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Neoisostegane, A New Bisbenzocyclooctadiene Lignan Lactone from Steganotaenia araliacea Hochst.

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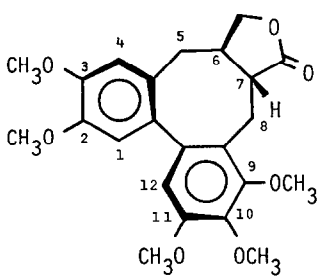
Abstract: The structure of neoisostegane, 1, a new bisbenzocyclooctadiene lignan lactone was elucidated from its ^{1}H and ^{13}C NMR spectra and by chemical correlation with known steganes.

In the course of isolating known steganes from Steganotaenia araliacea Hochst. (Apiaceae)[1,2] for use in another study, neoisostegane, 1, a new bisbenzocyclooctadiene lignan lactone, was isolated.[3] Unlike the homologous lignans previously isolated by Kupchan and coworkers,[2] neoisostegane, 1, bears no functionality at C-5. Thus, it is the first naturally occurring stegane to be reported.[4]

An ethanolic extract of S. araliacea was prepared and fractionated according to previously established protocols.[2] Preparative thin-layer chromatography of a fraction derived from the chloroform partition layer yielded 1 as a crystalline solid, mp 107 - 108° (EtOH). High resolution mass spectrometry established the molecular formula of 1 as $C_{23}H_{26}O_7$ (m/e 414.1702, calc'd. 414.1679), and the IR spectrum displayed a band at 1776 cm^{-1}, indicating the presence of a lactone. The 90 MHz $^{1}HNMR$ spectrum ($CDCl_3$)[5] showed 3 one-proton singlets at δ6.72, 6.69, and 6.52, 3 three-proton singlets at δ3.96, 3.89, and 3.86, and 1 six-proton singlet at δ3.94. These data suggested that 1 was a bisbenzocyclooctadiene lignan lactone bearing five methoxyl groups rather than the more usual three methoxyl groups and one methylenedioxy moiety.



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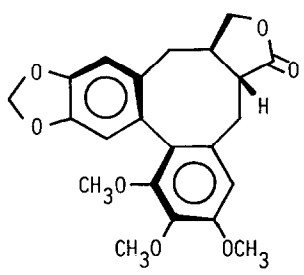


2

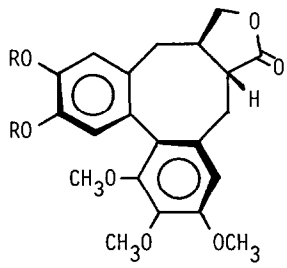
Examination of the 360 MHz ^{1}HNMR spectrum (C_6D_6)[5] indicated that the lactone must be trans-fused since the 6H, 7H coupling constant was found to be 13.0 Hz. This is similar to the spectra of isostegane, 2, and stegane, 3, whereas in the spectra of picrostegane and isopicrostegane which have a cis-fused lactone the coupling constant is approximately 8 Hz.[6-8] The coupling constants between the 5α-H and the 6-H and the 8α-H and the 7-H were both 0 Hz, indicating the presence of the iso-biaryl conformation.[6-8]

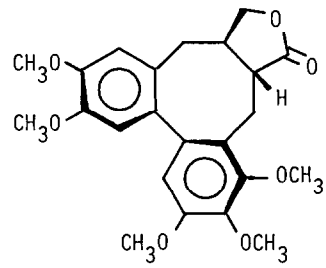
The positions of the methoxyl groups were inferred from the chemical shifts of the methoxyls, aromatic protons, 5α-H, and 8β-H signals. These was no upfield methoxyl singlet for a C-12 methoxyl (found at ca. δ3.6 in other steganes). The aromatic protons all appeared as singlets, indicating that no two were ortho or meta to each other and, as a consequence, that one aromatic ring must bear two methoxyls while the other ring must bear three. The chemical shift of the C-1 proton (δ6.38) was virtually identical to the equivalent signal in the spectrum of 2, as was the chemical shift of the 5α-H (δ2.69) which implied that there was no methoxyl at C-1 or C-4. There was a significant downfield shift of the 8β-H (δ3.63) which was even more pronounced in C_6D_6 (δ4.05). This indicated that the 8β-H was affected by the proximity of some other substituent, with the most likely explanation being the presence of a methoxyl at C-9.

