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ANTITUMOUR AND CYTOTOXIC XANTHONES OF *PSOROSPERMUM FEBRIFUGUM*

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Key Word Index—*Psorospermum febrifugum*; Guttiferae; Vismieae; xanthones; cytotoxic and antitumour xanthones.

Abstract—Activity-directed fractionation of the ethanolic extract of *Psorospermum febrifugum* has led to the isolation and structural elucidation of eight new dihydrofuranoxanthones. Six compounds were found to exhibit significant *in vitro* cytotoxic activity against 9PS cells in culture. A new dihydrofuranoxanthone epoxide, related to psorospermin exhibited activity both *in vitro* and *in vivo*.

INTRODUCTION

Psorospermum febrifugum is an African tropical woody plant. Previous chemical investigation of different species of this genus has led to the isolation of vismiones [1–4, 5], anthraquinones [4, 6], anthrones [5,7–9], and xanthonolignoids [10]. The cytotoxic and antitumour activity associated with the ethanolic extract of *Psorospermum* has led to extensive work to isolate the active compounds. The first account of the isolation of bioactive compounds from *Psorospermum febrifugum* was reported by Kupchan [11]. The furanoxanthone, psorospermin (**1**), was found to possess significant cytotoxic and antitumour properties. In connection with this study the structure, stereochemistry, synthesis and interconversion among members of this interesting class of compounds have been thoroughly investigated [12–15]. In addition, activity-directed fractionation of the plant extract has yielded a number of active fractions which do not contain psorospermin and from which a series of cytotoxic and antitumour xanthones has been isolated. This paper summarizes the isolation, structural elucidation and activity for eight psorospermin analogues obtained by fractionation and chromatographic analysis of the plant extract.

RESULTS AND DISCUSSION

The ethanolic extract of the root bark of *P. febrifugum* was partitioned, as described in the Experimental, so as to concentrate the activity in the chloroform fraction II. Chromatographic investigation of this active fraction led to the isolation of psorospermin (**1**), psorospermin diol (**2**), and psorospermin chlorohydrin [13]. More polar fractions that were eluted after psorospermin were found to contain both *in vitro* cytotoxic and *in vivo* antitumour activity. These fractions were analysed by chromatography on silica gel and as a result eight (**3–10**) xanthones were isolated in quantities sufficient for their analysis. Their identification was based on UV, IR, MS, ^{1}H NMR and ^{13}C NMR data. The IR spectra showed absorptions around 1640 cm^{-1} reminiscent of a xanthone carbonyl while their UV spectra indicated a polyoxygenated xanthone nucleus. Mass spectral analysis was very informative in suggesting the substitution pattern among different analogues in this series. The major fragment was produced by the loss of the entire side chain to generate the stable furanoxanthone carbonium ion (Fig. 1) [14]. These fragmentation patterns were found to be of value in predicting the substitution pattern on the furanoxanthone backbone and in correlating the structures among members of this class. The ^{1}H NMR data (Table 1) indicates the similar substitution pattern around the xanthone nucleus for all the isolated compounds. These data showed in common the presence of signals for three adjacent aromatic protons for a mono substituted ring A of the xanthone and a high field aromatic singlet for the C-2 proton on the electron rich phloroglucinol ring C. In addition, their spectra had in common an ABX system reminiscent of the dihydrofuran protons [12]. All analyses were confirmed by decoupling experiments. The molecular formula of $C_{18}H_{16}O_7$ was established for both compounds **3** and **4** through exact mass measurements which suggested that they were lower homologues (CH_2 unit less) of 3′,4′-deoxypsorospermin-3′,4′-diol (**2**, $C_{19}H_{18}O_7$). Since a major ion fragment was observed at *m/z* 269 in their MS spectra (cf 283 for **2**), it was suggested that the difference in their structures was derived from differences in the substituents on the xanthone nucleus. A 60 nm aluminium trichloride induced bathochromic shift in the UV spectrum of **3** and **4** indicated the presence of a hydroxyl group peri to the xanthone carbonyl. The absence of any methoxyl signals in the ^{1}H NMR spectra and the appearance of, instead, a far downfield signal at δ 13.24, D_2O exchangeable, together with the formation of a triacetate derivative (see Experimental) upon treatment of either **3** or **4** by acetone/pyridine confirmed the presence of a C-1 hydroxyl group. ^{13}C NMR analysis of **3** indicated the presence of only 18 carbons. Shifts observed

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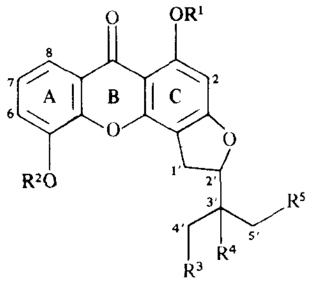
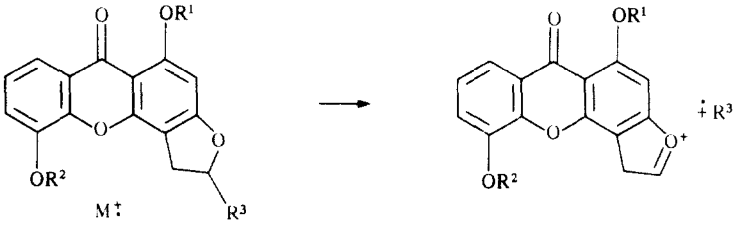


