PSOROSPERMIN, A NEW ANTILEUKEMIC XANTHONE FROM *PSOROSPERMUM FEBRIFUGUM*[1]

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ABSTRACT.—An ethanolic extract of *Psorospermum febrifugum* was fractionated with antileukemic activity *in vivo* in the P388 lymphocytic leukemia in mice and *in vitro* in the KB cell culture system used as a guide. The new antileukemic xanthone psorospermin **1** was isolated, and its structure was elucidated. The chlorohydrin of *O*-methylpsorospermin **2** was also isolated after treatment of the fraction containing psorospermin chlorohydrin **6** with diazomethane. Psorospermin **1** was demonstrated to have significant antitumor activity in the P388 *in vivo* system as well as cytotoxicity against the KB *in vitro* system.

Psorospermum febrifugum Sprach. (Guttiferae) is a woody plant of tropical Africa which has been used as a febrifuge, a leprosy treatment, a poison antidote, and a purgative (1, 2). The only previous chemical studies on *Psorospermum* species resulted in the isolation of an anthraquinone pigment from *P. guineense* (3). An ethanolic extract of *P. febrifugum* was found to exhibit significant activity *in vivo* against the P-388 lymphocytic leukemia (3PS) in mice and *in vitro* against a cell culture derived from a human epidermoid carcinoma of the nasopharynx (9KB) (4). Consequently, an investigation, guided by biological activity, was undertaken to isolate and characterize the antileukemic compounds of *P. febrifugum*.

The dried ground roots of *P. febrifugum* were extracted with ethanol. The resulting gum was partitioned between chloroform and water with the activity being concentrated in the chloroform layer. The chloroform soluble material was then partitioned between petroleum ether and aqueous methanol (CH_3OH-H_2O, 9:1). The biologically active methanol fraction was subjected to column chromatography on SilicAR CC-7 and eluted with chloroform followed by chloroform containing increasing amounts of methanol. The activity was concentrated in a fraction eluted with 2% methanol in chloroform. This material was subjected to low-pressure liquid chromatography on silica gel and eluted with benzene containing increasing amounts of ethyl acetate. The active fraction which eluted with 60% ethyl acetate in benzene was crystallized from ethanol and acetone-hexane to produce the new antileukemic xanthone, psorospermin **1**.

Analysis of the fraction eluted from the SilicAR column immediately following **1** by tlc suggested that it contained a compound related to psorospermin **1**. Low-pressure liquid chromatography of this fraction on silica gel with acetone in hexane as eluent partially purified the material, but isolation was difficult due to the fraction's very limited solubility. Thus the decision was made to isolate this material as its methyl ether. Methylation of the partially purified fraction with diazomethane in ether, followed by preparative tlc, first on silica gel developed with methanol-chloroform, then on ChromAR with ethyl acetate-dichloromethane as eluent, gave, after crystallization from ethyl acetate, compound **2**.

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[1]Tumor Inhibitors, Part 129. For the previous paper in this series, see S. M. Kupchan, H. Meshulam and A. T. Sneden, *Phytochemistry*, **18**, 767 (1978).

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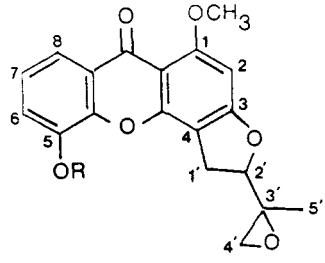
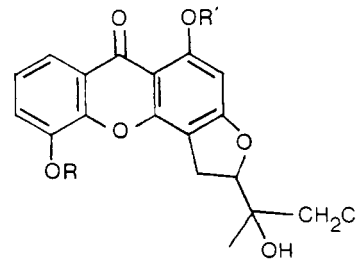
Elemental analysis and high resolution mass spectrometry established a molecular formula of $C_{19}H_{16}O_6$ for psorospermin **1**. A strong band at 1635 cm^{-1} in the infrared spectrum together with the ultraviolet spectrum [λ max (ε) 247 nm (40,900), 310 (17,200)] of **1** suggested that **1** was a xanthone. The nmr spectrum of **1** con-





