenedioxy group (11). The resulting diphenol, **5**, was then treated with CH_2N_2 to prepare **4**. Comparison of the pmr and cmr spectra of **4** with those of **1** confirmed that neoisostegane (**1**) did not correspond to **4**. Thermal isomerization of **4** (12, 13) proceeded in poor yield but did not give any material corresponding to **1** by analytical tlc. To confirm these results, neoisostegane (**1**) was also subjected to thermal isomerization. The resulting neostegane (**6**) was chromatographically different from **4**, and the pmr spectrum of **6** was also significantly different from the spectrum of **4**. In particular, no high field methoxyl was observed.

The results described above, a summary of data obtained from both laboratories on two geographically different but botanically identical samples, served to establish the structure of neoisostegane as **1**. Neoisostegane (**1**) was found to be weakly cytotoxic against the KB cell culture ($\text{ED}_{50} = 6.6 \mu\text{g/ml}$) (14), thus confirming the necessity for a C-5 substituent for significant cytotoxicity in this class of lignans.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—For the isolation of neoisostegane [LSO]: mps were measured on a Reichert Melting Point Microscope apparatus and are uncorrected. Pmr spectra were recorded at 400 and 500 MHz in Fourier transform mode, on Bruker WM400 and WM500 instruments, respectively, at the University of Paris VI (France). 250 MHz ^1H -spectra were recorded in Continuous Wave mode on a Cameca 250 instrument at the University of Nantes (France). The ir spectra were obtained on a Perkin-Elmer model 297 in CH_2Cl_2 as solvent. Mass spectra were obtained at the Service Central de Microanalyse du CNRS (Lyon-Vernaison). Optical rotation was measured on a Jobin Yvon micropolarimeter.

For the isolation of neoisostegane [VCU]: all mps are uncorrected. Pmr and cmr spectra were recorded on a JEOL FX90Q or a Nicolet 360 MHz spectrometer (at the University of Virginia) in CDCl_3 or C_6D_6 with TMS as an internal standard. The ir spectra were measured on a Perkin-Elmer model 283 instrument and the uv spectra were measured on a Beckman Acta MVII recording spectrophotometer. Mass spectra were obtained at the University of Pennsylvania Mass Spectrometry Center. Silica gel 60 for column chromatography and prepared tlc plates were from EM labs.

PLANT MATERIAL [LSO].—Stem wood and stem bark of *S. araliacea* were collected in the Republic of Guinea (Timbo, Fouta Djallon) during April 1980. A voucher specimen representing the collection is deposited in the herbarium of the Museum d'Histoire Naturelle in Paris (JPR 13).

PLANT MATERIAL [VCU].—Dried stem wood and stem bark of *S. araliacea* (B643452, PR-20777) collected in Ethiopia in 1971 was supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, Maryland, where voucher specimens are preserved.

ISOLATION OF NEOISOSTEGANE (1) [LSO].—The dried, ground stem wood and stem bark of *S. araliacea* (10.2 kg) were cold percolated with 65 liters of 95% EtOH, flash evaporated at 20° , and partitioned between CH_2Cl_2 and H_2O as described by Kupchan and co-workers (3,4). A sample (1 g) of the resulting active CH_2Cl_2 extract (NSC B847587)⁶ was submitted to medium pressure liquid chromatography over Silica gel 60 and eluted with 1% MeOH in CH_2Cl_2 . The fractions were monitored by tlc, and those containing **1** were combined and subjected to preparative tlc on silica gel 60 precoated plates developed three times with 50% EtOAc in hexane to give neoisostegane **1** as a major compound (55 mg). Crystallization of crude **1** from Et_2O yielded a pure analytical sample of **1**, mp $71\text{--}74^\circ$; $[\alpha]^{20}_{\text{D}} +65^\circ$ (c 0.35, CHCl_3); ir ν_{max} (CHCl_3) 1776 cm^{-1} (lactone C=O); pmr (CDCl_3) δ 6.72 (s, 1H), 6.69 (s, 1H), 6.52 (s, 1H), 3.96 (s, 3H), 3.94 (s, 6H), 3.89 (s, 3H), 3.86 (s, 3H), see Table 1 for aliphatic protons; mass measurement m/z 414.1671 ($\text{C}_{23}\text{H}_{26}\text{O}_7$ requires 414.1679).

ISOLATION OF NEOISOSTEGANE (1) [VCU].—The dried, ground stem wood of *S. araliacea* (3.8 kg) was percolated with 18 liters of 95% EtOH in a Soxhlet extractor for 18 h. The resulting extract was partitioned as described by Kupchan and co-workers (3), and a 1.2 g portion of the final CHCl_3 partition layer was subjected to low pressure liquid chromatography over silica gel 60 eluted with 20% EtOAc in petroleum ether. Fractions containing **1** were combined by analytical tlc to give fraction A (251 mg). Fraction A was subjected to preparative tlc on silica gel 60 prepared plates developed three times with 40% EtOAc in

⁶This extract exhibited a $\text{T/C} = 132\%$ against the P-388 lymphocytic leukemia in mice (12).