	R^1	R^2	R^3	R^4	R^5	9PS $\mu g/ml$	3PS T/C %
1	Me	H	---O---		H	1×10^{-4}	160/8 mg
2	Me	H	OH	OH	H	4×10^{1}	inactive
3	H	H	OH	OH	H	1×10^{-2}	inactive
4	H	H	OH	OH	H	1×10^{-2}	NT
5	Me	Me	OH	OH	H	9×10^{1}	NT
6	Me	H	OMe	OH	H	2×10^{-4}	NT
7	Me	H	OEt	OH	H	3×10^{-3}	NT
8	H	Me	OH	OH	OH	3×10^{0}	NT
9	Me	H	---O---		OH	7×10^{-5}	136/16 mg
10	Me	Me	Cl	OH	OH	$\ll 10^{-5}$	NT

NT = not tested



m/z	Compound
269 ($C_{15}H_9O_5$)	**3**,**4**
283 ($C_{16}H_{11}O_5$)	**1**,**2**,**6**,**7**,**8**,**9**
297 ($C_{17}H_{13}O_5$)	**5**,**10**

Fig. 1.

for the carbons of **3** were comparable to those reported for compound **2** [15]. The downfield shift (7 ppm) of the xanthone carbonyl and the slight paramagnetic shifts on C-1 and C-2 and a diamagnetic shift on C-4a and C-9a, when compared with the shifts of equivalent carbons of **2** is rationalized by the presence of a hydroxyl group at C-1 instead of a methoxyl group and also due to the chelation of the hydroxyl group with the xanthone carbonyl (Table 2). These data establish the structures of **3** and **4** as *O*^{1}-demethyl-3′,4′-deoxypsorospermin-3′,4′-diol. The very similar fragmentation behaviour and ^{1}H NMR data of these two compounds suggested the existence of a stereoisomeric relationship between them. The ^{1}H NMR spectrum of the triacetate derivative of **3** showed the C-4′ protons as a pair of doublets at δ4.34 and 4.11 ($\Delta\delta = 0.23$) while in the case of **4** the signals were observed at δ4.22 and 4.18 ($\Delta\delta = 0.04$). The optical rotation for **3** was found to be $-50°$ and **4** showed $+65.4°$ which suggests that these compounds differ in the stereochemistry at the C-2′ and/or C-3′ centres. Both **3** and **4** were found to be highly cytotoxic with an ED$_{50}$ of 1×10^{-2} μg/ml against 9PS cells in culture while compound **3** was found to be inactive in 3PS.

High resolution mass analysis indicated that compounds **5** and **6** were higher homologues of **2** as both of them had the molecular formula of $C_{20}H_{20}O_7$. The ion fragment corresponding to the furanoxanthone skeleton was observed at *m/z* 297 for compound **5** and at *m/z* 283 for compound **6** which suggested that the additional CH_2 unit was added onto the xanthone nucleus of **5** and on the R side chain of **6**. The ^{1}H NMR spectrum of **5** indicated similar signal patterns to the previously discussed furanoxanthones. In addition a singlet at δ3.96 was present which can be accounted for by the presence of a methoxyl group at C-5 (cf OH for **2**). The structure of **5** was confirmed as *O*^{5}-methyl-3′,4′-deoxypsorospermin-3′,4′-diol by methylation of compound **2** with diazomethane to yield **5** (see Experimental). On the other hand, the

Table 1. ^{1}H NMR spectral data of the dihydrofuranoxanthones

Compound	1-Methoxy or hydroxy	1-Methoxy or hydroxy	H-2	H-6	H-7	H-8
3	13.24 *s* –OH	8.32 *s* –OH	6.28 *s*	7.33 *dd* (7.8, 1.2)	7.24 *t* (7.8)	7.53 *dd* (7.8, 1.2)
4	13.24 *s* –OH	8.32 *s* –OH	6.30 *s*	7.31 *dd* (7.8, 1.2)	7.24 *t* (7.8)	7.52 *dd* (7.8, 1.2)
5	3.84 *s* –OMe	3.96 *s* –OMe	6.53 *s*	7.39 *dd* (7.9, 1.3)	7.30 *t* (7.9)	7.61 *dd* (7.9, 1.3)
6	3.83 *s* –OMe		6.52 *s*	7.20 *dd* (7.8, 1.6)	7.14 *t* (7.8)	7.47 *dd* (7.8, 1.6)
7	3.83 *s* –OMe		6.52 *s*	7.20 *dd* (7.8, 1.2)	7.19 *t* (7.8)	7.44 *dd* (7.8, 1.2)
8	13.08 *s* –OH	3.97 *s* –OMe	6.30 *s*	7.50 *d* (7.8)	7.38 *t* (7.8)	7.68 *d* (7.8)
9	3.83 *s* –OMe		6.56 *s*	7.21 *dd* (7.8, 1.6)	7.15 *t* (7.8)	7.47 *dd* (7.8, 1.6)
10	3.84 *s* –OMe	3.95 *s* –OMe	6.58 *s*	7.39 *dd* (7.9, 1.1)	7.29 *t* (7.9)	7.60 *dd* (7.9, 1.1)

