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(50) Deceptively simple triplet.
(51) D_2O + 1% (w/w) sodium 2,2-dimethyl-2-silapentane-5-sulfonate; 98.8% D.

Nonenolizable Podophyllotoxin Derivatives

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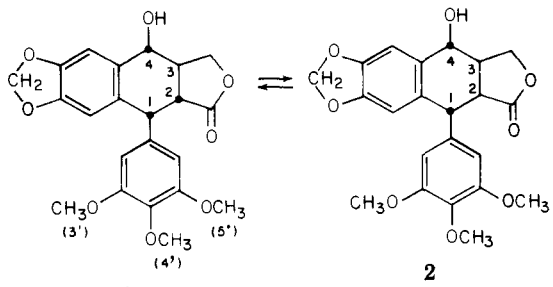
To block epimerization and the resulting biological deactivation of podophyllotoxin compounds, the lactone carbonyl group has been changed to methylene. Syntheses of several of these delactonized derivatives are described, all with modifications in the original lactone ring and some without the 4-hydroxyl group. Several biological assays show that most of the nonenolizable derivatives retain activity.

Podophyllotoxin (1) and several of its analogues and derivatives are cytostatic spindle poisons;[1] they have also received considerable attention as antitumor agents,[2-6] some at the clinical level. Most of these compounds contain a trans-fused highly strained γ-lactone system,[3]



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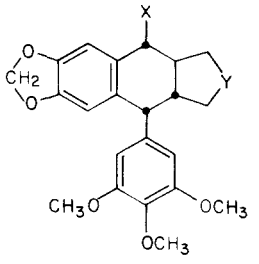
a feature that correlates with the smooth isomerization of podophyllotoxin (1) to its thermodynamically stable cis epimer, picropodophyllin (2).[7] The biological activity of picropodophyllin as well as of the other cis analogues is either much lower than that of the trans isomers or is lacking altogether.[4,8,9] Since the epimerization conditions are very mild, the possibility has been considered that, when exposed to podophyllotoxin, the cell makes use of this process for detoxication. A search of the literature has uncovered scattered reports that, in fact, are consistent with the idea of detoxication by epimerization.[9]

From the chemotherapeutic point of view, the epimerization is undesirable, since limiting the physiological lifetime of the compounds would set an upper limit to their biological effectiveness. To get rid of this built-in disadvantage, we initiated a program of synthesizing derivatives in which epimerization is precluded, thus allowing the maximum effect and the full potential of the agents to be realized. Replacing the hydrogen at the 2 position with a suitable group would provide the kind of compounds we were after, and, with this in mind, we have tried to insert a fluorine at this position but, so far at least, have not succeeded.[10] Substituting with a carboxyl or carbomethoxyl group was possible but unfortunately was accompanied by inversion, so that the derivatives were obtained in the unfavorable cis-fused picropodophyllin (2) configuration instead of in the trans-fused podophyllotoxin (1) form.[11] A quite different way of blocking epimerization would be to eliminate the lactone group altogether, since without an enolization mechanism, the cell could not bring about the mild inversion. Accordingly, we have now prepared a series of "delactonized" derivatives **3**, in which the original carbonyl group has been changed to methylene,

and in which X is H or OH, and Y is O, S, SO_2, CO, or CH_2. The present paper describes the syntheses and reports some assay results.



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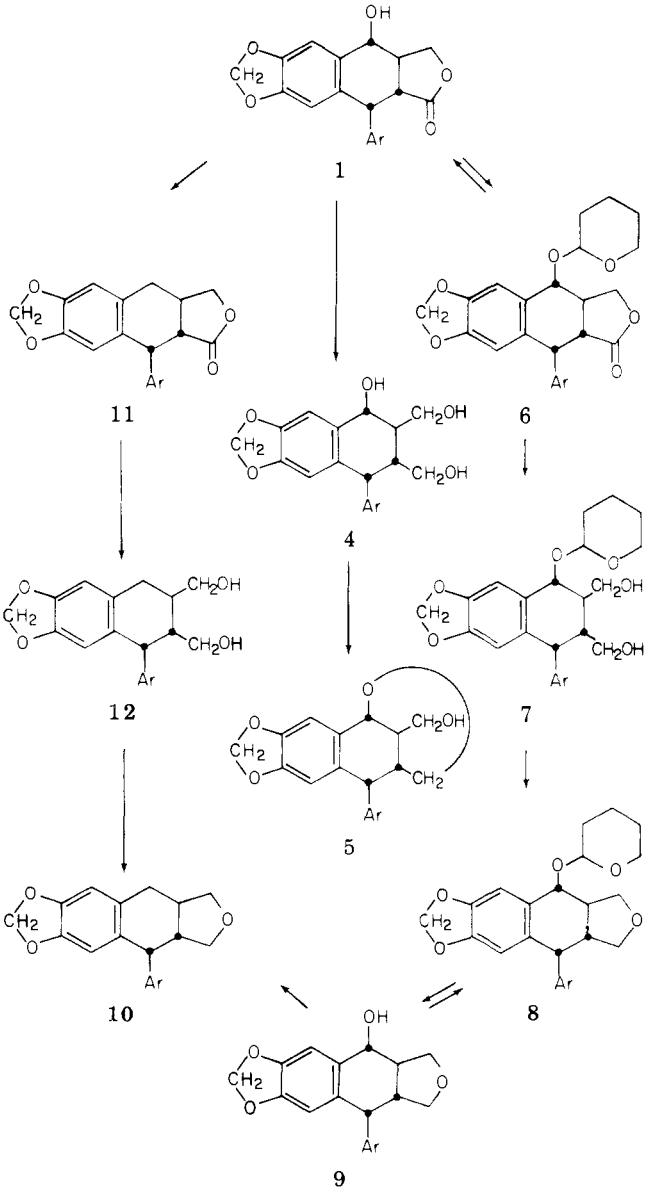
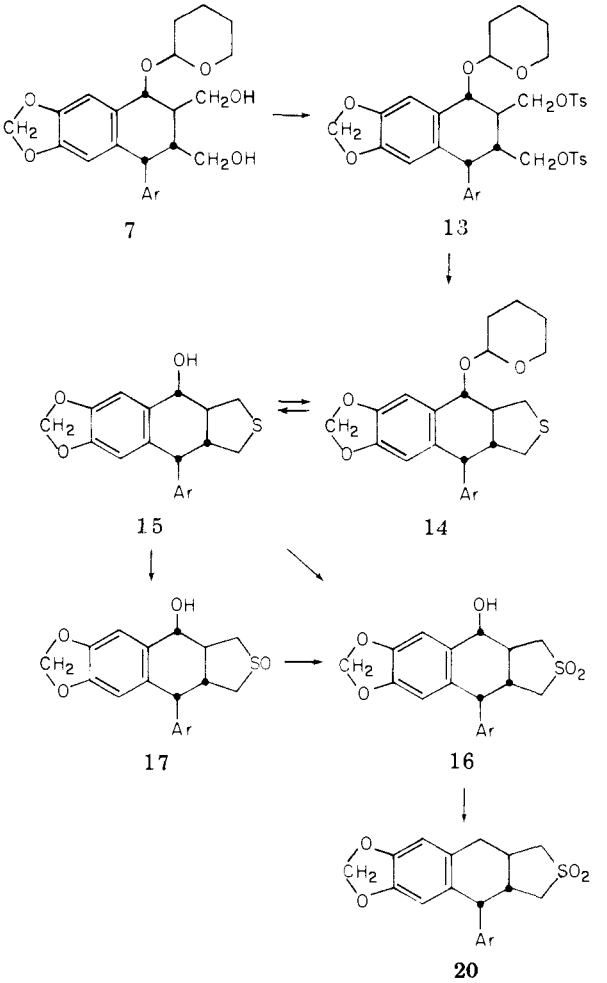
Syntheses. Lithium aluminum hydride can reduce podophyllotoxin (1) to the trihydroxy compound, podophyllol (4).[12-14] Earlier attempts at cyclizing podophyllol to anhydropodophyllol (9) were frustrated by involvement of the hydroxyl group at the 4 position, giving neoanhydropodophyllol (5) instead of 9.[14] To prevent the 2–4 mode of closure, we started with tetrahydropyranylpodophyllotoxin (6), in which the 4-hydroxyl group is blocked. Reduction of 6 produced 4-tetrahydropyranylpodophyllol (7), which under mild cyclization conditions afforded tetrahydropyranylanhydropodophyllol (8). The desired anhydropodophyllol (9) was obtained when the blocking tetrahydropyranyl group was removed.