petroleum ether. Isolation of the major band gave fraction B (58 mg). Crystallization of fraction B from EtOH-H₂O yielded neoisostegane (**1**) (28 mg), mp 107-108°; uv, λ max (EtOH) 250 (ϵ 3543), 277 (2300) nm; ir, ν max (KBr) 2940, 2850, 1776, 1605, 1518, 1495, 1403, 1258, 1118 cm⁻¹; pmr (CDCl₃) see above; (C₆D₆) δ 6.68 (s, 1H), 6.52 (s, 1H), 6.38 (s, 1H), 4.05 (d, J =13.0, 1H), 3.87 (s, 6H), 3.61 (dd, J =7.1, 7.8, 1H), 3.56 (s, 3H), 3.43 (s, 3H), 3.37 (s, 3H), 2.99 (dd, J =8.2, 11.4, 1H), 1.99 (d, J =5.7, 2H), 1.94 (dd, J =9.3, 13.0, 1H), 1.83 (dd, J =9.3, 13.0, 1H), 1.65 (m, 1H); cmr see Table 2; mass measurement m/z 414.1706, (C₂₃H₂₆O₇ requires 414.1679); ms m/z 414 (M⁺), 399, 315, 298, 207.

MEASUREMENT OF CD SPECTRA OF **1**, **2**, AND **3**.—The spectra were measured on a recording single-beam cd/mcd spectrometer constructed in our (DDS) laboratory (15) from 300-210 nm. A [θ]_m value of 7775 deg. cm² decimole⁻¹ (16) for D-(+)-10-camphorsulfonic acid in H₂O (c =0.44 mg/ml) was used for calibration of the spectra. The solvent used for **1**, **2**, and **3** was EtOH (abs.) and the concentrations were: **1**, 0.6 mg/ml; **2**, 0.8 mg/ml; **3**, 0.1 mg/ml.

PREPARATION OF DIPHENOL (**5**).—Stegane (**3**), (9.6 mg) prepared from steganacin (**6**), was heated at 70° with 32.4 mg of phenol in 3 ml 85% phosphoric acid and 2 ml of HOAc. After 2 h, the reaction mixture was poured over ice and extracted with two 10-ml portions of Et₂O. The combined Et₂O extracts were washed with two 10-ml portions of 5% NaHCO₃ and one 10-ml portion of H₂O and dried over anhydrous Na₂SO₄. The Et₂O was removed *in vacuo* to give a brown oil that was subjected to preparative tlc on silica gel 60 developed with 50% Et₂O in C₆H₆. Isolation of the two major bands gave 5.1 mg of recovered **3** and 4.3 of **5** as an amorphous solid; ir, ν max (CHCl₃) 3300 (br), 2950, 2860, 1600, 1460, 1125 cm⁻¹; pmr (CDCl₃) δ 6.69 (s, 1H), 6.64 (s, 1H), 6.48 (s, 1H), 4.24 (m, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.70 (m, 1H), 3.55 (s, 3H), 3.0-1.9 (m, 6H).

PREPARATION OF **4**.—Diphenol **5** (7.0 mg) was dissolved in 6 ml anhydrous MeOH and treated with excess ethereal CH₂N₂. After stirring at room temperature for 12 h, 10 drops of HOAc were added to destroy any unreacted CH₂N₂, and the reaction mixture was diluted to 25 ml with Et₂O. The ethereal solution was washed with two 20-ml portions of 5% NaHCO₃ and one 10-ml portion of H₂O and dried over anhydrous Na₂SO₄. The Et₂O was removed *in vacuo* to yield 8.2 mg of crude **4**. This material was subjected to preparative tlc on silica gel 60 (EM Labs) developed three times with 20% Et₂O in C₆H₆. Isolation of the major band gave 3.7 mg of **4**; ir, ν max (CHCl₃) 2945, 2850, 1779, 1605, 1465, 1409, 1125, 1095 cm⁻¹; pmr (CDCl₃) δ 6.64 (s, 2H), 6.50 (s, 1H), 4.3 (m, 1H), 3.90 (s, 6H), 3.87 (s, 3H), 3.85 (s, 3H), 3.60 (s, 3H), 3.5 (m, 1H), 3.1-2.2 (6H); cmr see Table 2; mass measurement m/z 414.1686 (C₂₃H₂₆O₇ requires 414.1679); ms m/z 414 (M⁺), 399, 368, 315, 299, 271, 207.

ISOMERIZATION OF **4**.—A sample of **4**, (3.7 mg) was heated at 195° for 2 h under N₂. Preparative tlc of the residue of this reaction on silica gel 60 developed in 20% Et₂O in C₆H₆ gave less than 0.2 mg of a product that was not identical (Rf 0.32) to **1** (Rf 0.41) or **4** (Rf 0.34) by analytical tlc on silica gel 60 developed with 10% Et₂O in C₆H₆ (4 \times).

ISOMERIZATION OF NEOISOSTEGANE (**1**).—Neoisostegane (**1**) (4.7 mg) was heated at 200° for 4 h under N₂, and the residue subjected to preparative tlc on silica gel 60 developed with 20% Et₂O in C₆H₆ (5 times) to yield 0.4 mg **6**; pmr (CDCl₃) δ 6.76 (s, 1H), 6.60 (s, 1H), 6.45 (s, 1H), 3.96 (m, 1H), 3.93 (s, 6H), 3.90 (s, 3H), 3.85 (s, 6H), 3.62 (m, 1H).

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ERRATUM

In the article entitled "Scabrosidol, a New Highly Oxygenated Iridoid Glucoside from *Deutzia scabra*," *J. Nat. Prod.*, **46**, 614 (1983), the listing of authors should read as follows:

Paola Esposito, Carlo Iavarone, Alina Sen, and Corredo Trogolo†

†In alphabetical order