Compound	H-1′a	H-1′b	H-2′	H-4′a	H-4′b	H-5′
3	3.31 *dd* (8.7, 13)	3.44 *dd* (8.7, 13)	5.00 *t* (8.7)	3.55 *d* (10.4)	3.30 *d* (10.4)	1.09 *s*
4	3.34 *dd* (10, 14)	3.45 *dd* (7, 14)	4.99 *dd* (10, 7)	3.55 *d* (10.4)	3.29 *d* (10.4)	1.11 *s*
5	3.28 *m*	3.28 *m*	5.00 *t* (9)	3.56 *dd* (10.3, 6)	3.29 *dd* (10.3, 6)	1.11 *s*
6	3.23 *dd* (9, 14)	3.28 *dd* (9, 14)	4.94 *t* (9)	3.49 *d* (10.5)	3.28 *d* (10.5)	1.13 *s*
7	3.27 *dd* (9, 13)	3.35 *dd* (9, 13)	4.95 *t* (9)	3.53 *d* (8.9)	3.3 *d* (8.9)	1.13 *s*
8	3.21 *dd* (9, 15)	3.39 *dd* (9, 15)	5.07 *t* (9)	3.39 *dd* (10.8, 5.6)	3.50 *dd* (10.8, 5.6)	3.54 *dd* 3.56 *dd* (9.1, 5.6)
9	3.38 *dd* (8.4, 15)	3.32 *dd* (15, 10)	5.11 *dd* (8.4, 10)		3.82 *s* (*br*)	3.52 *dd* 3.47 *dd* (11, 5)
10	3.27 *dd* (8.5, 15)	3.31 *dd* (10, 15)	5.11 *t* (8.5, 10)	3.81 *d* (9)	3.81 *d* (9)	3.45 *dd* 3.52 *dd* (11.2, 5.4)

presence of a singlet at δ3.31 in the ^{1}H NMR spectrum of **6** indicated the presence of an aliphatic methyl ether function on the side chain. The fact that compound **6** produced only the monoacetate derivative upon acetylation with acetic anhydride and pyridine eliminated the possibility of the presence of the ether function at the C-3′ position and established the structure of **6** as $O^{4'}$-methyl-3′,4′-deoxypsorospermin-3′,4′-diol. Although compound **6** was found to have significant cytotoxic activity against 9PS cells in culture with an ED$_{50}$ of 2×10^{-4} µg/ml, compound **5** was found to be completely inactive against the same cell line.

Compound **7** was shown to have a molecular formula of $C_{21}H_{22}O_7$ as deduced by accurate mass measurement. The additional C_2H_4 unit in the molecular formula of **7** compared to that of **2** was apparently associated with the side chain since compound **7** gave the typical value of *m/z* 283 for the monomethoxy-hydroxy-furanoxanthone skeleton. The presence of an additional two carbons in the ^{13}C NMR spectrum, one at δ15.1 for a methyl group and the second at δ74.7 for a methylene attached to oxygen, indicated the presence of an ethoxyl group (Table 2). These findings are supported by the presence of a quartet, in the ^{1}H NMR spectrum of **7**, centred at δ1.11 which is coupled with a triplet located at δ3.48 and confirming the presence of an ethoxyl function. The ^{1}H NMR spectrum of **7** showed a pattern typical of a psorospermin analogue. The ethoxyl group can be placed at the 4′-carbon since the compound yielded only the monoacetate derivative upon acetylation (see Experimental). Compound **7** was found to be highly cytotoxic in the 9PS system with an ED$_{50}$ of 3×10^{-3} µg/ml. Tandem MS analysis indicated that compounds **6** and **7** are naturally occurring and exist in the *Psorospermum* root bark material, excluding the possibility that they are artifacts produced during the processing steps [14].

UV, IR, MS and ^{1}H NMR data of compounds **8**–**10** also revealed the presence of the dihydrofuranoxanthone skeleton. Their ^{1}H NMR spectra contained spins for four aromatic protons of the xanthone nucleus typical of

Table 2. ^{13}C NMR chemical shifts

C	3',4'-Deoxy-psorospermin-3',4'-diol (**2**)	**3**	**7**	**9**
1	162.2	163.4	162.1	162.1
2	90.3	92.8	90.4	85.4
3	165.9	167.9	165.8	165.4
4	105.5*	104.4	105.5*	105.2
4a	153.5	151.6	153.4	153.4
10a	143.6	144.6	143.6	143.5
5	145.8	146.2	145.8	145.8
6	119.2	120.7	119.3	119.3
7	123.2	124.2	123.5	123.5
8	115.4	114.7	115.3	115.4
8a	123.7	120.8	123.6	123.6
9	173.5	180.2	173.5	173.5
9a	105.4*	102.4	105.3*	105.7
1'	26.3	25.7	26.2	26.5
2'	88.2	88.4	88.2	86.4
3'	72.9	72.6	71.9	74.8
4'	66.0	66.6	66.2	46.3
5'	20.9	20.8	21.5	61.4
1 OMe	56.3		56.3	56.6
O–$\underline{C}H_2$Me			74.7	
O–$CH_2\underline{Me}$			15.1	