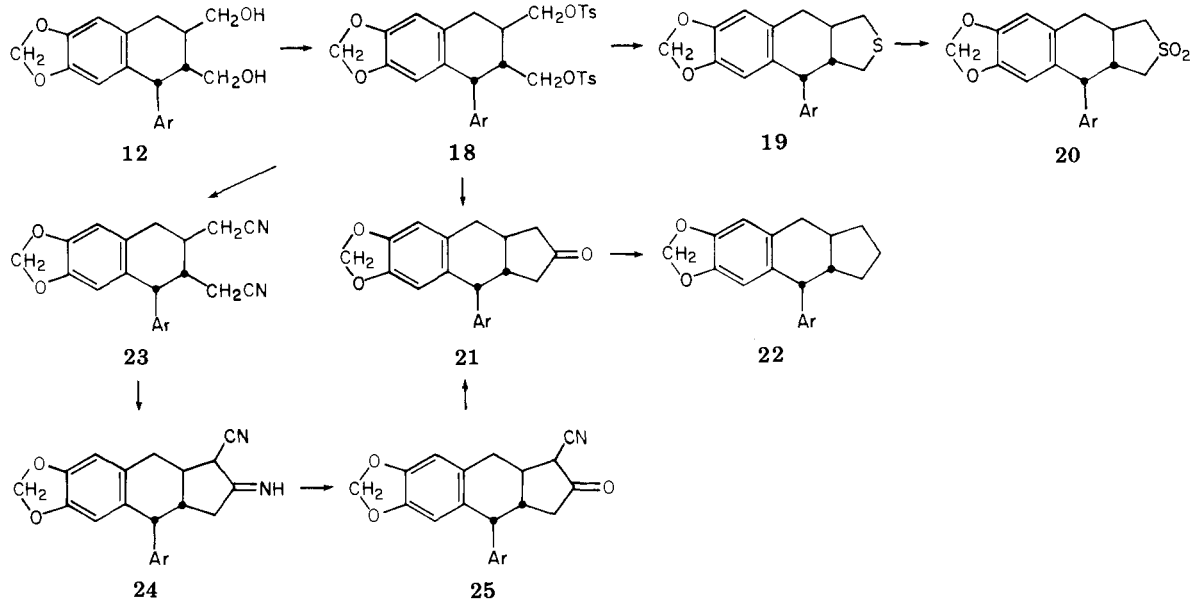
Hydrogenolysis of anhydropodophyllol (9) eliminated the benzylic hydroxyl group at position 4 and furnished 4-deoxyanhydropodophyllol (10). This was identical with the product emerging from the sequence: hydrogenolysis of podophyllotoxin (1) to deoxypodophyllotoxin (11),[11,15] lithium aluminum hydride reduction to 4-deoxypodophyllol (12), and, finally, cyclization to 10.[14-16]

The ditosylates 18 and 13 from deoxypodophyllol (12) and tetrahydropyranylpodophyllol (7), respectively, served as starting points for the remaining syntheses. Thus, treatment of the ditosylates with sodium sulfide gave the corresponding cyclic sulfides, 19 and 14. Mild hydrolysis removed the protective tetrahydropyranyl group from 14 to yield hydroxy cyclic sulfide 15, which is seen to be a sulfur isostere of anhydropodophyllol (9). Sulfides 19 and 15 reacted with *m*-chloroperbenzoic acid to give the corresponding sulfones 20 and 16, whose structural relation was shown by hydrogenolytic removal of the 4-hydroxyl group in 16, whereupon deoxy sulfone 20 was formed. In the hydroxy series, oxidation of cyclic sulfide 15 with periodate afforded cyclic sulfoxide 17 as a mixture of two stereoisomers. The sulfoxides were readily oxidized to sulfone 16 with peracid.





The approach to cyclopentanone 21 relied on a Thorpe sequence,[17] which converted ditosylate 18 first to the corresponding dinitrile, then to iminonitrile 24 (probably a mixture), and finally by hydrolysis (to 25) and decarboxylation to ketone 21. A shorter and more satisfactory way made use of disodium tetracarbonylferrate,[18] which



in a one-flask process converted ditosylate **18** directly to ketone **21**. The ketone could be reduced smoothly to cyclopentane **22** with zinc dust in ether saturated with hydrogen chloride.[19]

Stereochemistry. Since our goal was to prepare delactonized derivatives with the same molecular shape as podophyllotoxin (**1**), preserving the stereochemistry at the several asymmetric centers (in podophyllotoxin, 1*R*, 2*R*, 3*R*, 4*R*[20]) was important. The following considerations support the configurations as assigned.

Although there are a few scattered reports[21] of lithium aluminum hydride acting as a basic material, so that during the reduction of deoxypodophyllotoxin **11** the compound could conceivably epimerize at its 2 position, this in fact does not occur, since the single product, deoxypodophyllol (**12**), is different from the known epimer, deoxypicropodophyllol.[14,16] Likewise deoxyanhydropodophyllol (**10**) is different from its 2-epimer, deoxyanhydropicropodophyllol.[16] We have therefore accepted **12** and **10** as having the same configuration as in deoxypodophyllotoxin (**11**). Deoxypodophyllol (**12**) is converted to its ditosylate **18** under mild conditions. Neither in this step nor in any of those coming after, and using the ditosylate as starting material, are there reasonable mechanisms by which inversions at any of the asymmetric centers might occur. Accordingly, the same configurations have been assigned to sulfide **19**, sulfone **20**, cyclopentanone **21**, and cyclopentane **22** as in starting material **11**.

Hydrogenolysis of podophyllotoxin (**1**) gives the single product deoxypodophyllotoxin (**11**), free of the known epimer, deoxypicropodophyllin (**2** minus OH).[15] Similar hydrogenolyses directly relate anhydropodophyllol (**9**) to deoxyanhydropodophyllol (**10**) and hydroxy sulfone **16** to deoxy sulfone **20**. Thus, in the hydroxy series as in the deoxy series, the configurations at the 1, 2, and 3 positions are correctly assigned.

In considering the configuration of the 4-hydroxyl group, we relied on the fact that in the conversion of podophyllotoxin (**1**) to its tetrahydropyranyl derivative **6** and further in the sequence **6**–**16**, none of the conditions would be expected to promote inversions at position 4. Also, significantly, mixtures that might have to be explained by such inversions are not encountered. Further, for three of the steps, where strong acid is involved, there is direct evidence that product and starting material are configurationally related. Thus, **6** can be reconverted to **1**,[22] **9** can be reconverted to **8**, and **15** can be reconverted to **14**. For these reasons, all asymmetric centers in the compounds investigated have been accepted as having the same configuration, respectively, as in podophyllotoxin (**1**).

In the present series, circular dichroism and optical rotatory dispersion properties are not (with the exception of the 1 position) sensitive functions of stereochemistry,[13,14,23] so that the measurements provided only limited information about configurational relationships. All the compounds furnish curves that, although varying somewhat in the numerical values of the dispersion and the molecular ellipticity, are more or less similar in shape. All the ORD curves show a deep trough at 285–283 nm (negative rotation) and a peak at lower wavelengths in the 270–260-nm region. Generally a slowly rising positive, approximately plain curve on the high wavelength side of the trough (~300–280 nm) is noted, but this aspect is not pronounced, and in several of the compounds—including podophyllotoxin and deoxypodophyllotoxin—other features appear, superimposed on this "plain" curve. For this reason, we are reluctant to emphasize the 300–280-nm part of the curve as "the positive first Cotton effect" as has been customary in this family of compounds.[23] In fact, in a number of the curves, if the minor upward trend is disregarded as the ORD curve goes to lower wavelengths in the 300–290-nm region and if the marked extrema at 285–283 and at 270–260 nm are considered together, the curves might better be described as having a high-amplitude *negative* Cotton effect in the 290–260-nm region.[24] Not unexpectedly the ORD curves for the ketonic compounds **21** and **25** show additional detail at 340–310 nm.

Assay Results. The delactonized compounds were tested for their cytotoxicity and for their ability to interact with the highly specialized protein, tubulin. Table I shows that none of the compounds are more cytotoxic than the parent podophyllotoxin. The relative values obtained from three different tumor cultures tend to run parallel, with anhydropodophyllol (**9**) and deoxyanhydropodophyllol (**10**) the most effective of the delactonized derivatives and the sulfones **16** and **20** the least. Even the cyclopentane derivative **22** shows significant activity, and in one of the assays, cyclopentanone **21** has as much as 20% of the potency of podophyllotoxin. A limited number of in vivo assays were also performed, mainly with anhydropodophyllol (**9**) or deoxyanhydropodophyllol (**10**), using TLX-5

Table I. Assay Results

No.	Compound	Cytotoxicity[a] P-185[b]	L-5178Y[d]	TA·3[e]	Binding to tubulin[f]	Inhibn of microtubule assembly[a,g]
1	Podophyllotoxin	0.13[c]	0.14	3.0	2	6
11	Deoxypodophyllotoxin	c			2-4	
9	Anhydropodophyllol	0.59	1.3	6.0	0.2-0.26	10
10	Deoxyanhydropodophyllol	0.6[c]	1.4	6.0	0.4	8
15	Hydroxy sulfide	4.3	14	60	Small	100
19	Deoxy sulfide	6.0	15	50	Small	100
16	Hydroxy sulfone	>220	>220	>100	Small	>1000
20	Deoxy sulfone	69	139	>100	Small	>500
21	Cyclopentanone	1.0	15	15	0.02	50
22	Cyclopentane	6.5	16	50	0.02	50

