mmol) in EtOH (50 mL) under stirring. The solution was refluxed for 24 h, then a second portion of KOH (0.26 g, 4 mmol) was added, and the mixture was refluxed for additional 2 h. The mixture was filtered until hot to remove crude **34** (ca. 5%), and the filtrate was kept overnight in a refrigerator. The crystalline precipitate that deposited was collected by filtration, washed with water, and recrystallized from DMF to give **36** in 15% yield.

Reaction of 7 with 1,3-Dibromopropane. The general procedure was followed except for the substitution of 1,3-dibromopropane (10 mol). After removal of ca. 500 mL of solvent from the reaction mixture, the residue was kept overnight in a refrigerator. The crystalline material that precipitated was collected by filtration and recrystallized from DMF to give 1,10-dithia[4.4](3,5)-1,3,4-thiadiazolinophane-6,15-dithione (**37**) as yellowish prisms: 0.85 g, 45%; mp 210–212 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.44 (m, $SCH_2CH_2CH_2N$, 4 H), 3.39 (dd, J = 5.5 $SCH_2CH_2CH_2N$, 4 H), and 4.24 (t, J = 5.5, $SCH_2CH_2CH_2N$, 4 H); ^{13}C NMR (Me_2SO-d_6) δ 21.5 ($SCH_2CH_2CH_2N$), 27.3 ($SCH_2CH_2CH_2N$), 50.2 ($SCH_2CH_2CH_2N$), 161.5 (C_8 = C_{17}), and 186.3 (C_6 = C_{15}); MS (18 eV), m/z 380 (M^+, 5). Anal. Calcd for $C_{10}H_{12}N_4S_6$: C, 31.55; H, 3.18; N, 14.72. Found: C, 31.68. H, 3.12, N, 14.45.

4,5-Dihydro-1,3-thiazino[2,3-*b*][1,3,4]thiadiazolium Bromide (40). Solutions of 2-mercapto-5-(methylthio)-1,3,4-thiadiazole sodium salt (0.31 g, 1.68 mmol), generated from **3** by treatment with 1 equiv of EtONa, and 1,3-dibromopropane (0.34 g, 1.68 mmol) in absolute EtOH (10 mL) were dropped separately but synchronously from two dropping funnels into absolute EtOH (10 mL) under stirring. The solution was heated at reflux for 17 h. After cooling, the mixture was concentrated to dryness and extracted with benzene. Evaporation of the solvent left crude crystals of **13** (0.17 g, 60%). The residue was extracted several times with hot $CHCl_3$. Concentration of the chloroform solution gave a crystalline material, which did not redissolve in $CHCl_3$. It was recrystallized from EtOH–AcOEt to give white crystals of **40**: 0.15 g, 31%; mp 195–200 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 2.46 (m, $SCH_2CH_2CH_2N^+$, 2 H), 2.76 (s, SCH_3, 3 H), 3.50 (dd, J = 7.5, $SCH_2CH_2CH_2N^+$, 2 H), and 4.52 (br t, J = 6.5, $SCH_2CH_2CH_2N^+$, 2 H); ^{13}C NMR (Me_2SO-d_6) δ 17.1 (SCH_3), 21.5 ($SCH_2CH_2CH_2N^+$), 28.3 ($SCH_2CH_2CH_2N^+$), 52.0 ($SCH_2CH_2CH_2N^+$), 167.2 (C=N), and 171.6 (C=N^+); MS, m/z 284 (M^+, 9). Anal. Calcd for $C_6H_9BrN_2S_3$: C, 25.26; H, 3.18; N, 9.82. Found: C, 25.04; H, 3.02; N, 10.14.

2,5-Bis(methylthio)-3-methyl-1,3,4-thiadiazolium Iodide (41). A mixture of **17** (1.78 g, 10 mmol) and methyl iodide (2 mL) was heated at 50 °C for 20 h in a stoppered flask. The crystalline quaternary salt was collected by filtration, washed thoroughly with anhydrous Et_2O, and dried: 2.4 g, 75%; ^{1}H NMR (Me_2SO-d_6) δ 2.76 (s, C_5—SCH_3, 3 H), 3.03 (s, C_2—SCH_3, 3 H), and 4.05 (s, NCH_3, 3 H); ^{13}C NMR (Me_2SO-d_6) δ 16.7 (C_5—SCH_3), 20.7 (C_2—SCH_3), 41.9 (NCH_3), 167.9 (C_5), and 177.9 (C_2). Anal. Calcd for $C_5H_9IN_2S_3$: C, 18.75; H, 2.83; N, 8.75. Found: C, 18.85; H, 2.87; N, 8.71.

On heating for several hours in absolute EtOH, salt **41** was converted almost quantitatively to **23**, identical in all respects with an authentic sample.

1-[(3-Methyl-2-thioxo-1,3,4-thiadiazolin-5-yl)thio]-3-[5-(methylthio)-2-thioxo-1,3,4-thiadiazolin-3-yl)]propane (42). A mixture of salt **40** (142 mg, 0.5 mmol), thiol **22** (82 mg, 0.5 mmol), and EtONa (34 mg, 0.5 mmol) in absolute EtOH (10 mL) was heated at reflux under stirring for 17 h. The solvent was evaporated in vacuo to give a residue, which was extracted with benzene and chromatographed on silica gel, eluting with cyclohexane–ethyl acetate (5:1) to afford **42** as a pale yellow oil: 83 mg, 45%; ^{1}H NMR ($CDCl_3$) δ 2.33 (p, J = 7, $SCH_2CH_2CH_2N$, 2 H), 2.61 (s, SCH_3, 3 H), 3.20 (t, J = 7, $SCH_2CH_2CH_2N$, 2 H), 3.83 (s, NCH_3, 3 H), and 4.42 (t, J = 7, $SCH_2CH_2CH_2N$, 2 H); ^{13}C NMR ($CDCl_3$) δ 15.4 (SCH_3), 27.6 ($SCH_2CH_2CH_2N$), 32.9 ($SCH_2CH_2CH_2N$), 38.7 (NCH_3), 49.3 ($SCH_2CH_2CH_2N$), 154.8, 157.3 (C—S), and 185.9, 186.0 (C=S); MS (18 eV), m/z 368 (M^+, 16). Anal. Calcd for $C_9H_{12}N_4S_6$: C, 29.32; H, 3.28; N, 15.20. Found: C, 29.17; H, 3.33; N, 15.27.

Reaction of 7 with 1,4-Dibromobutane. The general procedure was followed except for the substitution of 1,4-dibromobutane (10 mmol). General workup afforded 1,6,12,17-tetrathia[6.6](2,5)-1,3,4-thiadiazolophane (**43**) as colorless prisms: 0.24 g, 12%; mp 206–208 °C ($CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.96 (m, SCH_2CH_2, 8 H), and 3.30 (m, SCH_2CH_2, 8 H); MS (18 eV), m/z 408 (M^+, 100). Anal. Calcd for $C_{12}H_{16}N_4S_6$: C, 35.27; H, 3.95; N, 13.71. Found: C, 35.38; H, 3.87; N, 13.85.