Similar structural conclusions were drawn from examination of the ^{13}CNMR data.[5] In the spectrum of stegane, 3, the resonances for C-6 and C-7 appear at 40.1 and 43.2 ppm, respectively, while in the spectrum of isostegane, 2, these resonances occur at 47.1 and 50.1 ppm. The C-6 and C-7 resonances in the ^{13}CNMR spectrum of 1 were located at 47.1 and 50.0 ppm, thus confirming that 1 had the same stereochemistry as isostegane, 2. The resonance for C-8 in the spectrum of 1 (24.3 ppm) was shifted upfield from the position found in the spectra of 2 and 3, again implying a slightly different environment for C-8. This could best be explained by a methoxyl substituent at C-9.

In order to confirm the position of the methoxyl substituent at C-9, the pentamethoxystegane, 4, was synthesized from stegane, 3.[9] Treatment of 3[10] with a mixture of phenol, phosphoric acid, and glacial acetic acid at 70° yielded diphenol 5, which was converted to 4 with ethereal diazomethane. The ^{1}HNMR spectrum of 4[13] exhibited a three-proton singlet at δ3.60 corresponding to the C-12 methoxyl. This, in conjunction with other resonances, confirmed that 1 was not simply the homologue of stegane. Thermal isomerization of 4 at 195° under nitrogen[11,12] gave material that did not match 1 by analytical tlc. To further confirm that 1 was not isopentamethoxystegane, 1 was also subjected to thermal isomerization conditions to yield neoisostegane, 6. The ^{1}HNMR spectrum[14] showed three methoxyl signals (δ3.93, 3.89, 3.85), but none at δ3.60, thus confirming that the isomerized product was not pentamethoxystegane. As a result of this data, neoisostegane was assigned structure 1.

Neoisostegane, 1 was found to be weakly cytotoxic (ED_{50} = 6.6 μg/ml) against the KB cell culture system.[15] This again confirms the requirement for a substituent at C-5 in order for these lignans to exhibit significant cytotoxicity.





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3 R = $-CH_2-$

4 R = $-CH_3$

5 R = -H

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References and Notes

1. Stem wood and bark of S. araliacea was collected in Ethiopia in 1971 and provided by the Medicinal Plant Resources Laboratory of the USDA.
2. S. M. Kupchan, R. W. Britton, M. F. Ziegler, C. J. Gilmore, and R. F. Bryan, J. Am. Chem. Soc., 95, 1335 (1972).
3. Neoisostegane, 1, was isolated simultaneously from a Guinean variety of S. araliacea by Dr. J.-P. Robin and coworkers who have named it.
4. Previously steganes have only been obtained synthetically. Recent reference: R. S. Ward, Chemical Society Reviews, 75 (1982). Also see references 7, 11, and 12.
5. 1: ^{1}HNMR ($CDCl_3$) δ6.72 (s, 1H), 6.69 (s, 1H), 6.52 (s, 1H), 4.38 (dd, J = 7.2, 8.4, 1H), 3.96 (s, 3H), 3.94 (s, 6H), 3.89 (s, 3H), 3.86 (s, 3H), 3.68 (d, J = 13.7, 1H), 3.6 - 3.8 (1H), 2.69 (d, J = 13, 1H), 2.5 - 1.8 (4H); (C_6D_6) δ6.68 (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); ^{13}CNMR ($CDCl_3$) δ176.0 s, 151.8 s, 150.7 s, 149.2 s, 147.7 s, 141.9 s, 136.3 s, 132.8 s, 131.2 s, 126.8 s, 114.5 d, 112.7 d, 110.1 d, 70.0 t, 61.1 q, 60.8 q, 56.3 q, 49.8 d, 47.0 d, 34.4 t, 24.3 t.

6. Personal communication from Dr. J.-P. Robin.
7. E. Brown and J.-P. Robin, Tetrahedron Lett., 3613 (1980).
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11. J.-P. Robin, O. Gringore, and E. Brown, Tetrahedron Lett., 2709 (1980).
12. K. Tomioka, H. Mizuguchi, and K. Koga, Tetrahedron Lett., 1409 (1979).
13. 4: ^{1}HNMR ($CDCl_3$) δ6.64 (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).
14. 6: ^{1}HNMR ($CDCl_3$) δ6.76 (s, 1H), 6.60 (s, 1H), 6.45 (s, 1H), 3.96 (m, 1H), 3.93 (s, 6H), 3.89 (s, 3H), 3.85 (s, 6H), 3.62 (m, 1H).
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