[a] The results for the cytoxicity assays and for the microtubule assembly inhibition are given in molar concentrations; all the numbers in these groups should be multiplied by 10^{-7}. [b] These values are based on results obtained at the Pharmaceutical Division of Sandoz, Ltd., Basle, and refer to molar concentrations producing 50% arrest (at metaphase) of mitotic activity in cultures of mouse (P-815) mastocytoma leukemia cells.[4,6,14] [c] Values obtained before at Sandoz Laboratories are 0.12×10^{-7} M for podophyllotoxin (**1**), 0.05×10^{-7} M for deoxypodophyllotoxin (11), and 0.52×10^{-7} M for deoxyanhydropodophyllol (**10**). Pertinently, the corresponding values for picropodophyllin (**2**), which is the cis isomer of podophyllotoxin (1), are 1.9×10^{-7} M and for deoxypicropodophyllin (2 minus OH), 1.3×10^{-7} M (see ref 5). [d] The values refer to molar concentrations that cause 50% inhibition of the cell count increase in suspensions of growing mouse leukemia cells (L-5178Y). The assays were performed at Sloan-Kettering Institute for Cancer Research in N.Y. [e] Molar concentrations at which protein production in tissue cultures of mouse mammary carcinoma (TA-3) is decreased by 50%. The assays were performed at Roswell Park Memorial Institute in Buffalo. [f] The numbers give the reversible tubulin association constant (K_A) in liters per mole. All values should be multiplied by 10^6. The measurements, which as supplied by Leslie Wilson of Stanford Medical School are preliminary, come from competitive binding studies against radioactive colchicine, using tubulin from chick embryo brain. Note that Wilson and Bryan (see ref 8) find K_A for picropodophyllin (**2**) to be 0.037×10^6 (grasshopper embryo tubulin) and thus estimate that picropodophyllin is approximately 100 times less strongly bound than podophyllotoxin (1). [g] In the microtubule assembly assay,[25] the tabulated numbers (preliminary results) are equilibrium concentrations (molarities) that at 37 °C and over a period of 30 min inhibit formation of microtubules from chicken brain tubulin by 50%. The increase in optical density as the result of increased light scattering serves as the experimental measure. The work was done at Albert Einstein College of Medicine in N.Y.

ascites, L1210 leukemia, and TA-3 carcinoma in mice as test systems. None of the compounds examined showed significant in vivo activity. However, where tested, podophyllotoxin also was not particularly effective, so that these assays may not be the most appropriate for the podophyllotoxin family.

In the tubulin studies,[1,25] Table I again shows that none of the delactonized derivatives are more active than podophyllotoxin (1) or deoxypodophyllotoxin (11). However, the binding strength of anhydropodophyllol and of deoxyanhydropodophyllol is still appreciable, with values 13 and 20% as great, respectively, as that of podophyllotoxin. Even ketone **21** and cyclopentane **22** show significant binding tendencies. Interestingly, the deoxy members of the pairs, **11** and **1**, **10** and **9**, and **19** and **15**, appear to be held more tightly than the hydroxy members. In an assay based on the closely related effect of inhibiting the polymerization of tubulin to microtubules,[25,26] anhydropodophyllol and deoxyanhydropodophyllol emerge as highly active, with 60 and 75% of the potency of podophyllotoxin, respectively. In the assembly assay as in the binding studies, the sulfones are essentially devoid of activity. Finally, in a preliminary in vivo study,[27] the minimum concentration of anhydropodophyllol (**9**) necessary to delay regeneration of the ciliated oral apparatus (i.e., to inhibit microtubule assembly) in *Stentor coeruleus* that had been stripped of its oral membranellar band was found to be 20×10^{-6} M as compared with 4×10^{-6} M for podophyllotoxin. In other words, in this in vivo process, anhydropodophyllol has 20% of the activity of podophyllotoxin.

Discussion

This work was directed to the synthesis of defunctionalized podophyllotoxin derivatives **3** which, because they could not epimerize to the inert picropodophyllin configuration, were expected to show enhanced biological properties. Disappointingly, none of the new compounds proved to be more effective than podophyllotoxin. Nevertheless, some of them, notably the cyclic ethers **9** and **10**, did show considerable activity, enough to make further inquiry into other delactonized compounds attractive. Along these lines, we are considering possibilities such as the heterocyclic nitrogen analogues (**3** with Y = NR), as well as the delactonized forms of α and β-peltatin,[3] 4′-demethylepipodophyllotoxin, β-apopicropodophyllin,[5] steganacin,[28] and the even more intriguing glucoside derivatives.[29]

A side result from this work pertains to the biochemical mechanism by which the podophyllotoxin compounds act. The presence of a highly strained lactone system with an open, easily accessible carbonyl group invites the hypothesis that these compounds react by lactone acylation with ring opening. Chemically, release of ring strain and the change of a rigid system to a more flexible one would favor the process. Biochemically, covalent bond formation producing an ester, thioester, or amide could destroy the activity of an essential cell constituent.[30] There are, however, some results in the literature[31] suggesting that this mechanism might not be correct. And in the present work, the significant activity observed in compounds containing no lactone function provides strong additional evidence, at least for the processes examined, that acylation need not be involved.

A different proposal that the mode of action involves *alkylation* of a cell constituent by podophyllotoxin compounds at their 4 position[32] is untenable, since it could not be reconciled with the recognized biological effectiveness of several podophyllotoxin analogues having no substitutable (solvolyzable) oxygen function at the 4 position. A remaining, still acceptable hypothesis involves noncovalent binding in which the podophyllotoxin com-

pound with or without a lactone function or a replacable group at the 4 position fits tightly on the surface of some key biological macromolecule and thereby interferes with its essential function.

Experimental Section

General. Melting points were taken using open capillary tubes and are uncorrected. Nuclear magnetic resonance curves were determined at 60 MHz, with chemical shifts reported as parts per million downfield from (internal) tetramethylsilane. Optical rotatory dispersion and circular dichroism were taken at 23–25 °C with a Cary-6002 recording spectropolarimeter.[24] Low-boiling solvents were routinely stripped from solutions by distillation under reduced pressure at the temperatures specified. A number of compounds were obtained as noncrystalline or poorly crystalline solids, a problem observed before with members of the podophyllotoxin family. There is also a marked tendency to hold on to the last traces of solvent.[3,14,16,33,34] An estimate of residual solvent (0–2%) was obtained routinely, with NMR integration values for selected solvent protons serving as the basis for the determinations. Yield figures are given on a solvent-free basis. Galbraith Laboratories, Inc., Knoxville, Tenn., reported all the analytical values except for the ditosylate of the tetrahydropyranyl podophyllol (13) which was obtained by Carol K. Fitz, Carlisle, Mass. Analytical TLC was done with glass plates coated with silica gel (0.25 mm, Merck), preparative layer chromatography with 2-mm layers of silica, and column chromatography with a packing of Merck 0.05–0.2-mm silica gel. TLC plates were examined either under ultraviolet light or after exposure to iodine vapors. The petroleum ether referred to is the bp 30–60 °C fraction.

Podophyllotoxin (1) and Deoxypodophyllotoxin (11). The starting material for this work was podophyllotoxin, available as a white powder, mp 183–184 °C (lit.[3] 183–184 °C for solvent-free material). Another sample showing mp 158–159 °C corresponded probably to a different modification of podophyllotoxin:[3] $[\alpha]_{589}$ −111 to −114°; $[\alpha]_{546}$ −157° (both *c* 0.02, dioxane). The starting material gave only a single spot on TLC with R_f 0.39–0.43 (chloroform–ethyl acetate, 1:9). The NMR spectrum was consistent with the structure and compared well with the published information.[35]

Hydrogenolysis of podophyllotoxin (1) over palladium on carbon[11] furnished thick white needles of deoxypodophyllotoxin (11) in 80% yield: mp 165–166 °C; homogeneous by TLC with R_f 0.41 (chloroform–ethyl acetate, 4:1); NMR spectrum in agreement with earlier determinations;[35,36] $[\alpha]_{589}$ −171° or $[\alpha]_{546}$ −229° (both *c* 0.02, dioxane) [lit. mp 166–168 °C,[11] 167–168 °C,[3] 168–169 °C;[15,36] $[\alpha]_D$ −115° (chloroform);[15,36] and −181° (pyridine)]. Note that the epimeric deoxypicropodophyllin shows mp 164–165 or 170–171 °C and $[\alpha]_D$ +34 (chloroform) or +43° (pyridine).[15,36,37]