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Registry No. 1, 1072-71-5; **3**, 6264-40-0; **4**, 96993-48-5; **5**, 103639-76-5; **6**, 105784-95-0; **7**, 4628-94-8; **8**, 62601-22-3; **9**, 103639-76-5; **10**, 105784-96-1; 11, 91021-38-4; **12**, 105784-97-2; **13**, 105784-98-3; **14**, 105784-99-4; **15**, 105785-00-0; **16**, 105785-01-1; **17**, 7653-69-2; **18**, 2245-12-7; **19**, 91021-39-5; **20**, 91021-35-1; **21**, 91021-36-2; **22**, 29546-26-7; **23**, 33682-80-3; **24**, 91021-40-8; **25**, 90567-39-8; **26**, 105785-02-2; **30**, 105785-03-3; **31**, 105785-04-4; **32**, 105785-05-5; **33**, 105785-06-6; **34**, 105785-07-7; **35**, 105785-08-8; **36**, 105785-09-9; **37**, 105785-10-2; **40**, 105785-11-3; **41**, 104249-18-5; **42**, 105785-12-4; **43**, 105817-44-5; CH_2BrCl, 74-97-5; $HOCH_2CH_2Br$, 540-51-2; CH_2Br_2, 74-95-3; $Br(CH_2)_2Br$, 106-93-4; $Br(CH_2)_3Br$, 109-64-8; $Br(CH_2)_4Br$, 110-52-1; CH_2O, 50-00-0; CH_3I, 74-88-4.

Structure and Stereochemistry of Psorospermin and Related Cytotoxic Dihydrofuranoxanthones from *Psorospermum febrifugum*

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Chemical studies of the cytotoxic extract of the plant *Psorospermum febrifugum* (Guttiferae) have led to the reisolation of the antileukemic xanthone psorospermin (**1**) and the discovery of a series of novel bioactive analogues. These analogues include 3′,4′-deoxypsorospermin (**2**), 3′,4′-deoxypsorospermin-3′,4′-diol (**3**), 3′,4′-deoxy-4′-chloropsorospermin-3′-ol (**4**), and O^5-methyl-3′,4′-deoxypsorospermin-3′-ol (**8**). The absolute stereochemistry of **1** was assigned by ORD, ^{1}H NMR, and X-ray studies of **1** and the epimeric epoxytubaic acids (**13a**, **13b**) and epoxyrotenones (**11a**, **11b**). The structures and stereochemistry of **2**–**4** and **8** were established by analysis of MS and ^{1}H NMR data and chemical correlation.

The observation that the extracts of the tropical African plant *Psorospermum febrifugum* Spach. (Guttiferae) exhibited cytotoxic and in vivo antitumor activity in the P388 mouse leukemia assay has stimulated a detailed study to determine the components responsible for these effects. Bioassay-directed chemical studies have led to the isolation

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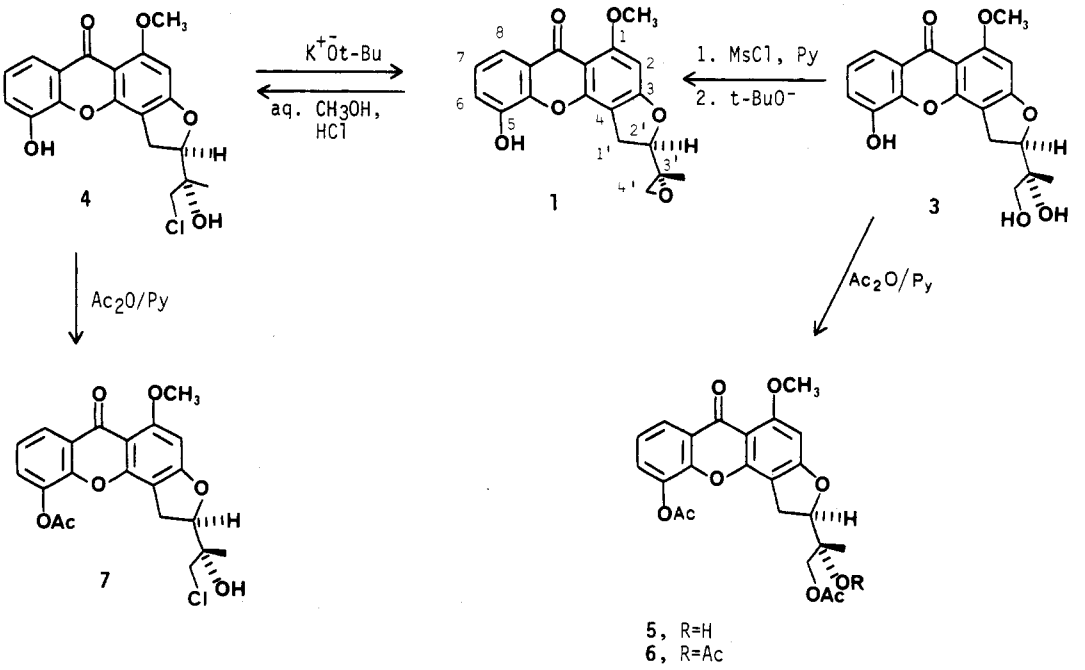
Scheme I. Structures and Chemical Interrelations among Natural Xanthones from *P. febrifugum*

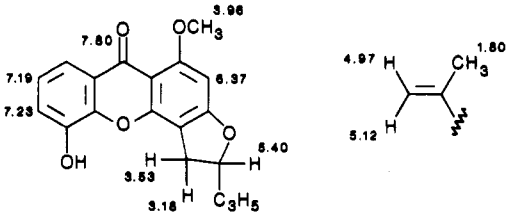


of a series of cytotoxic xanthones. Kupchan and coworkers reported the isolation of the first active constituent in this series, which was named psorospermin (**1**).[1,17] Psorospermin was cytotoxic and showed activity in the P388 mouse leukemia, mammary (CD), and colon (C6) models.[2,3] Investigations in our research group have led to the assignment of the absolute stereochemistry of **1**, as well as the isolation of a bioactive anthrone[4] and a series of dihydrofuranoxanthones[5] including 3′,4′-deoxypsorospermin (**2**), 3′,4′-deoxypsorospermin-3′,4′-diol (**3**), 3′,4′-deoxy-4′-chloropsorospermin-3′-ol (**4**), and O^5-methyl-3′,4′-deoxypsorospermin-3′-ol (**8**). The identification of **2**–**4** and **8** and the stereochemistry of **1**–**4** and **8** are now reported in detail.

Results and Discussion

Fractionation of the ethanolic extract, with in vitro and in vivo activities as a guide for subsequent purification steps, revealed that the cytotoxic activity concentrated in the chloroform fraction.[6,7] Flash column chromatography on silica gel and alumina followed by crystallization gave **1** and the four novel compounds **2**–**4** and **8**.