*These assignments may be interchanged.

psorospermins and an aliphatic ABX system reminiscent of the C-1' and C-2' protons of the dihydrofuran ring (Table 1). The striking difference in the ^{1}H NMR spectra of **8**–**10** that distinguishes them from the spectra of the previously reported compounds is the disappearance of the upfield signal of the C-5' methyl protons and the appearance of a new ABX system that collapse into an AB pattern after D_2O shake and which suggest the oxidation of the 5'-methyl group to a hydroxymethyl group.

A molecular formula of $C_{19}H_{18}O_8$ was established for compound **8** by high resolution MS which suggested compound **8** to be an analogue of 3',4'-deoxypsorospermin-3',4'-diol (**2**) with the 5'-methyl being oxidized to a hydroxymethyl function. This structure is confirmed by the presence of three ABX systems in the ^{1}H NMR spectrum of **8** (Table 1). One ABX system accounts for the C-1' and C-2' protons while the other two ABX patterns account for the 4' and 5' protons being coupled with the hydroxyl protons. The collapse of these two ABX patterns to AB patterns in the ^{1}H NMR spectrum of the triacetate derivative of **8** supports the presence of two primary hydroxy groups at the 4' and 5' positions. The most downfield signal at δ13.08 in the ^{1}H NMR spectrum of **8** indicates the presence of an hydroxyl group peri to the xanthone carbonyl which suggests the presence of a 1-hydroxy-5-methoxy-substitution pattern on the xanthone nucleus. This is confirmed by a 46 nm aluminium trichloride induced bathochromic shift in the UV spectrum of **8**. Compound **8** was found to have borderline activity against 9PS cells.

Compound **9** has exhibited potent cytotoxic and borderline *in vivo* antitumour activity. A molecular formula of $C_{19}H_{16}O_7$ was established for this compound by HRMS measurements, which also suggested a psorospermin analogue with an additional oxygen atom. UV, IR and ^{1}H NMR data indicated a dihydrofuranoxanthone framework. The absence of signals for the 5'-methyl in the ^{1}H NMR spectrum indicated the presence of a hydroxymethyl group at that position and accounts for the additional oxygen atom. The assignment of **9** as 5'-hydroxypsorospermin is further confirmed by ^{13}C NMR since carbon shifts observed with 5'-hydroxypsorospermin were comparable to those reported for psorospermin [15]. The absence of any signals for a methyl carbon and the appearance of a carbon signal at δ60.4 for a methylene carbon adjacent to oxygen confirms the presence of a hydroxyl function at the C-5' position.

Mass spectral analysis of compound **10** indicated a chlorine-containing compound with a molecular formula of $C_{20}H_{19}O_7Cl$. Similarly, compound **10** was identified as a dihydrofuranoxanthone derivative with two methoxyl groups on the xanthone ring since the ion fragment corresponding to the furanoxanthone skeleton was observed at m/z 297. The ^{1}H NMR of compound **10** showed the presence of two methoxy signals at δ3.95 and 3.84 and exhibited the typical pattern of a dihydrofuranoxanthone structure. As mentioned earlier, the disappearance of a methyl signal and the appearance of a methylene signal coupled to a hydroxyl proton indicated the presence of a hydroxymethylene group at the 5'-position. Based on this a chlorine atom is expected to be present at the 4' position and a hydroxyl function at the C-3' position. The existence of a tertiary hydroxyl group is also suggested by the formation of a monoacetate upon acetylation with acetic anhydride and pyridine. Compound **10** was found to be highly cytotoxic against 9PS cells with an ED_{50} of less than 10^{-5} μg/ml.

EXPERIMENTAL

Plant material. *Psorospermum febrifugum* Spach. roots were collected in Tanzania by Mr Leonard Mwasumbi of the University of Dar-es-Salam. Specimens were authenticated at the Economic Botany Laboratory, Beltsville Agricultural Research Center, Beltsville, Maryland, where a voucher specimen is on deposit.