Tetrahydropyranylpodophyllotoxin (6). An earlier preparation[22] was modified as follows. A paste of podophyllotoxin (3.4 g, 8.2 mmol) in 10 mL of reagent dihydropyran plus several small crystals of *p*-toluenesulfonic acid monohydrate was stirred for 1 h. Five milliliters of 3% aqueous bicarbonate was introduced, volatiles were stripped at temperatures below 45 °C, and the residual oil was extracted with ether. After being rinsed with water and dried, the solution was concentrated to about 30 mL and then dropped into 600 mL of ice-cold petroleum ether. The resulting amorphous, white tetrahydropyranylpodophyllotoxin (6) after drying in vacuo to constant weight (3.7 g, 91%) showed mp 90–100 °C (shrinking from 70 °C): single spot on TLC, R_f 0.62 (chloroform–ethyl acetate, 1:9); IR ($CHCl_3$) 1075, 1150 and 1775 cm^{-1}, but no significant absorption at 3540 cm^{-1}; $[\alpha]^{24}_{589}$ −155° (*c* 0.015, dioxane); NMR ($CDCl_3$) δ 1.7 [m, 6, tetrahydropyranyl $C(CH_2)_3C$], 2.84 (m, 2, H-2 and 3), 3.77 (s, 3′,5′-dimethoxy), 3.83 (s, 4′-methoxy), 3.3–4.3 (m, H-1 plus THP CH_2O), 4.4–5.1 [m, 4, H-1 plus lactone CH_2 plus THP $HC(O)_2$], 5.98 [s, 2, $(CH_2)_2O_2$], 6.45 (s, H-2′ and 6′), 6.53 (s, H-8), 6.95 and 7.22 ppm (2 s, H-5, 1). Integration between 3.77 and 4.3 ppm corresponded to 12 protons and at 6.45–6.54 ppm to three protons [lit.[22] mp 74–97 °C; $[\alpha]_D$ −103° (*c* 1, chloroform)]. Tetrahydropyranylpicropodophyllin prepared in a similar way showed mp 207–227 °C, R_f 0.34 (chloroform–ethyl acetate, 4:1), which may be compared with R_f 0.40 for tetrahydropyranpodophyllotoxin (6) in the same solvent [lit.[22] mp 219–220 °C; $[\alpha]_D$ −20° (*c* 1, chloroform)].

4-Tetrahydropyranylpodophyllol (7) from Tetrahydropyranylpodophyllotoxin (6). A solution of tetrahydropyranylpodophyllotoxin (6) (3.4 g, 6.7 mmol) in 30 mL of dry tetrahydrofuran was added slowly to a stirred, ice-cold suspension of lithium aluminum hydride (0.30 g, 7.9 mmol) in 20 mL of tetrahydrofuran. Use of ether solvent here was also satisfactory. After 3.5 h of stirring at room temperature, the mixture was cooled to 0 °C and then treated first with a few drops and then with 10 mL of water. Solids were removed, and the filtrate was concentrated in vacuo. The oily residue was dissolved in chloroform, and the solution was washed with water, dried, and then stripped of all volatiles. When the residual oil as an ether solution was dropped into cold vigorously stirred petroleum ether (600 mL), an amorphous solid precipitated. Drying this product 7 to constant weight afforded 4-tetrahydropyranylpodophyllol (3.0 g, 89%) as a highly electrostatic white powder: mp 75–90 °C; $[\alpha]_{589}$ −109° and $[\alpha]_{546}$ −130° (both *c* 0.02, dioxane); IR ($CHCl_3$) 3450–3425 and 1589 cm^{-1}, but no maxima at 1775 cm^{-1}; NMR ($CDCl_3$) δ 1.5–2.5 [m, 8, H-2 and 3 plus THP $C(CH_2)_3C$], 3.0–4.4 (m, H-1 plus THP CH_2O plus $2CH_2OH$), 3.78 (s, 4′-CH_3O), 3.83 [s, 3′,5′-$(CH_3O)_2$], 4.5–5.1 [m, 2, H-4 plus THP $HC(O)_2$], 5.90 [s, 2, $CH_2(O)_2$], 6.37 (s, H-2′ and 6′), 6.41 (s, H-8), 6.78 and 7.33 ppm (2 s, 1, H-5). Integration at 3.0–3.83 ppm corresponded to 18 protons and at 6.37–6.41 ppm to three protons. In one experiment, the product showed two spots, R_f 0.28 and 0.21 (chloroform–ethyl acetate, 1:9); in another run material was obtained with only a single spot, R_f 0.18, in the same solvent, but when this spot was developed seven times in succession[38] it separated into two spots, R_f 0.73 and 0.64. Anal. Calcd for $C_{27}H_{34}O_9$: C, 64.57; H, 6.82. Found: C, 64.49; H, 6.89.

4-Deoxypodophyllol (12)[16] from Deoxypodophyllotoxin (11). Reducing deoxypodophyllotoxin (11) in either ether or tetrahydrofuran gave an 81% yield of crystalline 4-deoxypodophyllol (12): mp 148–149 °C; single spot on TLC, R_f 0.18 (chloroform–ethyl acetate, 1:9); $[\alpha]_{589}$ −185° and $[\alpha]_{546}$ −235° (both *c* 0.02, dioxane) [lit.[15,16] mp 148–150 °C; $[\alpha]_D$ −158° (*c* 0.74, chloroform)]; IR ($CHCl_3$) 3400 cm^{-1} (br), but no maxima at 1775 or 1765 cm^{-1}; NMR ($CDCl_3$) δ 1.95–2.35 (m, 2, H-2 and 3), 2.50–4.25 (m, 2 CH_2OH plus H-4), 3.27 [s, 3′,5′-$(CH_3O)_2$], 3.30 (s, 4′-CH_3O), 4.0–4.25 (s, H-1), 5.87 [s, 2, $CH_2(O)_2$], 6.27 (s, H-2′ and -6′), 6.40 (s, H-5), 7.13 ppm (s, 1, H-8). Integration at 2.5–4.25 ppm showed 18 protons and at 6.27–6.40 ppm, three protons.

4-Tetrahydropyranylanhydropodophyllol (8). After a solution of *p*-toluenesulfonyl chloride (1.3 g, 6.7 mmol) in 5 mL of dry pyridine was dropped into a stirred solution of 4-tetrahydropyranylpodophyllol (7, 2.8 g or 5.6 mmol) in 20 mL of pyridine, the mixture was kept at 53 °C for 4.5 h. Atmospheric moisture was carefully excluded. More *p*-toluenesulfonyl chloride (0.1 g) was added, and the reaction continued at the same temperature for another 4.5 h. Distillation under reduced pressure (bath <45 °C) removed most of the pyridine. Alcohol (50 mL) plus water (100 mL) was added to the remaining oil, and the mixture was distilled (<50 °C) while gradually introducing water to maintain the volume. The solids that had formed were collected, dissolved in chloroform, and dried. Removing all solvent left a pale yellow oil, which was chromatographed through a column of silica gel (300 g) by starting with chloroform as the eluting solvent and gradually introducing increasing amounts of ethyl acetate. 4-Tetrahydropyranylanhydropodophyllol (8), emerging as the first major fraction, was isolated as a solvent-free gummy oil (0.86 g, 32%). This product was homogeneous according to TLC but still showed twinned NMR signals for H-5, an indication that two isomers were obtained as expected. Subsequent fractions contained some anhydropodophyllol (9, 0.12 g or 5.5%), and other eluate fractions furnished mixtures (0.5 g total) from which additional amounts of the separated materials were obtained.

One of the stereoisomers of 4-tetrahydropyranylanhydropodophyllol (8) was isolated in crystalline form by recrystallization of the gummy oil from ether or absolute alcohol. The data given below refer to the crystalline product (R_f 0.50 with chloroform–ethyl acetate, 1:9), which, in contrast to the oil, had little tendency to lose the masking tetrahydropyranyl group on standing: mp 170–171 °C; $[\alpha]_{589}$ −88° (*c* 0.01, dioxane); IR ($CHCl_3$) 1590 cm^{-1} but little absorption at 3425 cm^{-1}; NMR ($CDCl_3$) δ 1.72 [m, 6, THP $C(CH_2)_3C$], 2.50 (m, 2, H-2 and 3), 2.9–4.4 (m, $3CH_2O$'s

plus H-1), 3.80 [s, 3′,5′-$(CH_3O)_2$], 3.86 (s, 4′-CH_3O), 4.6–4.9 [m, 2, H-4 plus $HC(O)_2$ in THP], 5.97 [s, 2, $CH_2(O)_2$], 6.21 (s, 2, H-2′ and 6′), 6.48 (s, 1, H-8), 7.28 ppm (s, 1, H-5). Note that the gummy oil showed exactly the same NMR spectrum except that the signal for H-5 instead of a one-proton singlet at δ 7.28 ppm appeared as a one-proton twin-peaked signal at δ 6.95 and 7.28 ppm. Anal. Calcd for $C_{27}H_{32}O_8$: C, 66.93; H, 6.66. Found: C, 66.80; H, 6.62.