Compound **2** gave a molecular formula of $C_{19}H_{16}O_5$ on the basis of high-resolution chemical ionization mass spectra (CIMS) data. The 470-MHz ^{1}H NMR showed the existence of spin systems containing three adjacent aromatic protons [7.80 (dd, 7.8 and 1.8 Hz), 7.23 (dd, 7.8 and 1.8 Hz), 7.19 (t, 7.8 Hz) ppm], an aromatic proton flanked by two ortho oxygen substituents [6.37 (s) ppm], an aromatic methoxy group [3.96 (s) ppm], and an aliphatic ABX spin system [5.40 (dd, 9.0 and 7.5 Hz), 3.53 (dd, 15.5 and 7.5 Hz), 3.18 (dd, 15.5 and 9.0 Hz)]. All spin analyses were



further confirmed by decoupling experiments. Comparison of the chemical shifts and coupling patterns with those of psorospermin (**1**; Table I) supported a dihydrofuranoxanthone skeleton. The structure of the remaining C_3H_5 moiety was established by analysis of the ^{1}H NMR signals at 5.12 (1 H, d, 1.0 Hz) and 4.97 (1 H, d, 1.0 Hz) ppm and a three-proton signal at 1.80 (s) ppm. These data support the presence of the isopropenyl group. Thus, the ^{1}H NMR, CIMS, IR, and UV (Table I; Experimental Section) support the structure of 3′,4′-deoxypsorospermin for **2**.

By a similar approach, compound **3** could also be characterized as an analogue of psorospermin (Table I). The molecular formula $C_{19}H_{18}O_7$ was established by high-resolution exact mass measurement. High-field ^{1}H NMR revealed the dihydrofuranoxanthone moiety. The most prominent ^{1}H NMR spectral differences between compounds **2** and **3** were the disappearance of the two geminal olefinic protons (5.12, 4.97 ppm) and the appearance of a new ABX spin system [3.44 (dd, 10.6 and 5.0 Hz), 3.57 (dd, 10.6 and 5.0 Hz), 4.82 (t, 5.0 Hz, D_2O exchangeable) ppm], suggesting the presence of a primary alcohol group.

This ABX spectral pattern was changed to an AB pattern [4.15 (d, 11.3 Hz), 4.34 (d, 11.3 Hz) ppm] upon acetylation. All these spectral data suggested the structure **3**, 3′,4′-deoxypsorospermin-3′,4′-diol, which was unambiguously verified by chemical correlation with psorospermin (**1**) as shown in Scheme I.

The CIMS data for compound **4** revealed the existence of one chlorine atom. Comparison of the high-resolution

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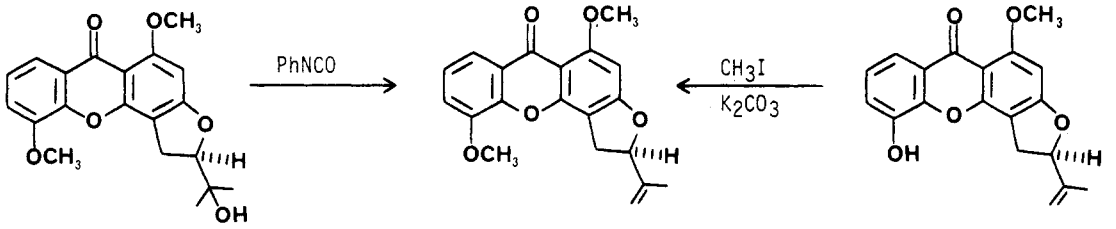
Table I. ^{1}H NMR Spectral Data

	1[a]	2[a]	3[b]	4[b]	8[a,c]
OCH_3-1	3.96, s	3.96, s	3.84, s	3.84, s	3.95, s
H-2	6.37, s	6.37, s	6.51, s	6.51, s	6.34, s
H-6	7.22, dd (7.8, 1.7)	7.23, dd (7.8, 1.8)	7.21, dd (7.3, 2.1)	7.21, dd (7.3, 2.1)	7.13, dd (8, 1)
H-7	7.19, t (7.8)	7.19, t (7.8)	7.15, t (7.3)	7.15, t (7.3)	7.28, t (8)
H-8	7.80, dd (7.8, 1.7)	7.80, dd (7.8, 1.8)	7.48, dd (7.3, 2.1)	7.48, dd (7.3, 2.1)	7.85, dd (8, 1)
H-1′a	3.49, dd (15, 9.9)	3.53, dd (15.5, 7.5)	3.31, d (8.7)	3.30, d (8.7)	3.38, dd (15.3, 8.8)
H-1′b	3.30, dd (15, 7.2)	3.18 dd (15.5, 9.0)	3.31 d (8.7)	3.30, d (8.7)	3.32, dd (15.3, 8.8)
H-2′	4.85, dd (9.9, 7.2)	5.40, dd (9.0, 7.5)	5.00, t (8.7)	5.08, t (8.7)	4.85, t (8.8)
H-4′a	2.98, d (4.5)	5.12, d (1.0)	3.57, dd (10.6, 5.0)	3.80 d (10.7)	1.37, s
H-4′b	2.73, d (4.5)	4.97, d (1.0)	3.44, dd (10.6, 5.0)	3.63, d (10.7)	
H-5′	1.43, s	1.80, s	1.11, s	1.24, s	1.27, s

[a] Spectra were recorded in $CDCl_3$ on the 470-MHz instrument. [b] Spectra were recorded in Me_2SO-d_6 on the 200-MHz instrument. [c] Chemical shifts are given (ppm) relative to $(CH_3)_4Si$. Coupling constants in parentheses are in hertz. Multiplicity: d, doublet; t, triplet; s, singlet. [d] (OCH_3-5): δ 3.97 (s).

Scheme II. Interrelationship of O^5-Methyl-3′,4′-deoxypsorospermin-3′-ol (8) and 3′,4′-Deoxypsorospermin (2)



8 9 2

MS measurement of compound **3** ($C_{17}H_{18}O_7$) with that of compound **4** ($C_{19}H_{17}O_6Cl$) suggested the substitution of a hydroxy group by a chlorine atom. Acetylation of **4** gave monoacetate **7**. The aromatic proton chemical shifts of **7** (6.35, 7.30, 7.30, 8.17 ppm) are similar to those of **5** (6.31, 7.33, 7.33, 8.17 ppm). The ^{1}H NMR spectral pattern of compound **4** is almost completely identical with that of compound **3** except for the AB spin systems at 3.80 (d, 10.7 Hz) and 3.63 (d, 10.7 Hz) ppm. Analysis of these data indicated that **4** was 3′,4′-deoxy-4′-chloropsorospermin-3′-ol. This was further substantiated by chemical interconversion between **1** and **4** as shown in Scheme I.

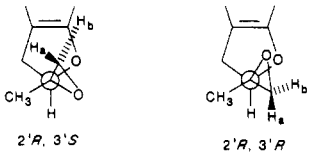
The molecular formula of $C_{20}H_{20}O_6$ for compound **8** was established by high-resolution exact mass measurement. The 470-MHz ^{1}H NMR spectrum of **8** (Table I) showed the existence of two *C*-methyl groups at 1.37 (s) and 1.27 (s) ppm, probably attached to a hydroxy-substituted quaternary carbon. The existence of an aliphatic ABX spin system was reminiscent of the dihydrofuran spin system in psorospermin. The ^{1}H NMR spectrum also revealed the absence of an AB spin system of the epoxide ring in psorospermin and the presence of two aromatic *O*-methyl groups, suggesting the conversion of the 5-hydroxyl group in **1** to a methoxyl group in **8**. This conversion was further confirmed by the typical ortho upfield shift (0.09 ppm) for H_6 and the meta downfield shift for H_7 (0.09 ppm) from **1** to **8**. All the above ^{1}H NMR spectral data pointed to the structure of O^5-methyl-3′,4′-deoxypsorospermin-3′-ol for compound **8**, which was substantiated by chemical correlation with 3′,4′-deoxypsorospermin (**2**) as shown in Scheme II. The optical rotation for compound **9** derived from either **8** or **2** is identical, indicating the same stereochemistry at the 2′-position for **8** and **2**.