Extraction and isolation. The large scale extraction and fractionation was carried out at Polysciences Laboratory, Warrington, Pennsylvania to generate all the fractions for further separation. The chipped woodey roots (133 kg) were defatted by percolation with hexane then dried by warm air. The dried chips were then ground and defatted again by successive steeping and percolation with hexane. The defatted powder was extracted with 95% EtOH and the extract was stripped almost to dryness to yield 19 gallons of thick black tar. The crude extract was triturated with 37 gallons of Me_2CO, the Me_2CO insoluble material was filtered while the Me_2CO extract was concd under red. pressure to yield 10.5 gallons of the Me_2CO extract. The Me_2CO extract was triturated with 56 gallons of $CHCl_3$ with stirring, the mixture was then allowed to stand and then filtered through celite. The $CHCl_3$ extract was evapd to dryness to yield 2.99 kg of reddish-black tar. The material from $CHCl_3$ was dissolved in 11 gallons of MeOH and then triturated with 4.7 gallons of H_2O. The MeOH–H_2O (7:3) extract was filtered to give a clear amber coloured soln. The MeOH–H_2O soln was then purified by shaking with 16 l of toluene–hexane (1:1) × 3. The aq. MeOH soln was then concd to 9 gallons to remove most of the MeOH, and then extracted × 5 with 3 gallons of $CHCl_3$. The combined extract II was evapd to dryness to yield 712.2 g of reddish-brown residue (0.54%).

The $CHCl_3$ extract II (200 g) was dissolved in Me_2CO and the nonpolar materials were removed by extraction with hexane. The Me_2CO extract (100 g) was chromatographed on a 3200 g silica gel low pressure column (9.5 × 100 cm). Elution was initially started with toluene–hexane–EtOAc (3:3:1), 13 l, followed by hexane–EtOAc (1:1), 32 l, hexane–EtOAc (2:3), 32 l, hexane–EtOAc (1:4), 32 l, and EtOAc, 20 l. Finally the column was eluted with increasing percentages of MeOH in EtOAc, 10% (20 l), 20% (20 l), 50% (20 l) and then washed with MeOH. A total of 220 fraction of 1 l each were collected. Fractions were analysed by TLC and pooled into 19 major fractions with F numbers from F1-F19.

All mp: uncorr. ^{1}H NMR spectra were obtained in the solvent indicated at 470 MHz. ^{13}C NMR spectra were recorded at either 50.31 MHz or 50.16 MHz in DMSO-d_6. Chemical shifts are given in ppm and spectra were referenced to the solvent peak. The P388 mouse leukemia assays were carried out at the Illinois Institute of Technology, Life Sciences Division.

A portion of F9 (4 g) was triturated with MeOH. The MeOH extract was then filtered. The MeOH soluble material (3.7 g) was partitioned between 5% aq Na_2CO_3, 100 ml, and EtOAc 3 × 100 ml. The combined EtOAc extract was washed with 10 ml H_2O, dried over Na_2CO_3 and *in vacuo* to yield 1.8 g of the neutral fraction A_1. The neutral fraction A_1 was chromatographed on an 80 g low pressure silica gel column (2.5 × 100 cm). Elution was started by $CHCl_3$ and polarity was increased gradually by increasing the percentage of added MeOH. Fractions of 20 ml vol were collected, analysed by TLC and pooled into 7 major fractions A_3–A_9.

(−)O^1-*Demethyl-3′,4′-deoxypsorospermin-3′,4′-diol* (**3**). Trituration of the fraction A_8 with yielded 88 mg of a yellow deposit which was then crystallized from MeOH to give solid S. TLC of Solid S (20 mg) 0.25 mm silica gel developed by $CHCl_3$–MeOH (97:3) gave **3** (higher R_f) and **4** (lower R_f).

Compound **3** was eluted from silica gel and crystallized from MeOH to give fine yellow needles, mp 220–221° (MeOH); $[\alpha]_D^{20} = -50°$ (MeOH; *c* 0.01); UV λ_{max}^{MeOH} nm (log ε): 245 (4.33), 260 (sh), 323 (4.05), 359 (3.45); λ_{max} (MeOH + $AlCl_3$) 228, 248 (sh), 278, 341 and 420 nm; IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1640, 1570, 1250 and 1120; CIMS (CH_4), *m/z* (rel. int.): 345 $[M+H]^+$ (100), 327 $[M+H-H_2O]^+$ (21), 269 (13); EIMS, obsd 344.089 $[M]^+$ calcd for $C_{18}H_{16}O_7$, 344.090; obsd 269.045, calcd for $C_{15}H_9O_5$, 269.045; ^{1}H NMR (470 MHz, DMSO-d_6): see Table 1; ^{13}C NMR (50 MHz, DMSO-d_6): see Table 2: $ED_{50} = 1 \times 10^{-2}$ μg/ml in 9PS, inactive in 3PS when tested at doses 20, 10, 5 and 2.5 mg/kg.