1 R = H

3 R = CH_3

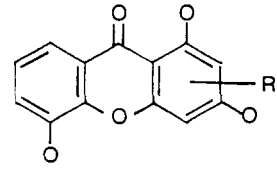
4 R = $COCH_3$

2 R = CH_3; R′ = CH_3

5 R = CH_3; R′ = H

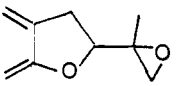
6 R = H; R′ = CH_3

tained signals for four protons in the aromatic region, a low field doublet of doublets (J = 2.3 and 7.0 Hz) at δ 7.79, a two proton multiplet at ca. δ 7.2, and a high-field, one proton singlet at δ 6.26. The nmr data confirmed the xanthonic nature of **1** and indicated that oxygenation was at C-1, C-3, and C-5, a common oxygenation pattern among the xanthones. The high field singlet was assigned to a proton on the electron-rich phloroglucinol ring. This ring, therefore, had to contain an alkyl substituent at C-4 or C-2 in order for this signal to appear as a singlet. The low field doublet of doublets was attributed to the C-8 hydrogen which is deshielded by the xanthone carbonyl (5). In the nmr spectrum of **1** obtained in pyridine-d_5, the C-6 and C-7 protons, which were unresolved in deuterochloroform, were resolved into separate resonances, clearly demonstrating the presence of three vicinal aromatic protons. This data may be accounted for by partial structure A.



A

The nmr spectrum of **1** also contained a doublet of doublets (J = 7.0 and 9.3 Hz) at δ 4.87, coupled to a two-proton multiplet centered at δ3.37, forming an ABX pattern (J_{AB} = 15.0 Hz) and peaks corresponding to an aromatic methoxyl (δ 3.92), a methyl singlet at δ 1.39, and a pair of doublets (J = 4.5 Hz) at δ 2.68 and δ 2.93. The latter signals are characteristic of the geminal protons of an α,α-disubstituted epoxide. This data plus the requirements of structure A could be accounted for by combining the R group and one oxygen in a dihydrofuran ring as in partial structure B.



B

Since two of the three oxygen substituents on the xanthone nucleus had been accounted for (one in the dihydrofuran ring and one as a methoxyl) and all of the carbons were accounted for, the remaining oxygen had to be present as a hydroxyl. Methylation of **1** with ethereal diazomethane gave a monomethyl ether **3**, establishing that the hydroxyl group was not at C–1, since hydroxyl groups at this position are unreactive toward diazomethane due to hydrogen bonding to the xanthone carbonyl (5). Treatment of psorospermin **1** with pyridine-acetic anhydride gave the acetate, **4**. A comparison of the nmr spectra of **1** and **4** revealed that the C–8 hydrogen signal of **4** had shifted to lower field by ca. 0.4 ppm. This paramagnetic shift indicated that acetylation had occurred in the same ring as C–8 and, therefore, allowed the unambiguous assignment of the free hydroxyl group in **1** to C–5 (6).

The relative positions of the methoxyl and the dihydrofuran ring were determined from studies of the nuclear Overhauser effect (NOE). NOE has been used to distinguish linear and angular pyranoxanthones (7). In the latter case, irradiation of the C–1 methoxyl causes a 31% enhancement in the singlet assigned to the C–2 proton; while in the former case only a 10% enhancement in the C–1′ doublet is seen. In the present case, irradiation of the methoxyl signal of a degassed deuterochloroform sample of psorospermin acetate **4** resulted in a 30% enhancement of the aromatic singlet at δ 6.37. Thus, this singlet could be unambiguously assigned to a proton at C–2 and, as a consequence, the dihydrofuran ring must be at C–3 and C–4, which means that psorospermin must have structure **1**.