Because the stereochemistry of compounds with an (epoxyisopropyl)dihydrofuran system has not yet been defined, the assignment of **1** had to be made by using compounds with an isopropenyldihydrofuran system such as (−)-rotenone (**10**). The absolute configuration of compound **10** was defined[8] and confirmed by X-ray[9,10] as 6a*S*,12a*S*,2′*R*. In order to make a valid comparison of optical activities, rotenone was converted into epoxides **11a** and **11b** and was also degraded to (*R*)-(−)-tubaic acid[11] (**12**), which has one chiral center (Scheme III).

Table II. ^{1}H NMR Chemical Shift Correlation of Epimeric Epoxides

compd	4′aH[a]	Δδ[b]	4′bH	Δδ[b]	3′CH_3
1	2.73		2.98		1.43
		0.02		0.11	
1b[c]	2.75		2.87		1.46
13a	2.70		2.94		1.38
		0.01		0.12	
13b	2.71		2.82		1.41
11a	2.67		2.96		1.40
		0.03		0.17	
11b	2.70		2.79		1.43

[a] The 4′a,4′b-protons are shown in the epimeric epoxides. The 4′aH is syn to the 3′-CH_3 in both epimers and away from the furan oxygen.



[b] Δδ is the difference in chemical shift between the H_a and H_b protons in the epimeric pairs. [c] Synthesis of (±)-(2′*R*,3′*S*)-psorospermin methyl ether (**1b**) has been completed.

Epoxidation of **12** with *m*-chloroperoxybenzoic acid (MCPBA) gave a pair of epimeric epoxytubaic acids that were separated by centrifugal thin-layer chromatography on a chromatotron with 2.5–10% ethyl acetate/hexane as the eluent to give the lower R_f (**13a**) and the higher R_f (**13b**)

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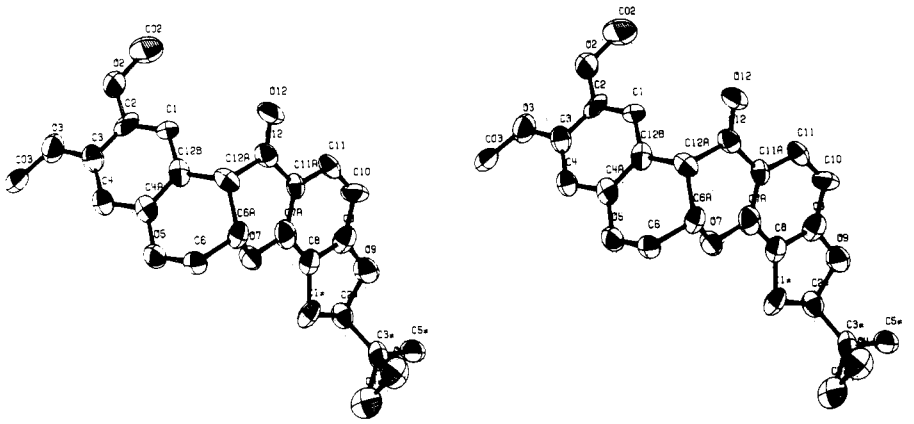
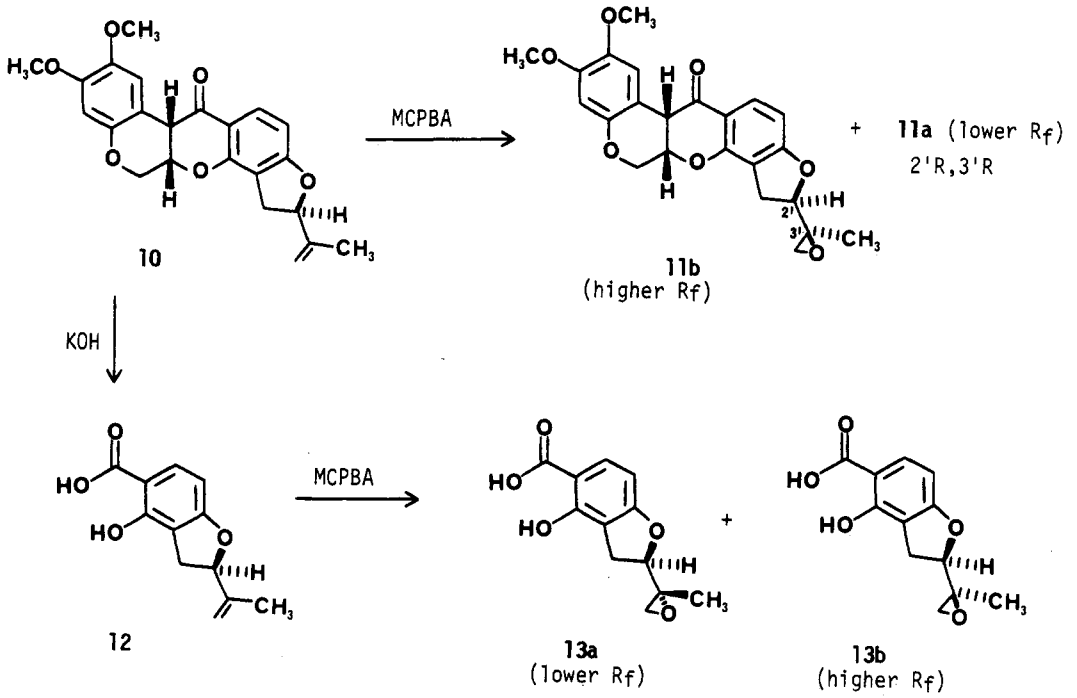


Figure 1. Stereoview of epoxyrotenone (**11b**).

Scheme III. Conversions of Rotenone for Stereochemical Correlation to 1



isomers. The plain Cotton curves of **1**–**4**, **12**, **13a**, and **13b** were all negative,[5] as were those of **11a** and **11b**.[13] Since **13a** and **13b** were 2′*R*, epimeric at the 3′-position, and displayed similar Cotton curves, it can be concluded that a negative Cotton curve shown by this class of compounds is consistent with the 2′*R* configuration. Therefore, the configurations of **1**–**4** and **8** are 2′*R*.