Acetylation of **3**. A soln of **3** (2 mg) in pyridine (0.5 ml) and Ac_2O (0.5 ml) was stirred overnight at room temp. The solution was evapd *in vacuo* and the residue was crystallized from MeOH to give white needles, mp 171–172°. EIMS, *m/z* (rel. it.): 470 $[M]^+$ (20), 428 $[M-Ac+H]^+$ (69), 386 $[428-2Ac+H]^+$ (8), 355 (27), 337 (58), 311 (71), 295 (45), 269 (100) and 241 (40); ^{1}H NMR (470 MHz, $CDCl_3$): δ1.23 (*s*, 3H, Me-5′), 2.13 (*s*, 3H, Ac-4′), 2.42 (*s*, 3H, Ac-5), 2.46 (*s*, 3H, Ac-1), 3.30 (*dd*, 1H, *J* = 15.2, 9.5 Hz, H-1′a), 3.42 (*dd*, 1H, *J* = 15.2, 9.5 Hz, H-1′b), 4.11 (*d*, 1H, *J* = 11.4 Hz, H-4′a), 4.34 (*d*, 1H, *J* = 11.4 Hz, H-4′b), 4.99 (*t*, 1H, *J* = 9.5 Hz, H-1′), 6.52 (*s*, 1H, H-2), 7.30 (*t*, 1H, *J* = 7.9 Hz, H-7), 7.41 (*dd*, 1H, *J* = 7.9, 1.6 Hz, H-6), 8.09 (*dd*, 1H, *J* = 7.9, 1.6 Hz, H-8).

(+)O^1-*Demethyl-3′,4′-deoxypsorospermin-3′,4′-diol* (**4**). This was crystallized from MeOH after elution from silica gel to yield yellow needles, mp 222–223° (MeOH); $[\alpha]_D^{20} = +65.4$ (MeOH; *C* 0.01); UV, IR and MS data are identical to those obtained for compound **5A**; ^{1}H NMR (470 MHz, DMSO-d_6): see Table 1; $ED_{50} = 1 \times 10^{-2}$ μg/ml in 9PS.

Acetylation of **4** (under the name conditions as those described for **3**). A soln gave white needles, mp 158–160°. CIMS, *m/z* (relative intensity): 471 $[M+H]^+$ (94), 429 $[M+H-Ac+H]^+$ (100) and 387 $[429-2Ac+H]^+$ (4); ^{1}H NMR (470 MHz, $CDCl_3$): δ1.23 (*s*, 3H, Me-5′), 2.13 (*s*, 3H, Ac-4′), 2.42 (*s*, 3H, Ac-5), 2.46 (*s*, 3H, Ac-1), 3.34 (*dd*, 1H, *J* = 16, 10 Hz, H-1′a), 3.42 (*dd*, 1H, *J* = 16, 8 Hz, H-1′b), 4.18 (*d*, 1H, *J* = 11.7 Hz, H-4′a), 4.22 (*d*, 1H, *J* = 11.7 Hz, H-4′b), 5.00 (*dd*, 1H, *J* = 10, 8 Hz, H-2′), 6.50 (*s*, 1H, H-2), 7.30 (*t*, 1H, *J* = 7.9 Hz, H-7), 7.41 (*dd*, 1H, *J* = 7.9, 1.6 Hz, H-6), 8.09 (*dd*, 1H, *J* = 7.9, 1.6 Hz, H-8).

O^5-*Methyl-3′,4′-deoxypsorospermin-3′,4′-diol* (**5**). The MeOH soluble material obtained by extracting F12 with 10 ml of MeOH was partitioned between 5% Na_2CO_3 (50 ml) and $CHCl_3$ (50 ml × 3). The $CHCl_3$ layer was separated, washed and the solvent removed *in vacuo*. The residue of neutral materials was fractionated by prep. TLC using three silica gel plates (2 mm thick, 20 × 20) into nine bands. Each band was eluted with $CHCl_3$–MeOH to yield 9 fractions A–I.

Crystallization of fraction E from MeOH yielded 8 mg of white needles, mp 235–236° (MeOH); $[\alpha]_D^{20}$ −111° (C 0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε): 248 (4.12), 312 (3.78) and 353 (3.27); IR ν_{max}^{KBr} cm^{-1}: 3320, 1620, 1570, 1470, 1450, 1250 and 1120; CIMS (CH_4), *m/z* (rel. int.): 373 $[M+H]^+$ (100) and 355 $[M+H-H_2O]^+$ (17), EIMS, obsd 372.119 (M^+) calcd for $C_{20}H_{20}O_7$, 372.121; obsd 297.076 calcd for $C_{17}H_{13}O_5$, 297.076; ^{1}H NMR (470 MHz, DMSO-d_6): see Table 1.

Methylation of 3′,4′-deoxypsorospermin-3′,4′-diol to **5**. A soln of 3′,4′-deoxypsorospermin-3′,4′-diol (2 mg) in 3 ml Et_2O was treated with CH_2N_2 and stirred for 1 hr. The Et_2O was evapd to dryness and the product crystallized from MeOH. EIMS, obsd 372.119 $[M]^+$ calcd for $C_{20}H_{20}O_7$, 372.121. Chromatographic analysis indicated that both the naturally occurring and the semisynthetic O^5-methyl-3′,4′-deoxypsorospermin-3′,4′-diol were identical.

O^4,-*Methyl-3′,4′-deoxypsorospermin-3′,4′-diol* (**6**). Fraction F10 (2.7 g) was triturated with MeOH, the MeOH-insoluble material D was filtered and recrystallized from MeOH. The MeOH soluble material M was added to the top of a 300 g silica gel gravity column, elution was started by $CHCl_3$, 15 ml fractions were collected. The polarity was increased gradually with increasing percentages of MeOH 1, 2, 5, 10, 20, 50% and finally washed with MeOH. The collected fractions were monitored by TLC and then pooled into 24 different fractions, S_1 to S_{24}.