The same material was obtained by allowing a solution of anhydropodophyllol (**9**) in dihydropyran (60 mg in 6 mL) containing several crystals of *p*-toluenesulfonic acid monohydrate to stand for 1 h. Removing the solvent left a glass, which when mixed with ether furnished crystalline tetrahydropyranylanhydropodophyllol (**8**) melting at 169–173 °C or, when admixed with the compound described above, at 172–174 °C. The glass as well as the crystalline product showed one spot on TLC, R_f 0.48 (chloroform–ethyl acetate, 1:9), the same as for the product **8** obtained by cyclizing **7**.

Anhydropodophyllol (9). A solution of tetrahydropyranylanhydropodophyllol **8** (0.69 g, 1.5 mmol) containing a little anhydropodophyllol in 25 mL of absolute ethanol containing 20 drops of concentrated hydrochloric acid was stirred at room temperature for 1 h. After neutralization to litmus with 3% aqueous sodium bicarbonate, the mixture was distilled in a 45 °C bath while slowly adding water. When the alcohol had all been removed, the remaining mixture was cooled, and the precipitated solids were collected. This product was dissolved in chloroform, and, after drying, the solution was stripped of all solvent. The yellow residual oil, dissolved in a small volume of ether, was dropped into 500 mL of cold petroleum ether. The precipitated anhydropodophyllol was removed and the precipitation procedure repeated to get additional amounts of product. Long exposure to vacuum gave white, amorphous solvent-free anhydropodophyllol (**9**) (0.44 g or 72%), mp 78–96 °C, a glass, which resisted crystallization. Thin-layer chromatography revealed a trace amount of impurity as a faint spot in addition to the predominant spot at R_f 0.30 (chloroform–ethyl acetate, 1:9). The product showed $[\alpha]_{589}$ −80° (*c* 0.01, dioxane); IR ($CHCl_3$) 3400, 1585 cm^{-1}; NMR ($CDCl_3$) δ 2.40 (m, 3, H-2 and -3 plus OH), 3.00 (distorted d, J = 6 Hz, 1, the THF CHO closest to pendant trimethoxyphenyl ring), 3.75 [s, 3′,5′-$(CH_3O)_2$], 3.82 (s, 4′-CH_3O), 3.3–4.4 (unresolved, H-1 plus the three remaining THF CH_2O protons), 4.57 (d, J = 6 Hz, 1, H-4), 5.92 [2, 2, $CH_2(O)_2$], 6.13 (s, 2, H-2′ and -6′), 6.43 (s, 1, H-8), 7.13 ppm (s, 1, H-5). The signals at 3.75–4.14 corresponded to a total of 13 protons. Anal. Calcd for $C_{22}H_{24}O_7 \cdot 0.5H_2O$: C, 64.50; H, 6.15. Found: C, 64.54; H, 6.06.

Neoanhydropodophyllol (5). In the cyclization of 4-tetrahydropyranylpodophyllol (**7**), when processing the reaction mixture included exposure to dilute hydrochloric acid in order to remove pyridine, the tetrahydropyranylanhydropodophyllol (**8**) was accompanied by a side product, which proved to be the same as neoanhydropodophyllol (**5**), prepared by cyclizing podophyllol (**4**).[14] After crystallization from ethanol, the neoanhydropodophyllol isolated in the present work gave a single TLC spot, R_f 0.18 (chloroform–ethyl acetate, 1:9), and showed mp 256–257 °C: $[\alpha]_{589}$ +15° (*c* 0.02, dioxane) [lit. mp 253.5–255.5, 254–256.5, and 256–257 °C; $[\alpha]_D$ 0° (*c* 0.3, chloroform)[3,12,14]]; NMR ($CDCl_3$) δ 2.70 (m, 2, H-2 and -3), 3.82 [s, 3′,5′-$(CH_3O)_2$], 3.88 (s, 4′-CH_3O), 3.5–4.1 (m, CH_2OH plus THF CH_2O), 4.41 (distorted d, J = 2 Hz, 1, H-1), 4.70 (d, J = 3 Hz, 1, H-4), 5.92 [s, 2, $CH_2(O)_2$], 6.38 (s, 2, H-2′ and -6′), 6.58 and 6.65 ppm (2 s, 2, H-8 and -5). Integration from 3.82–4.1 ppm showed 14 protons as required.

Deoxyanhydropodophyllol (10). A. From Hydrogenolysis of Anhydropodophyllol (9). Stirring a mixture of anhydropodophyllol (**9**, 0.14 g) dissolved in 20 mL of absolute ethanol containing 0.1 g of 5% palladium on carbon for 24 h under an atmosphere of hydrogen removed the hydroxyl group. The reaction mixture, when freed of catalyst and volatiles, left an oil, which was taken up in chloroform and dried. A concentrated solution of the product in chloroform was flooded with hexane to give amorphous deoxyanhydropodophyllol (**10**) (mp 56–70 °C): homogeneous by TLC, R_f 0.53 (chloroform–ethyl acetate, 1:9); $[\alpha]$ −148° (*c* 0.01, dioxane); IR ($CHCl_3$) 1590 cm^{-1} but no peak at 3500 cm^{-1}; NMR ($CDCl_3$) δ 2.0–3.7 (complex, H-2, -3, and -4 plus THF CH_2O closest to trimethoxyphenyl ring), 3.78 [s, 3′,5′-$(CH_3O)_2$], 3.83 (s, 4′-CH_3O), 3.7–4.4 (complex, H-1 and THF CH_2O), 5.92 [s, 2, $CH_2(O)_2$], 6.12 (s, 2, H-2′ and -6′), 6.48 (s, 1, H-8), 6.67 ppm (s, 1, H-5). Crystallization attempts did not succeed.

B. Deoxyanhydropodophyllol (10) by Cyclizing 4-Deoxypodophyllol (12).[16] A solution of deoxypodophyllol (**12**, ~0.4 g) in dry pyridine (30 mL) containing *p*-toluenesulfonyl chloride (0.3 g) was heated at 80 °C for 3.5–4 h. The crude cyclization product was chromatographed through silica gel (200 g) using chloroform at first and then chloroform containing increasing amounts of ethyl acetate as eluting solvent. Appropriate fractions (TLC monitoring) were combined and stripped of all volatiles. The resulting gum was dissolved in methanol and then forced out by adding water. The precipitated deoxyanhydropodophyllol (one spot on TLC, R_f 0.52, using chloroform–ethyl acetate, 1:9) showed mp 62–76 °C and gave an NMR curve identical with that described above [lit.[3,16] mp 65–85 °C; $[\alpha]_D$ −71° (chloroform)]. Note that 4-deoxyanhydropicropodophyllin has been reported[3,16] with mp 176 °C and $[\alpha]_D$ +20.5° (chloroform).

4-Tetrahydropyranylpodophyllol Ditosylate (13). 4-Tetrahydropyranylpodophyllol (**7**, 4.0 g or 7.6 mmol) in 7 mL of dry pyridine was added dropwise (cooling) to a solution of *p*-toluenesulfonyl chloride (6.0 g, 32 mmol) in 10 mL of pyridine. The faintly brown mixture was allowed to stand in the refrigerator for 2 days, during which time much solid formed. After most of the solvent had been distilled away (temperatures below 25 °C), 250 g of crushed ice was added and, after stirring the mixture for 1 h, it was filtered. The white solids were washed on the funnel with water and then dried in a current of air. This product (6.0 g) was chromatographed through silica gel, with chloroform–ethyl acetate (4:1), as solvent. The early fractions were combined (TLC monitoring) and were stripped of solvent, keeping the temperature no higher than 25 °C. The gummy residue was taken up in chloroform (20 mL), and the solution was dropped into 500 mL of cold, stirred petroleum ether. The precipitate was collected and dried to give 4.5 g (73%) of white tetrahydropyranylpodophyllol ditosylate (**13**): mp 76–83 °C; homogeneous by TLC, R_f 0.55 (chloroform–ethyl acetate, 4:1) and R_f 0.55 (carbon tetrachloride–ethyl acetate, 9:3); IR ($CHCl_3$) 1187 cm^{-1} for sulfonate S=O but no maxima at 4000–3050 cm^{-1}; $[\alpha]_{589}$ −146° and $[\alpha]_{546}$ −169° (both *c* 0.013, dioxane). The ditosylate is somewhat unstable in the air at room temperature but keeps well when sealed and stored at below 0 °C temperatures.

A small sample was crystallized by diluting an ether solution (0.2 g in 25 mL) with petroleum ether (30 mL) and concentrating the clear solution to about half its volume at temperatures below 25 °C. Anal. Calcd for $C_{21}H_{46}O_{13}S_2$: C, 60.73; H, 5.72; S, 7.91. Found: C, 61.1; H, 5.8; S, 7.6.