Further comparison of epimers of **1**, **11**, and **13** using ^{1}H NMR indicated that differences in the chemical shifts of the 4′-methylene protons could differentiate the chirality at 3′ (see Table II). In contrast with the 4′a-protons and the 3′-CH_3, which resonate within 0.03 ppm in all of the isomers, there is a clear difference among the 4′b-protons. In each pair of epimers there is a difference ranging from 0.11 to 0.17 ppm. This difference and the downfield position relative to the 4′a-protons must be due to the difference in chirality at 3′ and the proximity of the 4′b-protons to the unshared electron pair on oxygen in the dihydrofuran ring.[12] This distinction between the epimeric epoxyrotenones based on ^{1}H NMR has been previously noted.[13,14] By comparison, it is clear from the difference in chemical shifts that compound **1** has the same chirality at 3′ as **13a**. Therefore, it would be possible to assign the configuration of **1** when that of either **13a** or **13b** is assigned. However, recrystallization of either **13a** or **13b** or their derivatives did not yield any crystals suitable for X-ray analysis.

The use of **11a** or **11b** now becomes valid since the 2′-position of **1** has been defined. Therefore, **10** was epoxidized[14] with *m*-chloroperoxybenzoic acid to give a pair of epimers that were separated on LPS-1 silica gel with 40% ethyl acetate/hexane. Recrystallization of the higher R_f isomer (**11b**) from methanol gave a crystal suitable for X-ray analysis. As a result, the configuration of **11b** was assigned as 6a*S*,12a*S*,2′*R*,3′*S* (Figure 1). It follows the **1** must have the 2′*R*,3′*R* configuration. The fact that both **3** and **4** were converted to **1** establishes the configuration for **3** as 2′*R*,3′*R* and the configuration for **4** as 2′*R*,3′*S*.

The bond lengths (Table III; supplementary material) of epoxyrotenone **11b** are within 3 standard deviations of those for rotenone[12] except for the C8–C7a (1.34 Å) and CO2–O2 (1.38 Å), which differ by 6 standard deviations,

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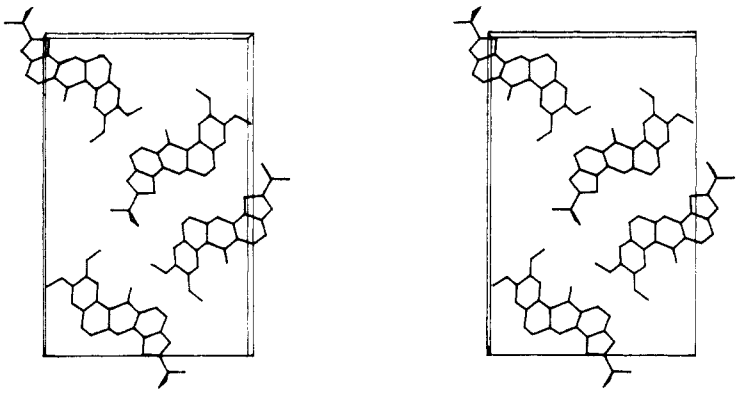

Figure 2. Stereoview of the crystal packing of epoxyrotenone (**11b**) Directions of the crystal axes: b, horizontal; c, verticle; a, out of the plane of the paper.

C12a–C12 (1.55), which differs by 5 standard deviations, and C6–O5, which differs by 4 standard deviations. The C8–C7a (1.34 Å) distance is unusually short for an aromatic C–C bond length.

All of the bond angles (Table IV; supplementary material) of epoxyrotenone **11b** are within 3 standard deviations of those of rotenone[12] except the angle C3′–C2′–O9 (107°), which differs by 4 standard deviations, and this angle is adjacent to the epoxy group. The torsion angles (Table V; supplementary material) of epoxyrotenone **11b** are similar to those of rotenone.[12] The largest differences are in the torsion angles C6–C6a–C12a–C12b, Δ = 6.8°; C6–C6a–C12a–C12b, Δ = 5.3°; O7–C7a–C11a–C12, Δ = 5.2°; C12a–C6a–O7–C7a, Δ = 5.7°; and C11a–C12–C12a–C6a, Δ = 10.8°. The angle between the plane of the A ring and D ring is 111° in **11b** and 105° in rotenone. The angle between the C3–O3–CO3 plane and the A ring is 8.1° in **11b** and 2.9° in rotenone. All of these differences probably reflect the effect of crystal packing on conformation. Larger differences in ring bowing angles of steroid polymorphs have been observed in our laboratory, again showing the effect of crystal packing on conformation.

The crystal packing of **11b** (Figure 2) and rotenone are quite different especially since rotenone contains CCl_4 solvent of crystallization. Epoxyrotenone **11b** appears to contain stacks of molecules rather than the nested packing arrangement observed for rotenone.

The psorospermin series is among a very limited number of naturally occurring xanthones with significant antileukemic activity. The results of testing this short series of natural analogues of **1** have established the importance of the configuration and functionality of the epoxydihydrofuran moiety to activity in vivo. Compound **1** is clearly more active than **2** or **4**; however, fractions containing **3** have demonstrated significant activity and further testing of **3** is underway. Further research is also under way to establish the importance of xanthone and dihydrofuran ring substituents to antileukemic activity in this series.

Experimental Section

Extraction and Fractionation. *P. febrifugum* Spach. (Guttiferae) roots were collected in Tanzania by Leonard Mwasumbi of the University of Dar-es-Salam. Specimens were authenticated at the Economic Botany Laboratory, Beltsville Agricultural Research Center, Beltsville, MD, where a voucher specimen is on deposit.

The large, woody roots were coarsely chipped, defatted by percolation with hexane, and then finely ground. The powdered plant material (10 kg) was extracted with hexane and then with 95% ethanol by slow percolation at room temperature. The extract was concentrated in vacuo at 40 °C to give a solid marc, 830 g. These solids were dissolved in 3.5 L of 95% ethanol to which 16 L of acetone was added with vigorous stirring. This mixture was allowed to stand until precipitation was complete; then, the precipitate was separated, and the filtrate was concentrated in vacuo, giving another precipitate. This procedure was repeated (4×), yielding precipitates totaling 245 g. The precipitates were tested and found to be inactive in the P388 in vivo test system.

The final filtrate was concentrated to 1 L, and further precipitation was induced by stirring with 8 L of chloroform. This procedure was repeated (3×), giving a solid residue totaling 390 g. This residue was tested against P388 in vivo and was toxic at 200 mg/kg and inactive at lower doses.

The chloroform fraction was concentrated to give 134 g of a solid residue that showed activity (132% T/C at 100 mg/kg) in P388 in vivo. This material was then dissolved in 1.5 L of methanol to which water was added to give a 30% aqueous solution. A resinous precipitate formed on standing that was separated from the supernatant by decantation. The precipitate weighed 91.5 g and was inactive in the P388 test system. The supernatant was then concentrated to 1 L, transferred to a separatory funnel, and extracted six times with an equal volume of hexane/toluene (1:1). The pooled hexane/toluene-soluble fraction weighed 15.5 g and showed no activity in P388 in vivo.

The remaining aqueous methanol phase was then extracted six times with 1 L of chloroform. The chloroform extracts were pooled and concentrated to give a solid (20.4 g) that showed activity (133% T/C at 25 mg/kg) in P388 in vivo and cytotoxicity (ED_{50} 1 × 10^{-2} μg/mL) in P388 in vitro. The material remaining in the aqueous methanolic solution was not active in P388 in vivo.