Crystallization of fraction S_{11} yielded compound **6**, 30 mg, mp 243–245° (MeOH); UV λ_{max}^{MeOH} nm (log ε): 244 (4.44), 312 (4.18) and 350 (3.68); IR ν_{max}^{KBr} cm^{-1} 3340, 1640, 1610, 1580, 1270 and 1110; CIMS (CH_4), *m/z* (rel. int.): 373 $[M+H]^+$ (100) and 283 (14); EIMS, obsd 372.120 $[M]^+$ calcd for $C_{20}H_{20}O_7$, 372.121; obsd 283.060, calcd for $C_{16}H_{11}O_5$, 283.061; ^{1}H NMR (470 MHz, DMSO-d_6): see Table 1; $E_{50} = 2 \times 10^{-4}$ μg/ml in 9PS.

O$^{4′}$-*Ethyl-3′,4′-deoxypsorospermin-3′,4′-diol* (**7**). The mother liquor of F8 from the isolation of psorospermin chlorohydrin, 100 mg, was subjected to prep. TLC on silica gel (2 mm, 20 × 20). The band corresponding to the blue fluorescent material with a little higher R_f value than psorospermin chlorohydrin was recovered and extracted with $CHCl_3$–MeOH. The brownish extract was crystallized from MeOH to give 30 mg of compound **7** as a white powder, mp 257–258°; $ED_{50} = 3 \times 10^{-3}$ μg/ml in 9PS;UV λ_{max}^{MeOH} nm (log ε): 247 (4.23), 312 (3.88), 350 (3.26); IR ν_{max}^{KBr} cm^{-1}: 3250, 1630, 1580, 1120, ^{1}H NMR (470 MHz, DMSO-d_6): see Table 1; CIMS (CH_4), *m/z* (rel. int.): 387 $[M+H]^+$ (100), 369 $[M+H-H_2O]^+$ (13), 341 (3.5), 283 (13), EIMS; obsd 386.137 $[M]^+$ calcd for $C_{21}H_{22}O_7$, 386.136, CMR (50 MHz, DMSO-d_6: see Table 2.

Acetylation of compound **7**. Compound **7** (5 mg) was acetylated under the same conditions as those described for **3** to give crystals, mp 110–112°. CIMS, *m/z* (rel. int.): 429 $[M+H]^+$ (100)

and 387 $[M+H-Ac]^+$ (8). ^1H NMR (470 MHz, $CDCl_3$): δ1.19 (*t*, 3H, $J = 7$ Hz, O–CH_2–Me), 1.26 (*s*, 3H, Me-5′), 3.23 (*dd*, 1H, $J = 10$, 15 Hz, H-1′a), 3.33 (*dd*, 1H, $J = 8$, 15, Hz, H-1′b), 3.46 (*d*, 1H, $J = 9.1$ Hz, H-4′a), 3.49 (*d*, $J = 9.1$ Hz, H-4′b), 3.53 (*q*, 2H, $J = 7$ Hz, O–CH_2–Me), 3.94 (*s*, 3H, OMe-1), 4.98 (*dd*, 1H, $J = 8$, 10 Hz, H-2′), 6.35 (*s*, H-2), 7.27 (*t*, 1H, $J = 7.8$ Hz, H-7), 7.36 (*dd*, 1H $J = 1.7$, 7.9 Hz, H-6), 8.14 (*dd*, 1H, $J = 1.7$, 7.9 Hz, H-8).

Isolation of 3′,4′-deoxyisopsorospermin-3′,4′,5′-triol (**8**). Crystallization of the residue from fraction D yielded 10 mg of fine yellow crystals, mp 215–216° (MeOH); $[\alpha]_D^{20}$ $-143°$ (MeOH c0.1); UV λ_{max}^{MeOH} nm (log ε): 246 (4.88), 322 (4.59) and 362 (4.03); UV (MeOH + $AlCl_3$) λ_{max} 236, 272, 280, 328, 342, 416 nm; IR ν_{max}^{KBr} cm^{-1}: 3475, 1640, 1600, 1570, 1470, 1250, 1120 and 1030; CIMS (CH_4), *m*/*z* (rel. int.): 375 $[M+H]^+$ (100), 357 $[M+H-H_2O]^+$ (4) and 283 (4), EIMS, obsd 374.099 $[M]^+$ calcd for $C_{19}H_{18}O_8$, 374.100, obsd 283.060 calcd for $C_{16}H_{11}O_5$, 283.061; ^1H NMR (470 MHz, DMSO-d_6): see Table 1.