It now remained to establish the relationship between **1** and **2**. Elemental analysis and high resolution mass spectrometry revealed the presence of chlorine in **2** and established a molecular formula of $C_{20}H_{19}O_6Cl$. The aromatic region of the nmr spectrum of **2** was very similar to that of **1**, showing a doublet of doublets ($J=2.7$ and 7.0 Hz) at δ 7.82, a two-proton multiplet at δ 7.2, and a one-proton singlet at δ 6.21. In addition, the nmr spectrum contained signals for two methoxyl groups (δ 3.86 and 3.93), a tertiary methyl (δ 1.37), and a one-proton triplet ($J=9$ Hz) at δ 5.08 coupled to a two-proton doublet at δ 3.38. The signals due to the epoxide proton of **1** were missing, however, and were replaced with a two-proton AB pattern ($J=8.2$ Hz) at δ 3.67 and 3.74. Consideration of the above spectral data led to the conclusion that **2** was the chlorohydrin of psorospermin methyl ether **3**. This was demonstrated by the conversion of **2** to **3** by treatment with base. The readily accomplished conversion of **2** to **3** and the resistance of **2** toward acetylation ruled out an alternate structure in which the hydroxyl and chlorine would be transposed. The reverse reaction, the conversion of **3** to the chlorohydrin **2**, was very facile and could be accomplished under a variety of acidic conditions. Partial conversion was even observed after prolonged treatment of **3** with chloroform. This raises the possibility that the parent compound from which **2** was derived was not a true natural product, but rather an artifact of the isolation process. This question has been investigated previously in relation to the sesquiterpene expoxide, euparotin, and its chlorohydrin eupachlorin (8), but similar studies were not carried out in the present case.

In order to establish the position of the methoxyl groups, **2** was treated with boron trichloride. Boron trichloride is known to selectively demethylate methoxyl

groups which are *ortho* to a carbonyl function (9). A C–1 methoxyl of a xanthone meets this requirement, and demethylation proceeds readily, leaving other methoxyl groups untouched (7, 9). The reaction of **2** with boron trichloride proceeded smoothly to give a product, **5**, which was shown by high resolution mass spectrometry and elemental analysis to be monodemethylated. The nmr spectrum of **5** confirmed that only demethylation had taken place. The spectrum was very similar to that of **2**, the only difference being that one of the methoxyl signals of **2** had disappeared and was replaced with a sharp one-proton singlet at δ 12.97, characteristic of the hydrogen bound hydroxyl proton of 1-hydroxyxanthones (10). Furthermore, **5** gave a strong positive ferric chloride test and showed a bathochromic shift in its ultraviolet spectrum upon addition of aluminum chloride, both diagnostic for 1-hydroxyxanthones. Chlorohydrin **2** was demonstrated, therefore, to contain a methoxyl group at C–1, and, owing to the interconversion of psorospermin **1** and **2**, confirms the conclusion that psorospermin **1** also contains a C–1 methoxyl. Consequently, **2** must be *O*-methylpsorospermin chlorohydrin and the compound from which **2** was derived by methylation must be psorospermin chlorohydrin **6**.

The stereochemistry of psorospermin **1** has not been determined. Chemical methods were precluded by lack of sufficient quantities of material, and a suitable crystal could not be obtained for X-ray crystallographic determination of the stereochemistry.

Psorospermin **1** is one the few naturally occuring xanthones to show significant antileukemic activity. It demonstrated P–388 activity (e.g. T/C 158% at 8 mg/kg) at doses ranging from 8 to 0.1 mg/kg and some cytotoxicity against the KB cell culture ($ED_{50}=10^{-1}$ μg/ml). It also was found to inhibit mitosis in sea urchin eggs at 10^{-6} M.

EXPERIMENTAL[4]

PLANT MATERIALS.—Dried roots of *Psorospermum febrifugum* Sprach. (B 676297, PR–40806), collected in October, 1973, in Tanzania, were supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, Maryland, where voucher specimens are preserved.