Deoxypodophyllol Ditosylate (18). In their essentials, the directions followed those described for compound **13**. Thus a mixture of 2.8 g (6.9 mmol) of deoxypodophyllol (**12**) with 6.0 g (31 mmol) of *p*-toluenesulfonyl chloride in 15 mL of pyridine was allowed to react and was processed as before. The chromatographed product was forced out of chloroform solution with pentane, and the white crystals were dried to constant weight. The ditosylate **18** obtained in this way weighed 4.2 g (85%) and showed one spot on TLC with R_f 0.57 (chloroform–ethyl acetate, 4:1), 0.21 (carbon tetrachloride–ethyl acetate, 9:3), and 0.28 (chloroform–ethyl acetate, 19:1): mp 132–134 °C dec; $[\alpha]_{589}$ −163° and $[\alpha]_{546}$ −188° (both *c* 0.02 in dioxane); IR ($CHCl_3$) 1177 and 1600 cm^{-1}; NMR ($CDCl_3$) δ 2.0–3.1 (m, H's at 2,3,4), 2.45 (s, 2 × CH_3Ar), 3.45–4.30 (m, 2 × CH_2OTs), 3.72 [s, 3′,5′-$(CH_3O)_2$], 3.78 (s, 4′-CH_3O), 4.18 (m, H-1), 5.80 [s, 2, $CH_2(O)_2$], 6.15 (s, 2, H-2′,6′), 6.30 and 6.43 (s, 2, H-8 and -5), 7.30 and 7.65 ppm (2 sets of m, 4 + 4, aromatic tosylate H's). Integration between 2.0 and 2.45 ppm indicated ten protons and between 3.45 and 4.2 ppm indicated 14 protons.

Evacuation of the analytical sample at 65 °C (0.1 mm) for 1 day removed 0.4% of pentane. Anal. Calcd for $C_{36}H_{38}O_{11}S_2$: C, 60.83; H, 5.39. Found: C, 60.84; H, 5.28.

Hydroxy Cyclic Sulfide 15. To a cold stirred solution of sodium sulfide monohydrate (4.0 g, 16.6 mmol) in 10 mL of water was slowly added dimethyl sulfoxide (35 mL) followed by 0.5-mL portions of a solution of 4.5 g (5.6 mmol) of tetrahydropyranylpodophyllol ditosylate (**13**) in 35 mL of dimethyl sulfoxide. The somewhat turbid mixture was stirred overnight at 25–30 °C. Dropwise addition to crushed ice (800 g) precipitated a faintly yellow solid, which was collected, washed with water, air-dried, and then chromatographed through silica gel using chloro-

form–ethyl acetate (19:1) as the developing solvent. Monitoring by TLC (visualization with the help of a spray of 10% palladium chloride in 6 M hydrochloric acid) guided the combination of fractions that contained only the desired product. When all solvent was stripped at temperatures below 35–40 °C, a fluffy solid was left, which was dissolved in 35 mL of dry ether and added dropwise to cold, stirred pentane (500 mL) to give white thioether 14 containing 6–7% of solvent. This product 14 was homogeneous by TLC with R_f 0.52 (chloroform–ethyl acetate, 19:1) and R_f 0.75 (CCl_4–ethyl acetate, 3:1): mp 127–136 °C; $[\alpha]_{589}$ −139° and $[\alpha]_{546}$ −164° (both *c* 0.02, dioxane); IR ($CHCl_3$) no significant absorptions at 1180–1195 or 3050–3600 cm^{-1}.

A sample for analysis was prepared by dissolving 0.1 g of thioether 14 (which was very soluble in ordinary laboratory solvents) in ~5 ml of ether, adding the solution to 20 mL of pentane, and evaporating about half the solvent at room temperature. Filtration afforded product 14, which showed mp 133–143 °C after evacuation for 24 h. Anal. Calcd for $C_{27}H_{32}O_7S$: C, 64.77; H, 6.40; S, 6.40. Found: C, 64.74; H, 6.56; S, 6.37.

To remove the masking tetrahydropyranyl group, a solution containing 2.0 g (4.0 mmol) of 14 plus 0.5 mL of concentrated hydrochloric acid in 100 mL of 9:1 acetone–water was allowed to stand at room temperature for 20 h. Concentration to a volume of 20 mL at temperatures below 25 °C afforded solids, which were collected, washed with water, and sucked dry.

The crude product (1.8 g) was chromatographed through silica gel (chloroform–ethyl acetate, 19:1), and the fractions showing a single TLC spot at R_f 0.16 (chloroform–ethyl acetate, 19:1) were pooled and stripped of solvent (<25 °C). The residual gummy solid, dissolved in 10 mL of chloroform, was added dropwise to cold pentane (500 mL), and the white precipitated hydroxy cyclic sulfide **15** was dried in the air (1.5 g, 90%). Crystallization was unsuccessful.

This product **15**, mp 116–120 °C, contained some pentane (2.3%) but was otherwise homogeneous, with R_f 0.16 (chloroform–ethyl acetate, 19:1) and R_f 0.33 (chloroform–ethyl acetate, 49:1): $[\alpha]_{589}$ −21° and $[\alpha]_{546}$ −26° (*c* 0.02, dioxane); IR ($CHCl_3$) broad band at 3400 cm^{-1}; NMR ($CDCl_3$) δ 2.10–3.00 (m, 6, tetrahydrothiophene H's), 3.25 (m, 1, OH), 3.75 [s, 3′,5′-$(CH_3O)_2$], 3.83 (s, 4′-CH_3O), 4.20 (br s, 1, H-1), 4.60 (br s, 1, H-4), 5.90 [s, 2, $CH_2(O)_2$], 6.15 (s, 2, H-2′,6′), 6.40 (s, 1, H-5), 7.08 ppm (s, 1, H-8). The two peaks at 3.83 and 4.20 ppm together came to nine protons.

A sample of hydroxy sulfide **15** was held at 0.1 mm overnight before submitting for analysis. Anal. Calcd for $C_{22}H_{26}O_6S$: C, 63.44; H, 5.81; S, 7.70. Found: C, 63.60; H, 6.00; S, 7.48.

To prove that no stereochemical change had occurred when the tetrahydropyranyl group was removed, the hydroxy sulfide **15** was reconverted to its tetrahydropyranyl derivative, as follows. A solution of freshly distilled dihydropyran (0.6 mL) and 80 mg of hydroxy sulfide **15** in 4 mL of chloroform containing a small amount of *p*-toluenesulfonic acid was allowed to stand at room temperature for 2 h. The reaction mixture was diluted with more chloroform before rinsing it with portions of 2% aqueous bicarbonate and then water. The dried solution was stripped of all volatiles at room temperature to leave a residue, which was dissolved in ether (1.5 mL) and added by drops to cold pentane. Cooling the slightly turbid solution by stages to −25 °C slowly precipitated a fluffy white solid, the desired tetrahydropyranyl derivative 14. The sample, homogeneous according to TLC with R_f 0.53 (chloroform–ethyl acetate, 19:1) and 0.75 (carbon tetrachloride–ethyl acetate, 3:1), showed mp 139–149 °C (shrinking) or, when mixed with the earlier material, mp 135–148 °C. Virtually superposable infrared and nuclear magnetic resonance curves, as well as the TLC properties, confirmed the identity of the regenerated tetrahydropyranyl derivative 14 with the same compound obtained by cyclization from **13**.

Cyclic Sulfide 19. The cyclization procedure was similar to that followed for the tetrahydropyranyloxy analogue **13**. A mixture, containing sodium sulfide monohydrate (4.0 g, 16.6 mmol) and deoxypodophyllol ditosylate **18** (2.8 g, 3.9 mmol) in 120 mL of dimethyl sulfoxide plus 25 mL of water, was allowed to react for 5 h. The crude dry thioether **19**, still containing traces of dimethyl sulfoxide, was chromatographed essentially as described above for the tetrahydropyranyl derivative. The pooled fractions showing only one spot on TLC were freed of all solvent, and a solution of the residue in 15 mL of chloroform was dropped into 200 mL of cold pentane. Slow evaporation of solvent at room temperature furnished cyclic sulfide **19** as a white powder (1.2 g or 77% after correction for 2% content of solvent), homogeneous by TLC with R_f 0.41 (chloroform–ethyl acetate, 19:1) and R_f 0.43 (carbon tetrachloride–ethyl acetate, 3:1), and showing mp 82–85 °C (shrinking at 75 °C): $[\alpha]_{589}$ −80° and $[\alpha]_{546}$ −133° (both *c* 0.023, dioxane); IR (CCl_4) 1589 cm^{-1} (aromatic C=C) but no significant absorptions at 3600–3200 or at 1177 cm^{-1}; NMR ($CDCl_3$) δ 2.00–3.45 (m, 8, all tetrahydrothiophene H's plus 2H-1), 3.75 [s, 3′,5′-$(CH_3O)_2$], 3.80 (s, 4′-CH_3O), 4.15 (distorted d, H-1), 5.85 [s, 2, $CH_2(O)_2$], 6.10 (s, 2, H-2′,6′), 6.40 (s, 1, H-8), 6.60 ppm (s, 1, H-5). The signals from 3.75 to 4.15 ppm corresponded to ten protons as required.

