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SPORANDOL: A NOVEL ANTIPARASITIC BINAPHTHALENE FROM *CHRYSOSPORIUM MERIDARIUM*

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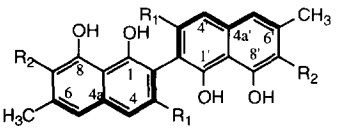
Abstract: Sporandol (7,7'-diacetyl-1, 1', 8, 8'-tetrahydroxy-3, 3'-dimethoxy-6, 6'-dimethyl-2, 2'-binaphthalene, **1**) a novel *endo* and *ecto* parasiticide has been isolated from *Chrysosporium meridarium*. This compound appears to have much less mammalian toxicity than other members of the binaphthalene class. Axial stereochemistry of sporandol was determined as *S* by CD measurements. © 1997 Elsevier Science Ltd.

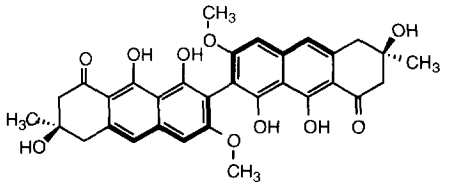
The search for new antiparasitic agents is an ongoing process driven by resistance development to those in current use.[1] The potent antiparasitic activity of some naturally occurring binaphthalenes has been known for some time.[2-5] These compounds are the active components of several traditional medicines used for such purposes.[2-5] Despite their effectiveness against parasites, widespread use of these has been limited due to mammalian neurotoxicity. Stypandrol (**2**) is the active component of several *Hemerocallis species* (day lilies) used in China for the treatment of schistostomiasis in humans. The treatment is marred with side effects, including occasional fatalities.[2] This perhaps is to be expected as stypandrol is also the active component of blind grass, *Stypandra imbricata*. Ingestion of the grass has resulted in a variety of toxic symptoms in Australian sheep herds including characteristic lesions in the nervous system and irreversible blindness.[3] *Diospyros molii* berries used in Thailand for their anthelmintic properties produce diospyrol (**3**) as the active component. This product was considered for a larger program but was abandoned when its toxicity became obvious.[4] Among other related compounds, the flavomannins (**4**) have been reported to cause nervous disorders;[5] antiparasitic activity and toxicity have also been reported for xanthomegnin.[6]



1 : R_1 = OCH_3; R_2 = $COCH_3$

2: R_1 = H; R_2 = $COCH_3$

3: R_1 = R_2 = H



4

There are significant differences in the nervous system of parasites and vertebrates, and as a result selective neurotoxicity has been used as the mechanism of action for development of very useful antiparasitic agents.[1,7] Whether one can take advantage of the antiparasitic properties of the binaphthalenes, in this light, remains to be seen. Sporandol, **1**, is a novel binaphthalene isolated from *Chrysosporium meridarium* using bioassays for antiparasitic activity. The isolation, structure elucidation, and biological activity of sporandol (**1**) are presented here.

The methyl ethyl ketone extract of a fermentation of *C. meridarium*[8] was initially chromatographed on a silica gel flash column using a methylene chloride-methanol gradient, followed by a second silica column, eluted with 1% methanol in methylene chloride, to give pure sporandol **1**, which crystallized from methylene chloride as yellow needles, mp >270 °C, $[\alpha]_{578} = 485$.[9]

Electron impact mass spectrometry suggested a molecular weight of 490, and by high resolution measurements the molecular formula was determined as $C_{28}H_{26}O_8$.[9] There are only 14 carbon signals in the ^{13}C NMR spectrum (Table 1) of **1** indicating that sporandol is a symmetrical dimer. In the UV spectrum absorption bands are observed at 393 and 277 nm and shoulders at 315 and 235 nm, suggesting a highly conjugated system.[9] The infra red spectrum shows absorption bands for hydroxy and acetophenone groups.[9] The ^1H NMR spectrum (Table 1) of **1** is extremely simple and shows signals for an aromatic methyl group coupled to an aromatic proton *via* four bonds, an acetophenone methyl, two aromatic protons one being coupled with the aromatic methyl, thus indicating an *ortho* relationship, an aromatic methoxy group, and two chelated hydroxy groups, one being at *peri* position to a carbonyl.

Table 1: ^1H and ^{13}C NMR Assignment of Sporandol (1) in CD_2Cl_2 Solutions.

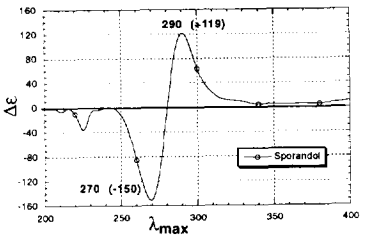
Position	δC	δH	HMBC
1,1'	157.3	---	1-OH
2,2'	108.0	---	H-4, 1-OH
3,3'	162.3	---	H-3a, H-4
3a,3a'	56.2	3.83, s	---
4,4'	97.8	6.65, s	H-5
4a,4a'	139.7	---	H-5
5,5	121.3	6.96, q, J = 1 Hz	H-4, H-6a
6,6'	135.0	----	H-6a
6a,6a'	25.5	2.66, d, J = 1 Hz	H-5
7,7'	112.8	---	H-5, H-6a, 8-OH
7a,7a'	204.5	---	H-7b
7b,7b'	32.1	2.74, s	---
8,8'	169.9	---	8-OH
8a,8a'	108.5	---	H-4, H-5, 1-OH, 8-OH
1,1'-OH	---	10.53, s	
8,8'-OH	---	17.83, s	