EXTRACTION AND PRELIMINARY FRACTIONATION.—The dried ground roots of *P. febrifugum* (5 kg) were extracted with 95% ethanol in a Soxhlet extractor, and the ethanol extract was evaporated under reduced pressure to yield a dark brown gum (A, 1160 g). Fraction A was partitioned between water (2.5 liters) and chloroform (2.5 liters, 3 x 1.5 liters), and the chloroform layer was evaporated to give fraction B (406 g). Partitioning of Fraction B between petroleum ether (6 x 2 liters) and 10% aqueous methanol (2 liters) afforded, after evaporation, fractions C (225 g) and D (105 g), respectively. Fraction D was subjected to column chromatography (SilicAR CC–7, 2.3 kg) with chloroform followed by chloroform containing increasing amounts of methanol as eluent. Elution with 2% methanol in chloroform gave fraction E (4.6 g) followed by fraction F (3.1 g).

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[4]Melting points were determined on a Mettler model FP2 hot stage and are uncorrected. Ultraviolet absorption spectra were determined on a Beckman model DK–2A recording spectrophotometer. Infrared spectra were determined on Perkin-Elmer model 257 and model 337 recording spectrophotometers. Nuclear magnetic resonance spectra were determined on a JEOL PS–100 pFTNMR spectrometer interfaced to a Texas Instrument JEOL 980A computer with tetramethylsilane as an internal standard. Mass spectra were determined on Hitachi Perkin-Elmer model RMU–6E and AEI Model MS–902 spectrometers. Values of $[\alpha]$ D were determined on a Perkin-Elmer model 141 automatic polarimeter. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Michigan, and Atlantic Microlab, Inc., Atlanta, Georgia. SilicAR CC–7 (Mallinckrodt) and silica gel 40 (EM Reagent) were used for column chromatography. ChromAR 7GF (Mallinckrodt) and silica gel 60 F254 (EM Reagent) prepared plates were used for preparative tlc. The P388 lymphocytic leukemia *in vivo* assays were performed at Raltech Associates, Madison, Wisconsin, and the 9KB cytotoxicity assays were performed at Arthur D. Little, Inc., Cambridge, Massachusetts.

ISOLATION OF PSOROSPERMIN **1**.—Low pressure liquid chromatography of fraction E (Silica Gel 40,200 g) with ethyl acetate in benzene as eluent, afforded fractions G (0.3 g) and H (0.15 g) upon elution with 60% ethyl acetate-benzene. Fraction G was crystallized from ethanol, combined with material from preparative tlc (silica gel, 5% methanol-chloroform) of fraction H, and recrystallized from acetone-hexane to give psorospermin **1**, 0.2 g, 0.004%: mp 280–282° (227–228° from ethanol); $[\alpha]^{21}D$ −71.3° (*c* 0.32, acetone); uv max (EtOH) λ (ε) 340 nm (sh), 310 (17,200), 300 (sh), 247 (40,900), 240 (sh); ir (KBr) 3500 (br), 2950, 1635, 1585, 1485, 1420, 1380, 1340, 1285, 1225, 1130, 1090, 1075, 1020, 970, 860, 810, 770 cm^{-1}; nmr ($CDCl_3$) δ 1.39 (3H, s, 5'-H), 2.68, 2.93 (ea 1 H, d, *J*=4.5 Hz, 4'-H), 3.24 (1H, dd, *J*=15 and 7 Hz, 1'-H), 3.49 (1H, dd, *J*=15 and 9.3 Hz, 1'-H), 3.92 (3H, s, $-OCH_3$), 4.87 (1H, dd, *J*=7 and 9.3 Hz, 2'-H), 6.29 (1H, s, 2-H), 7.2 (2H, m, 6- and 7-H), 7.79 (1H, dd, *J*=2.3 and 7.0 Hz, 8-H); mass spectrum 340.0943 (M^+, calcd for $C_{19}H_{16}O_6$, 340.0947).