A small sample was held at 65 °C (0.1 mm) for 15 h to get rid of solvent. Anal. Calcd for $C_{22}H_{24}O_5S$: C, 65.98; H, 6.09; S, 8.01. Found: C, 66.19; H, 6.22; S, 8.20.

Hydroxy Cyclic Sulfone 16. A solution of *m*-chloroperbenzoic acid (1.2 g of 75% material or 5.3 mmol) in 10 mL of chloroform was added in small portions to a cold solution of hydroxy sulfide **15** (1.05 g, 2.5 mmol) in 20 mL of chloroform, and the mixture at room temperature was set aside for 1 h. Vigorous stirring after adding saturated aqueous sodium sulfite decomposed all unchanged peracid, after which the organic layer was washed with 0.5 M aqueous bicarbonate and then with water and finally dried and stripped of volatiles. The residual gum was chromatographed on silica gel with chloroform–methanol (50:1) as solvent. Fractions homogeneous by TLC were combined and freed of all solvent (<40 °C). When the residual solid was dissolved in 10 mL of chloroform and the solution was dropped into cold pentane (200 mL), the hydroxy sulfone **16** was obtained as a fluffy white powder (0.79 g or 70% after correction for ~1% pentane), homogeneous by TLC, R_f 0.15 (chloroform–methanol, 9:1), with mp 140–143 °C (shrinking at 135 °C): $[\alpha]_{589}$ −381° and $[\alpha]_{546}$ −476° (*c* 0.02, dioxane); IR ($CHCl_3$) 3400, 1302 cm^{-1}; NMR ($CDCl_3$) δ 2.5–3.0 and 3.1–3.9 (br m, 7, all sulfone ring H's plus OH), 3.80 and 3.85 [s, 9, 3′,4′,5′-$(CH_3O)_3$], 4.2 (br, 1, H-1), 4.60 (br, 1, H-4), 5.95 [s, 2, $CH_2(O)_2$], 6.18 (s, 2, H-2′,6′), 6.42 (s, 1, H-8), 7.08 ppm (s, 1, H-5).

The sample for analysis was held at 0.1 mm for 15 h. Anal. Calcd for $C_{22}H_{24}O_8S$: C, 58.92; H, 5.30; S, 7.15. Found: C, 58.67; H, 4.98; S, 6.93.

Cyclic Sulfone 20. A. By Oxidation of Cyclic Sulfide 19. The reaction mixture was made up of 0.6 g of 75% *m*-chloroperbenzoic acid (2.8 mmol) and 0.50 g (1.2 mmol) of cyclic sulfide **19** dissolved in 16 mL of chloroform. The crude sulfone product was chromatographed through silica gel with chloroform–ethyl acetate (19:1) as developing solvent. Appropriate pooled fractions were freed of solvent at temperatures below 30 °C. Then a solution of the residual colorless gum in ether was slowly concentrated to a volume of 20 mL before flooding with pentane (70 mL). The cyclic sulfone **20** that precipitated was collected as a white granular solid (0.39 g, still with 1.8% of ether), mp 120–122 °C (to a glass), producing a single spot on TLC, R_f 0.53 (chloroform–methanol, 49:1): $[\alpha]_{589}$ −206° and $[\alpha]_{546}$ −266° (both *c* 0.02, dioxane); IR (CCl_4) 1295 cm^{-1}; NMR ($CDCl_3$) δ 2.3–3.6 (br m, 8, ring H's of the sulfone plus CH_2 at position 4), 3.78 and 3.83 [s, 9, 3′,4′,5′-$(CH_3O)_3$], 4.20 (br, 1, H-1), 5.90 [s, 2, $CH_2(O)_2$], 6.10 (s, 2, H-2′,6′), 6.40 (s, 1, H-8), 6.60 ppm (s, 1, H-5). Anal. (sample evacuated for 15 h at 75 °C): Calcd for $C_{22}H_{24}O_7S$: C, 61.09; H, 5.59; S, 7.41. Found: C, 61.27; H, 5.80; S, 7.68.

B. By Hydrogenolysis of Hydroxy Cyclic Sulfone 16. A suspension of 10% palladium on carbon (0.25 g) in methanol (5 mL) containing 0.19 g of hydroxy sulfone **16** (0.44 mmol) was stirred under H_2 at a small positive pressure. Processing the reaction mixture included silica gel column chromatography with chloroform–methanol (49:1). The chromatographed one-spot material (~0.85 g), as a solution in 1.5 mL of dry ether, was dropped into stirred cold pentane. Slow concentration of the solution to ~5 mL precipitated the desired cyclic sulfone **20**. This material showed the same TLC behavior with a variety of developing solvents as the above oxidation product **20**. A sample after drying overnight at 65 °C (0.2 mm) melted alone at 118–121 °C and when mixed with the oxidation sulfone at 118–123°. The two IR absorption spectra were essentially identical; the two NMR curves were almost superposable.

Hydroxy Cyclic Sulfoxide 17. Hydroxy sulfide **15** (0.30 g, 0.72 mmol) in 6 mL of methanol was mixed with sodium metaperiodate (0.16 g, 0.75 mmol) dissolved in 2 mL of water, and the heterogeneous system was stirred for 1 h. Adding more periodate (15 mg) and further stirring for 0.5 h still left detectable amounts of starting material. The solids were collected and rinsed on the funnel with absolute methanol. When the combined filtrate and rinsings were stripped of solvent (<40 °C) a colorless gum remained, which was extracted with portions of chloroform. The extract was washed with water, dried, and stripped of solvent, leaving a residue that was chromatographed through a small column of silica gel with chloroform–methanol (49:1) as solvent. When the starting material emerging in the eluate was exhausted, the solvent was switched to methanol, which removed the desired sulfoxide product. The combined fractions were freed of solvent, and the residue was forced out of a chloroform solution (6 mL) by dilution with cold pentane. The resulting white amorphous solid, after exposure to a 0.1-mm vacuum for 10 h, was taken as hydroxy sulfoxide **17** (0.22 g, 71%), mp 148–156 °C. TLC showed two spots with R_f 0.40 and 0.33 (chloroform–methanol, 9:1): IR (mineral oil mull) 3350 (br) and 1068 cm^{-1}; NMR ($CDCl_3$) δ 1.6–5.0 (br m for the cyclic sulfoxide H's) with signals superimposed at 3.77 and 3.82 [s, 3′,4′,5′-$(CH_3O)_3$] and at 4.0–5.0 (br, H-1, 4 plus OH), 5.92 [s, 2, $CH_2(O)_2$], 6.22 (d, J = 3 Hz, 2, H-2′,6′), 6.45 (s, 1, H-8), 7.14 ppm (s, 1, H-5). Integration from 1.6 to 5.0 indicated 18 protons. Anal. (long exposure to 65 °C at 0.1 mm): Calcd for $C_{22}H_{24}O_7S$: C, 61.09; H, 5.59. Found: C, 59.51; H, 5.48.

When a small sample of sulfoxide **17** was treated with *m*-chloroperbenzoic acid essentially according to the procedure followed in the oxidation of hydroxy sulfide **15** to sulfone **16**, the twin sulfoxide TLC spots at R_f 0.40 and 0.33 disappeared in favor of a single spot at R_f 0.51 (chloroform–methanol, 9:1), a value that matched that given by hydroxy sulfone **16**.

Cyclopentanone 21 from Ditosylate 18 by Reaction with Tetracarbonylferrate. Deoxypodophyllol ditosylate **18** (6.0 g, 8.5 mmol) in a three-necked flask was stirred for 15 min with *N*-methyl-2-pyrrolidone (50 mL) freshly distilled from calcium hydride. Dry nitrogen blanketed the mixture during this and all other phases of the reaction. Disodium tetracarbonylferrate, $Na_2Fe(CO)_4$ (8.0 g, 27 mmol), in a small flask was fitted to the reaction flask through a right-angle tube that swiveled in the greased joint to permit addition. The reagent was introduced in small portions over 45 min to the stirred pyrrolidone mixture, which was then stirred further for 2 h. More tetracarbonylferrate (0.5 g) was added, and stirring was continued for another 30 min. During this time the originally colorless solution gradually became deep purple.

The reaction mixture was poured into 300 mL of ethyl ether, and the solids, collected on a sintered glass funnel, were rinsed with ether. The combined filtrates were shaken with three portions of water. To recover small amounts of product, the water wash was reextracted with some ether, which was then returned to the main ether extract, and the whole mixture was dried.

Additional product was obtained by extracting the original precipitate with portions of chloroform, drying the green chloroform extracts over sodium sulfate, and removing the drying agent together with some dark-colored precipitated solids.