The active $CHCl_3$-soluble fraction (11.5 g) was flash chromatographed on a 7 × 45 cm column of EM 9385 silica, developed initially with hexane/toluene/ethyl acetate (3:3:1; 225 L), and increasing stepwise in polarity. Fractions (500 mL) were collected, concentrated in vacuo at 40 °C, and analyzed by TLC. On the basis of this analysis, 13 pooled fractions were formed from the 146 column cuts.

Psorospermin (1). Column fraction 4 (eluted in hexane/ethyl acetate (1:1), cuts 55–63, 426 mg) was triturated with acetone/hexane to remove yellow pigments. This residue was recrystallized alternately from methanol/ether or acetone/hexane to give 10 mg of psorospermin (1) as colorless needles: mp 229–230 °C (lit.[1] mp 227–228 °C, EtOH); ED_{50} = 1.5 × 10^{-2} μg/mL in 9KB and T/C 158% at 8 mg/kg in P388 mouse leukemia (NSC 266491). The UV, IR, and ^{1}H NMR spectra were identical with those published for psorospermin (1).[1]

3′,4′-Deoxypsorospermin (2). Column fraction 2 (eluted in hexane/toluene/ethyl acetate, cuts 17–22, 0.74 g) showed a major bright blue fluorescent spot on TLC. Flash chromatography through a short column of aluminum oxide with a gradient of increasing ethyl acetate in chloroform yielded 6 mg of 3′,4′-deoxypsorospermin (**2**). Compound **2** crystallized from methanol/ether as small needles: mp 228–230 °C; $[\alpha]^{20}_D$ −46° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 340 (sh), 310 (4.13), 247 (4.59), 239 (sh), nm; IR (KBr) ν_{max} 3300, 1647, 1600 cm^{-1}; ^{1}H NMR, see Table I; CIMS (CH_4) *m/z* (relative intensity) 325 ($M + H^+$, 100), 307 ($M + H^+ - H_2O$, 1), 279 (4), 181 (12); EIMS for $C_{19}H_{16}O_5$, obsd 324.0996 (M^+), calcd 324.0997; ED_{50} = 0.8 μg/mL in 9KB.

3′,4′-Deoxypsorospermin-3′,4′-diol (3). Column fraction 9 (eluted in ethyl acetate, cuts 111–128, 310 mg) was triturated with methanol to give crude **3**. Compound **3** crystallized as needles (225 mg) from MeOH/$CHCl_3$: mp 278–279 °C dec; $[\alpha]^{20}_D$ – 114° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 340 (sh), 310 (4.13), 247 (4.57), 239 (sh) nm; IR (KBr) ν_{max} 3300, 1640, 1580 cm^{-1}; ^1H NMR, see Table I; CIMS (CH_4) *m/z* (relative intensity) 359.1123 (observed M + H^+, 76), calcd for $C_{19}H_{19}O_7$, 359.1131, 257 (14), 108 (100); ED_{50} = 7 μg/mL in 9 KB.

Acetylation of 3′,4′-Deoxypsorospermin-3′,4′-diol. A solution of 3′,4′-deoxypsorospermin-3′,4′-diol (**3**; 10 mg) in anhydrous pyridine (0.5 mL) and acetic anhydride (0.5 mL) was stirred overnight at room temperature under nitrogen. The solution was evaporated in vacuo, and the residual pyridine and acetic anhydride were codistilled with toluene (3 × 1.5 mL). The resulting gummy residue (10 mg) was purified by preparative silica gel TLC (5% MeOH in $CHCl_3$). The lower R_f (0.5) diacetate (**5**) crystallized as tiny needles (7 mg) from $CHCl_3$/MeOH: mp 176–178 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 1.30 (s, 3 H, CH_3-5′), 2.14 (s, 3 H, $OCOCH_3$-4′), 2.41 (s, 3 H, $OCOCH_3$-5), 3.36 (dd, 1 H, *J* = 15.7, 8.4 Hz, H-1′a), 3.26 (dd, 1 H, *J* = 15.7, 8.4 Hz, *H*-1′b), 3.92 (s, 3 H, OCH_3-1), 4.15 (d, 1 H, *J* = 11.3 Hz, H-4′b), 4.34 (d, 1 H, *J* = 11.3 Hz, H-4′a), 4.98 (t, 1 H, *J* = 8.4 Hz, H-2′), 6.31 (s, 1 H, H-2), 7.33 (m, 2 H, H-6 and H-7), 8.17 (dd, 1 H, *J* = 2 and 7.5 Hz, H-8); CIMS (CH_4) *m/z* (relative intensity) 443 (M + H^+, 100), 401 (M + H^+ – $COCH_2$, 11), 383 (M + H^+ – AcOH, 8); EIMS for $C_{23}H_{22}O_9$, obsd 442.1256 (M^+), calcd 442.1263.

The higher R_f (0.77) triacetate **6** obtained as an amorphous powder (1.2 mg) from $CHCl_3$/MeOH: CIMS (CH_4) *m/z* (relative intensity) 482 (M + H^+, 35), 279 (17), 257 (8), 229 (100); EIMS for $C_{25}H_{24}O_9$, obsd 484.1381 (M^+), calcd 484.1368.

Conversion of 3 to Psorospermin (1). A solution of 3′,4′-deoxypsorospermin-3′,4′-diol (**3**; 8.5 mg) in pyridine (2 mL) was treated with methanesulfonyl chloride (5.5 mg, 3.7μL) at 0 °C for 4 h. More methanesulfonyl chloride (3.7 μL) was added, the reaction mixture stirred at room temperature overnight and quenched with H_2O (40 mL), and the product extracted into EtOAc (2 × 40 mL). The combined EtOAc extract was washed with aqueous saturated NaCl, dried (Na_2SO_4), and evaporated in vacuo. The crude mesylate [11.3 mg (93%)] without purification was treated with *t*-BuOK (16 mg) in *t*-BuOH (6 mL) at 40 °C for 1 h. The reaction mixture was diluted with 50 mL of $CHCl_3$, and the resulting solution was washed with 1:1 solution (20 mL) of H_2O and 2% acetic acid. The aqueous phase was reextracted with $CHCl_3$ (2 × 10 mL). The combined $CHCl_3$ extract was washed, dried (Na_2SO_4), and evaporated to give psorospermin (**1**) as a white solid [9 mg (91%)] that was identical with the natural sample by ^1H NMR (200 MHz) and TLC.

3′,4′-Deoxy-4′-chloropsorospermin-3′-ol (4). Column fraction 6 (eluted in hexane/ethyl acetate (4:6), cuts 71–79, 0.50 g) was repeatedly triturated with hexane/acetone to remove pigments. The residue was recrystallized from either methanol/ether or acetone/hexane to give 118 mg of **4** as small needles, mp 269–270 °C. Adjacent fractions yielded an additional 280 mg of **3**. Compound **4** gave an ED_{50} = 1 × 10^{-1} μg/mL in 9KB and a T/C 135% at 8 mg/kg in P388 mouse leukemia: $[\alpha]^{20}_D$ –114° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 340 (sh), 311 (4.26), 2.47 (4.67), 239 (sh) nm; IR (KBr) ν_{max} 3250, 1642, 1585 cm^{-1}; ^1H NMR, see Table I; CIMS (CH_4) *m/z* (relative intensity) 377.0786 (obsd, M + H^+, 36), calcd for $C_{19}H_{18}O_6Cl$ 377.0792, 341 (M + H^+ – HCl, 30).