Anal. Calcd for $C_{19}H_{16}O_6 \cdot 1.5\ H_2O$: C, 62.12; H, 5.21. Found: C, 62.22; H, 5.04.

ISOLATION OF CHLOROHYDRIN **2**.—Fraction F was subjected to low pressure column chromatography (Silica Gel 40,200 g) with acetone in hexane as eluent. Elution with 35% acetone-hexane yielded fraction I (0.4 g), which was stirred in excess ethereal diazomethane for 16 hr. Evaporation of the solvent, followed by preparative tlc, first on silica gel with 5% methanol-chloroform as eluent, then on ChromAR, developed with 50% ethyl acetate-dichloromethane, afforded a residue, which was crystallized from ethyl acetate to give **2**, 0.053 g, 0.001%: mp 218–220°; $[\alpha]^{28}D$ −132° (*c* 0.268, EtOH); uv max (EtOH) λ (ε) 310 nm (18,600), 300 (sh), 246 (44,500), 239 (37,100); ir (KBr) 3430, 1645, 1595 cm^{-1}; nmr ($CDCl_3$) δ 1.37 (3H, s, 5'-H), 3.38 (2H, d, *J*=9 Hz, 1'-H), 3.67, 3.74 (2H, ABq, *J*=8.2 Hz, 4'-H), 3.86, 3.93 (each 3H, s, $-OCH_3$), 5.08 (1H, t, *J*=9 Hz, 2'-H), 6.21 (1H, s, 2-H), 7.2 (2H, m, 6- and 7-H), 7.82 (1H, dd, *J*=2.7 and 7.0 Hz, 8-H); mass spectrum (chemical ionization: methane reagent gas) 391.0948 (M^++1, calcd for $C_{20}H_{19}O_6Cl$+H, 391.0948).

Anal. Calcd for $C_{20}H_{19}O_6Cl$: C, 61.47; H, 4.90; Cl, 9.07. Found: C, 61.39; H, 4.86; Cl, 8.98.

METHYLATION OF PSOROSPERMIN **1**.—A suspension of 32 mg of psorospermin **1** in 2 ml of ethereal diazomethane was stirred at room temperature for 16 hr. The solvent was evaporated, and the residue was subjected to preparative tlc on silica gel, developed with 5% methanol-chloroform. Elution of the major band, which had a bright blue fluorescense under long-wave uv light, gave a colorless glass (28 mg), which was crystallized from ethanol to afford **3**: mp 253–254°; $[\alpha]^{29}D$ −77.3° (*c* 0.194, $CHCl_3$); uv max (EtOH) λ (ε) 339 nm (6300), 309 (19,600), 246 (47,600), 240 (sh) (41,700); ir (KBr) 1655, 1635, 1595, cm^{-1}; nmr ($CDCl_3$) δ 1.41 (3H, s, 5'-H), 2.69, 2.94 (ea 1H, d, *J*=4.4 Hz, 4'-H), 3.29 (1H, dd, *J*=7.6 and 15.0 Hz, 1'-H), 3.54 (1H, dd, *J*=9.3 and 15.0 Hz, 1'-H), 3.94, 3.97 (ea 3H, s, $-OCH_3$), 4.84 (1H, dd, *J*=7.6 and 9.3 Hz, 2'-H), 6.36 (1H, s, 2-H), 7.2 (2H, m, 6- and 7-H), 7.88 (1H, dd, *J*=2.7 and 6.9 Hz, 8-H); mass spectrum (chemical ionization: methane reagent gas) 355.1177 (M^-+1, calcd for $C_{20}H_{15}O_6$+H, 355.1182).

Anal. Calcd for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.66; H, 5.12.