Acetylation of compound **8**. Under the same conditions on those used for **3** gave crystals mp 178–179°. CIMS (rel. int.); 501 $[M+H]^+$ (100), 459 $[M+H-Ac]^+$ (85), 441 (10) and 399 (8); ^1H NMR (470 MHz, $CDCl_3$): δ2.14 (*s*, 3H, OAc-5′), 2.14 (*s*, 3H, OAc-4′), 2.48 (*s*, 3H, OAc-1), 3.48 (*dd*, 1H, $J = 15.7$, 9.8 Hz, H-1′a), 3.63 (*dd*, 1H, $J = 15.7$, 9.8 Hz, H-1′b), 4.00 (*s*, 3H, OMe-5), 4.23 (*d*, 1H, $J = 11.5$ Hz, H-5′a), 4.23 (*d*, 1H, $J = 11.5$ Hz, H-5′b), 4.27 (*d*, 1H, $J = 11.8$ Hz, H-4′a), 4.50 (*d*, 1H, $J = 11.8$ Hz, H-4′b), 5.11 (*t*, 1H, $J = 9.8$, H-2′), 6.53 (*s*, 1H, H-2), 7.18 (*dd*, 1H, $J = 7.9$, 1.5 Hz, H-6), 7.26 (*t*, 1H, $J = 7.9$ Hz, H-7), 7.79 (*dd*, 1H, $J = 7.9$, 1.5 Hz, H-8).

5′-Hydroxypsorospermin (**9**). Crystallization of fraction S_{21} of F10 yielded 84 mg of white fine needles, mp 255–256° (MeOH); UV λ_{max}^{MeOH} nm^{-1} (log ε): 240 (4.33), 244 (4.32), 312 (4.18) and 355 (3.54); IR ν_{max}^{KBr} cm^{-1}: 3400, 3300, 1630, 1590, 1370, 1240, 1125 and 900; CIMS (CH_4), *m*/*z* (rel. int.), obsd. 357.090 $[M+H]^+$ (100) calcd. $[C_{19}H_{16}O_7+H]^+$, 357.090; 339 $[M+H-H_2O]^+$ (17), obsd. 283.061 (29) calcd. $C_{16}H_{11}O_5$, 283.061; ^1H NMR (470 MHz, DMSO-d_6): see Table 1; ^{13}C NMR (50 MHz, DMSO-d_6): see Table 2. $ED_{50} = 7 \times 10^{-5}$ g/ml in 9PS; 3PS T/C % 133/15, 133/7.5, 136/3.75, 126/1.88 mg/kg.

5′-Hydroxypsorospermin diacetate. A solution of 5′-hydroxypsorospermin (2 mg) in pyridine (0.5 ml) and Ac_2O (0.5 ml) was stirred overnight at room temp. The soln was evapd *in vacuo* and the residue was purified by prep. TLC on silica gel (5 × 20, 0.25 mm. The eluted material was crystallized from MeOH, mp 233–235°. EIMS (rel. int.): 440 (9), 283 (88), 265 (67) and 253 (100); ^1H NMR (470 MHz, $CDCl_3$); δ2.15 (*s*, 3H, Ac-5′), 2.39 (*s*, 3H, Ac-5), 3.25 (*dd*, 1H, $J = 14.90$, 9.8 Hz, H-1′a), 3.40 (*dd*, 1H, $J = 14.9$, 8.2 Hz, H-1′b), 3.85 (*t*, 2H, $J = 13$ Hz, H-4′a and H-4′b), 3.85 (*s*, 3H, OMe-1), 4.23 (*d*, 1H, $J = 11.7$ Hz, H-5′a), 4.35 (*d*, 1H, $J = 11.7$ Hz, H-5′b), 5.12 (*dd*, 1H, $J = 9.8$, 8.2 Hz, H-2′), 6.2 (*s*, 1H, H-2), 7.28 (*t*, 1H, $J = 7.8$ Hz, H-7), 7.36 (*dd*, 1H, $J = 7.8$, 2 Hz, H-6), 8.12 (*dd*, 1H, 7.8, 2 Hz, H-8).

4′-Chloro-5-O-methyl-3′,4′-deoxypsorospermin-3′,5′-diol (**10**). Trituration of fraction A_7 yielded a yellowish white powder. The precipitate was crystallized from MeOH to yield 25 mg of compound **4**, mp 228–229° (MeOH), UV (MeOH) λ_{max}^{MeOH} nm: (log ε) 243 (4.35), 310 (4.26), 341 (3.80); IR ν_{max}^{KBr} cm^{-1}: 3440, 3370, 1630, 1610, 1570, 1260, 1120; CIMS (CH_4), *m*/*z* (rel. int.): 407 $[M+H]^+$ (32), 371 $[M+H-HCl]^+$ (100), 297 (25); EIMS, obsd 406.0816 $[M]^+$ calcd for $C_{20}H_{19}O_7Cl$, 406.0819; obsd 370.105 $[M-HCl]^+$ calcd. for $C_{20}H_{18}O_7$, 370.105; obsd 297.076, calcd for $C_{17}H_{13}O_5$, 297.072; ^1H NMR (470 MHz, DMSO-d_6): see Table 1; $ED_{50} << 10^{-5}$ μg/ml in 9PS.

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