Acetylation of 3′,4′-Deoxy-4′-chloropsorospermin-3′-ol. A solution of 3′,4′-deoxy-4′-chloropsorospermin-3′-ol (**4**; 1.5 mg) in anhydrous pyridine (5 drops) and acetic anhydride (5 drops) was stirred overnight. The solution was evaporated in vacuo, and the residual pyridine and acetic anhydride were codistilled with few drops of toluene. The acetate (**7**; 1.5 mg) obtained as a colorless solid from MeOH: ^1H NMR (470 MHz, $CDCl_3$) δ 1.40 (s, 3 H, CH_3-5′), 2.45 (s, 3 H, $OCOCH_3$-5), 3.32 (dd, 2 H, *J* = 9.4 and 14 Hz, H-1′b), 3.37 (dd, 1 H, *J* = 9.4 and 14 Hz, H-1′a), 3.70 (d, 1 H, *J* = 11 Hz, H-4′b), 3.75 (d, 1 H, *J* = 11 Hz, H-4′a), 3.95 (s, 3 H, OCH_3-1), 5.10 (t, 1 H, *J* = 9.4 Hz, H-2′), 6.35 (s, 1 H, H-2), 7.30 (m, 2 H, H-6 and H-7), 8.17 (dd, 1 H, *J* = 1.5 and 8 Hz, H-8),; CIMS (CH_4) *m/z* (relative intensity) 419 (M + H^+, 100), 383 (M + H^+ – HCl, 23).

Conversion of 4 to Psorospermin (1). A solution of 3′,4′-deoxy-4′-chloropsorospermin-3′-ol (**4**; 5 mg) and *t*-BuOK (10 mg) in *t*-BuOH (10 mL) was stirred at 40 °C for 1 h under nitrogen atmosphere. The reaction mixture was partitioned between $CHCl_3$ (100 mL) and 0.2% acetic acid solution (50 mL). The aqueous phase was reextracted with $CHCl_3$ (2 × 50 mL), and the combined $CHCl_3$ extract was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and evaporated in vacuo. The crude product was recrystallized from acetone/hexane as tiny prisms [4.1 mg (90%)], which were identical with natural psorospermin by ^1H NMR (470 MHz) spectral data and thin-layer cochromatography.

Conversion of Psorospermin (1) to 3′,4′-Deoxy-4′-chloropsorospermin-3′-ol (4). A solution of psorospermin (**1**; 5.0 mg) in methanol (1.0 mL) and $CHCl_3$ (0.5 mL) was treated with 1 drop of concentrated HCl at room temperature under nitrogen atmosphere for 2.5 h. The reaction mixture was evaporated with a stream of nitrogen, and the resulting residue was dissolved in 1.0 mL of $CHCl_3$ and a few drops of methanol and passed over a small column of silica gel. The fraction after evaporation yielded the chlorohydrin as small colorless needles: 4.6 mg (84%); mp 270–272 °C; ^1H NMR (470 MHz), identical with that of the naturally occurring compound; CIMS, *m/z* 377 (M + $H)^+$, 359 (M – H_2O + $H)^+$, 341 (M – HCl + $H)^+$.

***O*^{5}-Methyl-3′,4′-deoxypsorospermin-3′-ol (8).** Column fraction 5 was further repeatedly separated by preparative silica gel TLC (first separation, 8% MeOH in $CHCl_3$; second separation, 5% MeOH in $CHCl_3$). The residue was recrystallized from MeOH to give 3.5 mg of pale yellow needles: mp 224–226 °C; $[\alpha]^{20}_D$ –82° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 341 (3.8), 311 (4.20), 246 (4.57), 239 (sh); ^1H NMR, see Table I; EIMS for $C_{20}H_{20}O_6$, obsd 356.1267 (M^+), calcd 356.1259.

Methylation of 3′,4′-Deoxypsorospermin (2).[15] A solution of 3′,4′-deoxypsorospermin (**3**; 3.0 mg) and anhydrous potassium carbonate (10 mg) in dry acetone (3 mL) was treated with 4 drops of methyl iodide at room temperature overnight under nitrogen atmosphere. The reaction mixture was filtered, and the filtrate was evaporated under vacuum. The residue thus obtained was purified by preparative silica gel TLC with 5% methanol in $CHCl_3$. The product crystallized as shining plates from $CHCl_3$/MeOH to give *O*^{5}-methyl-3′,4′-deoxypsorospermin (**9**): 1.2 mg; mp 202–204 °C; R_f 0.73 (10% MeOH in $CHCl_3$); $[\alpha]^{20}_D$ –75.5° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 340 (sh), 311 (4.16), 246 (4.52), 239 (sh); ^1H NMR (470 MHz, $CDCl_3$) δ 1.81 (s, 3 H, CH_3-5′), 3.23 (dd, 1 H, *J* = 15.3, 7.7 Hz, H-1′a), 3.58 (dd, 1 H, *J* = 15.3, 9.7 Hz, H-1′b), 3.98 (s, 3 H, OCH_3-5 or OCH_3-1), 3.99 (s, 3 H, OCH_3-1 or OCH_3-5), 4.97 (br s, 1 H, H-4′a), 5.13 (br s, 1 H, H-4′b), 5.40 (dd, 1 H, *J* = 9.7, 7.7 Hz, H-2′), 6.38 (s, 1 H, H-2), 7.14 (dd, 1 H, *J* = 8.0, 1.4 Hz, H-7), 7.23 (t, 1 H, *J* = 8.0 Hz, H-6), 7.87 (dd, 1 H, *J* = 8.0, 1.4 Hz, H-8); EIMS for $C_{20}H_{18}O_5$, obsd 338.1155 (M^+), calcd 338.1154.

Dehydration of *O*^{5}-Methyl-3′,4′-deoxypsorospermin-3′-ol (8).[16] A solution of 3′,4′-deoxypsorospermin-3′-ol (**8**; 2.8 mg) in 0.5 mL of phenyl isocyanate was refluxed under nitrogen atmosphere for 16 h. The reaction mixture was quenched with a few drops of dilute HCl, and the product was extracted into $CHCl_3$ (3 × 2.0 mL). The $CHCl_3$ extract was washed with saturated $NaHCO_3$ solution and water, dried, and evaporated under vacuum. The resulting residue was purified by prepararative silica gel TLC with 5% methanol in $CHCl_3$. The product crystallized from methanol as plates: mp 203–204 °C; EIMS for $C_{20}H_{18}O_5$, obsd 338.1150 (M^+), calcd 338.1154. It was identical with compound **9** by thin-layer cochromatography (silica gel; 10% MeOH in $CHCl_3$) and all spectral data.