ACETYLATION OF PSOROSPERMIN **1**.—A solution of 33 mg of psorospermin **1** in 0.5 ml pyridine containing 5 drops of acetic anhydride was allowed to stand at room temperature for 2.5 hr. The solvent was evaporated, and the residue was crystallized from acetone to give psorospermin acetate **4**: mp 190–192°; $[\alpha]^{21}D$ −93° (*c* 0.25, $CHCl_3$); uv max (EtOH) λ (ε) 339 nm (7100), 307 (14,900), 287 (9000), 254 (21,800), 237 (34,600); ir ($CHCl_3$) 3010, 2935, 2860, 1765, 1650, 1635, 1595; nmr ($CDCl_3$) δ 1.44 (3H, s, 5'-H), 2.43 (3H, s, -OAc), 2.47, 2.97 (ea 1H, d, *J*=4.8 Hz, 4'-H), 2.83 (1H, dd, *J*=8 and 15.5 Hz, 1'-H), 3.08 (1H, dd, *J*=10 and 15.5 Hz, 1'-H), 3.97 (3H, s, $-OCH_3$), 4.88 (1H, dd, *J*=8 and 10 Hz, 2'-H), 6.37 (1H, s, 2-H), 7.3 (2H, m, 6- and 7-H), 8.17 (1H, dd, *J*=2.0 and 6.3 Hz, 8-H); mass spectrum (chemical ionization: methane reagent gas) 383.1133 (M^-+1, Calcd for $C_{21}H_{18}O_7$+H, 383.1131).

Anal. Calcd for $C_{21}H_{18}O_7$: C, 65.97; H, 4.74. Found: C, 65.93; H, 4.75.

DEMETHYLATION OF **2**.—To a solution of **2** (28 mg) in 1.5 ml of dichloromethane, was added 1 ml of a 1 M solution of boron trichloride in dichloromethane. The reaction mixture turned orange immediately. After stirring at room temperature for 20 min, the reaction mixture was poured into 20 ml of water and extracted with 15 ml of chloroform. The chloroform layer was washed with water, evaporated, and subjected to preparative tlc on silica gel, developed with 3% methanol-chloroform. Elution of the ferric chloride-active band gave a yellow glass (19 mg), which was crystallized from methanol to give **5**: mp 180–182°; $[\alpha]^{29}D$ −89.3° (*c* 0.101, $CHCl_3$); uv max (EtOH) λ (ε) 319 nm (11,400), 257 (16,800), 244 (21,800), 238 (20,400); +$AlCl_3$, 338, 324, 278, 270, 248 (sh), 240 (sh), 231; ir (KBr) 3530, 3460, 1655, 1610, 1575 cm^{-1}; nmr ($CDCl_3$) δ 1.37 (3H, s, 5'-H), 3.37 (2H, d, *J*=9 Hz, 1'-H), 3.70 (2H, s, 4'-H), 3.97 (3H, s, $-OCH_3$), 5.09 (1H, t, *J*=9 Hz, 2'-H), 6.23 (1H, s, 2-H), 7.2 (2H, m, 6- and 7-H), 7.79 (1H, dd, *J*=3 and 6.5 Hz, 8-H), 12.97 (1H, s, 1-OH); mass spectrum (chemical ionization: methane reagent gas) 377.0787 (M^-+1, calcd for $C_{19}H_{17}O_6Cl$+H, 377.0792).

Anal. Calcd for $C_{19}H_{17}O_6Cl$: C, 60.57; H, 4.55. Found: C, 60.50; H, 4.57.

CONVERSION OF **2** TO **3**.—A mixture of dhlorohydrin **2** (5 mg) and potassium *tert*-butoxide (3.8 mg) were stirred in dry tetrahydrofuran (1 ml) at room temperature for 1 hr. A few drops of methanol were added, and the suspension was filtered through a small column of silica gel packed in 10% methanol-ethyl acetate. The chlumn was washed with additional methanol-ethyl acetate, and the combined filtrates were evaporated to give **3** (3.7 mg, 82%), which was identical (tlc behavior, nmr, ir , and mass spectra) with psorospermin methyl ether **3** obtained from methylation of psorospermin **1**.

ACKNOWLEDGMENTS

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