The proton carbon correlations (Table 1) were deduced from an HMQC experiment and the monomeric structure of sporandol was assembled from the HMBC ($^{n}J_{CH}$ = 7 Hz) experiment. The connectivities are listed in Table 1. For example, the methoxy protons at δ 3.83 gave correlations to δ 162.3 (C-3) thus it was readily distinguished from other down field oxygenated carbons. The methyl group protons gave correlations to three carbons (C-5, 6, 7) surrounding the methyl group. HMBC correlations from both aromatic protons (H-4 and H-5) and hydroxy protons (8-OH and 1-OH) established the complete substitution pattern of the binaphthalene including the site of dimerization at C-2. C-2 (δ 108.0) is a shielded quaternary carbon and thus must be substituted on both sides by an oxygen atom. This carbon was correlated to only two protons, one of which was the OH at C-1 (δ 10.53) and the other H-4 (δ 6.65), a clear indication that it is the site of dimerization.

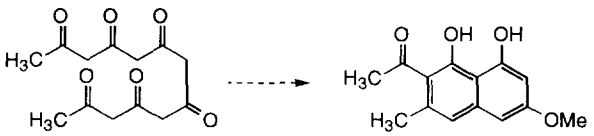
The structure of sporandol (**1**) was confirmed by X-ray crystallography. Crystallization from methylene chloride by slow evaporation at low temperature allowed formation of crystals of marginal quality. This was good enough to get X-ray crystallographic data to confirm the structure but did not give satisfactory refinement to a low R factor.

The strong positive first and negative second Cotton effects in the CD spectrum of sporandol (Figure 1), due to exciton coupling between the $^{1}B_{b}$ transitions of the naphthalene chromophores, the (S) axial configuration.[10] The chiroptical properties of sporandol are in close agreement with those reported in the literature for similar compounds e.g. flavomannin A_1 (**4**).[11]

Figure 1: CD Spectrum of Sporandol in CH_2Cl_2



Sporandol (**1**) is one of the simplest binaphthalenes possible. The monomer has minimal functionalization beyond the basic structure expected from heptaketide biosynthesis,[12] and it is surprising that it was not discovered sooner.



BIOLOGICAL ACTIVITY

Sporandol (**1**) is active against a variety of parasites including the endoparasite (fluke) *Fasciola hepatica* and the ectoparasite *Dipetalogaster maximus* in the mouse at 190 mg/kg.[13] There were no signs of gross toxicity to the mouse when a daily dose of 620 mg/kg was administered for six straight days.[13] Studies into the mechanism of action will be necessary to evaluate the potential promise of **1** as a useful antiparasitic agent.

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7. *Neuropharmacology and Pesticide Action*. Ford, M. G.; Lunt, C. G.; Reay, R. C.; Usherwood P. N. R. eds.; Horwood: Chichester 1986.
8. A spore suspension of *Chrysosporium meridarium* (ATCC 74009) was used to inoculate a seed medium, consisting of (per liter): corn steep liquor, 5 g; tomato paste, 40 g; oat flour, 10 g; glucose, 10 g; and trace elements ($FeSO_4$•$7H_2O$, 10 mg; $MnSO_4$•$4H_2O$, 10 mg; $CuCl_2$•$2H_2O$, 0.25 mg; $CaCl_2$•H_2O, 1.0 mg; H_3BO_3, 0.56 mg; $(NH_4)_6MoO_{24}$•$4H_2O$, 0.19 mg; and $ZnSO_4$•$7H_2O$, 2.0 mg) adjusted to pH 6.8. After 2 days shaking at 220 rpm, 25 °C, 10 -13 mL of the seed was used to inoculate six 2-liter flasks, each containing 70 grams vermiculite, over which was poured 250 ml of the production medium, consisting of (per liter): glucose, 150 g; urea, 4 g; NZ-amine Type A, 4 g; K_2HPO_4, 0.5 g; $MgSO_4$·$7H_2O$, 0.25 g; KCl 0.25 g; $ZnSO_4$·$7H_2O$, 0.9 g; and $CaCO_3$, 16.5 g (no pH adjustment). The flasks were shaken vigorously to insure homogeneous inoculation throughout the vermiculite, and the culture was incubated for 21 days without agitation at 22 °C.
9. HREIMS (*m/z*): 490.1626 (M^+, calcd for $C_{28}H_{26}O_8$:490.1630); $[\alpha]_{578}$ = 485 (c, 0.2, CH_2Cl_2); $[\alpha]_{546}$ =705 (c, 0.2, CH_2Cl_2); UV (CH_3CN) : λ_{max} (ε) : 393 (12,000), 315 (sh, 9,600), 277 (45,000), 235 (sh,25,000) nm; CD(CH_2Cl_2): λ_{max} ($\Delta\varepsilon$) 290 (+119), 270 (-150); IR (KBr pellet) ν_{max}: 3675, 3649, 1770, 1623, 1577, 1508, 1473, 1407, 1361, 1346, 1267, 1224, 1196, 1179, 1112, 1065, 1026, 988, 862, 812, 735, 688 cm^{-1}.
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