3′,4′-Epoxytubaic Acids 13. To a methylene chloride solution of **12** (0.22 g, 0.001 mol) obtained from **10** by degradation[11] was added *m*-chloroperoxybenzoic acid (0.35 g, 0.002 mol) in methylene chloride dropwise. The reaction was stirred at room temperature, for 24 h, then washed with 5% sodium bisulfite solution, distilled water, and saturated sodium chloride solution, dried with anhydrous sodium sulfate, and distilled in vacuo to give a solid (0.57 g), from which 0.49 g was chromatographed on a silica gel 60 silanized (RP2) column (20 g) in 10% ethyl acetate in hexane to

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(17) In this paper, numbering sequences in compounds do not agree with systematic approaches.

give the diastereomeric epoxide in 54% yield. Subsequent separation by centrifugal thin-layer chromatography (chromatotron) on a rotor coated with silica gel (2 mm thick) with 2.5–10% ethyl acetate in hexane resulted in three major fractions. The higher R_f fraction (0.03 g, 26% theoretical yield) was **13b**: mp 142–143 °C; ^{1}H NMR ($CDCl_3$, 470 MHz) δ 1.41 (s, 3 H), 2.71 (d, 1 H, J = 4.6 Hz), 2.82 (d, 1 H, J = 4.6 Hz), 3.07 (dd, 1 H, J = 7.6, 15.7 Hz), 3.23 (dd, 1 H, J = 9.9, 15.7 Hz), 4.83 (dd, 1 H, J = 7.6, 9.9 Hz), 6.41 (d, 1 H, J = 8.6 Hz), 7.76 (d, 1 H, J = 8.6 Hz); exact mass for $C_{12}H_{12}O_5$ (M^+), calcd 236.0685, found 236.0688. The middle fraction (0.03 g) was a mixture of the higher and lower R_f isomers. The lower R_f fraction (0.02 g, 17% of theoretical yield) was **13a**: ^{1}H NMR ($CDCl_3$, 470 MHz) δ 1.38 (s, 3 H), 2.7 (d, 1 H, J = 4.7 Hz), 2.94 (d, 1 H, J = 4.7 Hz), 3.11 (dd, 1 H, J = 7.4, 15.7 Hz), 3.30 (dd, 1 H, J = 9.9, 15.7), 4.78 (dd, 1 H, J = 7.4, 9.9 Hz), 6.40 (d, 1 H, J = 8.7 Hz), 7.76 (d, 1 H, J = 8.7 Hz); exact mass for $C_{12}H_{12}O_5$ (M^+), calcd 236.0685, found 236.0684.

3′,4′-Epoxyrotenone (11a,b). To a chloroform solution of **10** (19.72 g, 0.05 mol) was added *m*-chloroperoxybenzoic acid (17.3 g, 0.1 mol) in chloroform dropwise. The reaction was stirred at room temperature for 24 h and then worked up[13] to give a brown solid, 25.49 g. Separation on LPS-1 silica gel (1000 g) in a Michel–Miller column with 40% ethyl acetate in hexane gave **11b** as a yellow solid, 1.96 g (19% crude yield). Purification by trituration (ethanol and hexane) and recrystallization (methylene chloride and methanol) gave white needles: mp 179–181 °C; ^{1}H NMR ($CDCl_3$, 470 MHz), literature values; exact mass for $C_{23}H_{22}O_7$ (M^+), calcd 410.136, found 410.1350. Slow recrystallization from methanol at room temperature yielded the crystal for X-ray analysis.

Crystal Data: $C_{23}H_{22}O_7$, M = 410, orthorhombic, a = 4.536 (2) Å, b = 16.49 (2) Å, c = 25.31 (2) Å, V = 1894 (3) Å^3, Z = 4, ρ_{calcd} = 1.44 g/cm^3, $F(000)$ = 864, μ(Cu Kα) = 7.91, space group $P2_12_12_1$ from systematic absences.

Data Collection: The crystallographic data were collected by Cu Kα X-rays and a monochromator on a Syntex P3 four-circle diffractometer with the θ–2θ scan technique out to a 2θ of 116.0°. A variable scan rate was used with a maximum of 29.30°/min and a minimum of 7.32°/min. The scan range was from 1.2° less than $K\alpha_1$ to 1.2° more than $K\alpha_2$; the length of time the backgrounds at both ends of the scan range were counted was equivalent to the scan time. Three standard reflections were measured every 50 reflections. Of the 1578 reflections collected, 25 were rejected as systematically absent, leaving 1553 unique reflections, of which 915 met the condition $F_o > 5\sigma(F_o)$ and were considered observed. The structure was solved by the MULTAN80 program, and refined by SHELX76, giving a final R = 0.0703 with hydrogens fixed in calculated positions. A final difference map showed no peaks greater than 0.26 e/Å^3. Table VI (supplementary material) shows the final positional parameters.

All melting points are uncorrected and were obtained on a Laboratory Devices Mel-Temp apparatus. IR spectra were obtained in KBr on a Beckman IR-33 spectrophotometer. UV spectra were recorded, in the solvents indicated, on either a Cary 17 or Perkin-Elmer Coleman 124 double-beam spectrophotometer. Electron impact and chemical ionization mass spectra were obtained on a Finnigan Model 4023 mass spectrometer, and high-resolution accurate mass measurements were made on a Kratos MS 50 mass spectrometer. ^{1}H NMR spectra were obtained in the solvent indicated on either a Varian XL-200 or the Nicolet 470 MHz spectrometer at the Purdue University Biological Magnetic Resonance Laboratory. The P388 mouse leukemia assays were carried out at the Illinois Institute of Technology, Life Sciences Division.

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Supplementary Material Available: Tables III–VII listing bond lengths, bond angles, torsion angles, and positional and thermal parameters of epoxyrotenone (5 pages). Ordering information is given on any current masthead page.

A Method for the Preparation of Stereochemically Defined ψ[CH_2O] Pseudodipeptides[1]

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A short, stereochemically defined synthesis of (2*S*,5*S*)-ψ[CH_2O] pseudodipeptides (**7a, 7b, 10**) using commercially available, chiral amino acids has been developed. The key step of the synthesis is the intramolecular S_N2 displacement of bromine with alkoxide to give 1,4-oxazin-2-ones (**6a, 6b, 9**) that are subsequently hydrolyzed to the desired ψ[CH_2O] pseudodipeptides.

The development of peptides as potential therapeutic agents is an area of intense interest to many organic chemists.[2] A primary drawback to the use of many synthetic peptides is their rapid degradation in vivo by numerous peptidases.[3] One approach to avoiding the rapid hydrolysis of the peptide bond is to substitute nonhydrolyzable bonds for the peptide amide bond.[4] The subject of peptide backbone modifications has recently been reviewed extensively by Spatola.[5] Absent from this discussion, however, are ψ[CH_2O] pseudodipeptides. Only brief mention in the literature (with no experimental de-

(1) The ψ nomenclature has been accepted by IUPAC for peptide amide bond replacements. The unit inside the bracket following ψ is the unit substituting for the peptide amide (–CONH–) bond. IUPAC–IUB Joint Commission on Biochemical Nomenclature *Eur. J. Biochem.* **1984**, *138*, 9.

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