The combined ether and chloroform solutions were concentrated (40–45 °C), and the residual syrup was chromatographed on silica gel using chloroform–ethyl acetate (15:1) as solvent. After the first 400 mL of solvent had passed through, the next 400 mL was collected, stripped of solvent, and finally warmed at 61 °C (0.2 mm) for 36 h. Ketone **21** was obtained in this way as a white solid (2.3 g, 67%), melting indefinitely at 78–145 °C and showing one spot on several different TLC systems, e.g., R_f 0.21 (chloroform–ethyl acetate, 49:1), R_f 0.16 (carbon tetrachloride–ethyl acetate, 4:1), and R_f 0.36 (carbon tetrachloride–tetrahydrofuran, 4:1): IR ($CHCl_3$) 1735 cm^{-1}; $[\alpha]_{589}$ −27° and $[\alpha]_{546}$ −80° (*c* 0.015, dioxane); NMR ($CDCl_3$) δ 1.5–3.4 (br m, 8, all cyclopentanone H's plus CH_2 at position 4), 3.80 and 3.85 [2 s, 9, 3′,4′,5′-$(CH_3O)_3$], 4.60–4.75 (distorted doublet, 1, H-1), 5.93 [s, 2, $CH_2(O)_2$], 6.15 (s, 2, H-2′,6′), 6.48 (s, 1, H-8), 6.65 ppm (s, 1, H-5). Attempts at recrystallizing the product from a variety of solvents failed. Anal. Calcd for $C_{23}H_{24}O_6$: mol wt, 396; C, 69.48; H, 6.10. Found: mol wt (mass spectral), 396; C, 69.49; H, 6.04.

This cyclopentanone product **21** was the same as the product obtained from **25** by several TLC comparisons and by the same infrared absorption curves. With the exception of three minor extraneous signals given by the latter product, the two NMR curves were also identical.

Dinitrile 23 from Deoxypodophyllol Ditosylate (18). The ditosylate (0.90 g, 1.3 mmol) was added against a slow stream of dry argon to a stirred, homogeneous solution of sodium cyanide (0.22 g, 4.4 mmol) in dry dimethyl sulfoxide, and the mixture at 90–95 °C was stirred under argon for 1.5 h. The cooled solution was quenched over crushed ice (~100 g), and after stirring the mixture, it was filtered. The solids were washed with water and then taken up in chloroform. The solution was shaken with water, dried, concentrated to a small volume, and then dropped into cold pentane. The solids were dried to constant weight first in air and then for 16 h at 65 °C (0.1 mm). The faintly yellow dinitrile **23** (0.45 g, 84%) obtained in this way showed mp 85–100 °C and developed only single spots on TLC, R_f 0.31 (chloroform–ethyl acetate, 4:1) or R_f 0.32 (benzene–acetic acid–methanol, 17:1:1): $[\alpha]_{589}$ −119° and $[\alpha]_{546}$ −156° (*c* 0.02, dioxane); IR (CCl_4) 2250 cm^{-1} (weak); NMR ($CDCl_3$) δ 1.7–3.4 (br m, 8, H-2,3,4 plus 2 × CH_2CN), 3.80 and 3.85 [2 s, 9, 3′,4′,5′-$(CH_3O)_3$], 4.30 (br s, 1, H-1), 5.90 [s, 2, $CH_2(O)_2$], 6.35 (s, H-2′,6′), 6.45 (s, H-8), 6.63 ppm (s, H-5). Integration with the last three signals agreed with the required 4 H's. Anal. Calcd for $C_{24}H_{24}N_2O_5$: C, 68.55; H, 5.76. Found: C, 68.53; H, 5.98.

Ketonitrile 25. Podophyllol ditosylate (**18**, 1.2 g or 1.7 mmol) and sodium cyanide (0.29 g, 6.0 mmol) in dimethyl sulfoxide (10 mL) were allowed to react as before at 95 °C for 1.5 h. Then, with the minimum exposure to air, powdered sodium hydride (48 mg, 2.0 mmol) was added to the faintly brown solution at room temperature,[39] and heating and stirring were continued as before for another hour. The dark solution was poured over 100 g of crushed ice, and the mixture was stirred for 0.5 h. Filtration afforded the iminonitrile **24**, which after air-drying was obtained as a pale yellow solid with an intense IR absorption peak at 2250 cm^{-1} (nitrile).

In order to reach ketone **25**, a solution of this iminonitrile **24** in 15 mL of hot ethanol was diluted with 5 mL of 6 M hydrochloric acid and hydrolysis was allowed to proceed at room temperature for 1 h. The mixture was then stirred with crushed ice (50 g), and the precipitate was collected, rinsed with some water, and dried in the air. This crude ketonitrile **25** was chromatographed through silica gel using chloroform–ethyl acetate (4:1) as solvent and TLC monitoring. Pooled fractions were stripped of solvent (<50 °C), and a solution of the residual gum in 3 mL of chloroform was added dropwise to 100 mL of cold stirred pentane. The resulting precipitate was collected and dried to constant weight first in the air and then at 65 °C (0.1 mm) to give ketonitrile **25** (0.47 g, 70%), mp 140–145 °C (preliminary softening). Thin-layer chromatography results showed that this product was a mixture, with R_f 0.45 and 0.42 (chloroform–ethyl acetate, 4:1): IR ($CHCl_3$) 2250 and 1758 cm^{-1}; NMR ($CDCl_3$) δ 1.7–3.5 (br m, 7, all cyclopentanone H's plus CH_2 at position 4), 3.75 and 3.78 [2 s, 9, 3′,4′,5′-$(CH_3O)_3$], 4.10–4.35 (br s, 1, H-1), 5.84 [s, 2, $CH_2(O)_2$], 5.95 and 6.70 ppm (m, 4, H-2′,6′,5,8). Anal. Calcd for $C_{24}H_{23}NO_6$: C, 68.44; H, 5.51. Found: C, 68.32; H, 5.53.

Ketonitrile **25** dissolved in 10% aqueous sodium hydroxide and came out of solution on neutralization.

Cyclopentanone 21 from Ketonitrile 25. A stream of hydrogen chloride was bubbled into a solution of ketonitrile **25** (0.8 g, 1.9 mmol) in 80 mL of methanol for about 20 min, after which the reaction mixture was allowed to stand at room temperature for 15 h. The solution was concentrated to a small volume before quenching over ice. The mixture was sitrred for 1 h, and the resulting solid keto methyl ester was rinsed with water and dried.

This keto ester weighed 0.6 g and showed intense IR absorption maxima at 1755 and 1725 cm^{-1}. It went into solution with 5% aqueous sodium hydroxide and reprecipitated on acidification.

To remove the ester group, the keto ester (0.5 g) was refluxed for 15 h in a solution of methanol (8 mL) plus 20% aqueous potassium carbonate (20 mL). The dark solution was extracted with chloroform, and the extracts were rinsed first with several portions of 10% aqueous sodium hydroxide and then with water. The dried chloroform solution was stripped of solvent to leave a gum, which was column chromatographed through silica gel with

chloroform–ethyl acetate (20:1) as solvent. Fractions were combined on the basis of TLC results, and the combined eluates were stripped of all solvent. The product remaining was dissolved in ether and again freed of volatiles. The addition and removal of ether was repeated three times, and finally the still somewhat tacky solid ketone **21** was dried at 45 °C (1.5 mm).

This ketone **21** was homogeneous according to the single spots observed with several TLC systems. The mass spectral molecular weight was 396 as required, and with the exception of three unexplained minor signals at δ 6.33, 6.35, and 6.63 ppm, the NMR curve was the same as that obtained for the cyclization ketone **21**.

Cyclopentane 22 from Ketone 21. Zinc dust (4.5 g, 70 mmol) was added in small portions over a period of 20 min to a well-stirred solution of ketone **21** (1.5 g, 3.8 mmol) in dry ether (100 mL) that had been treated with a stream of hydrogen chloride gas at room temperature for 15 min. After 1.5 h of stirring, the ether was decanted and the remaining slurry was rinsed with additional ether. The combined ether solutions were washed with water, aqueous bicarbonate, and water and then dried. Removal of all solvent gave a brown gummy residue, which was chromatographed with chloroform–ethyl acetate (15:1) as solvent. Fractions showing one spot on TLC were combined and stripped of solvent (<45 °C). Crystallization of the residue from pentane gave the white cyclopentane product **22** (0.9 g, 61%) plus some additional amounts (7%) of slightly impure material.

Cyclopentane **22** showed mp 101–103 °C (shrinking at 98 °C) and produced single spots on TLC, R_f 0.60 with chloroform–ethyl acetate (15:1) and R_f 0.67 with acetone–hexane (2:1): $[\alpha]_{589}$ −187° and $[\alpha]_{546}$ −194° (*c* 0.016, dioxane); IR ($CHCl_3$) 1590 cm^{-1} but no maximum at 1735 cm^{-1}; NMR ($CDCl_3$) δ 0.60–3.0 (br m, 10, all cyclopentane H's plus CH_2 at position 4), 3.83 and 3.88 [2 s, 9, 3′,4′,5′-$(CH_3O)_3$], 4.10–4.30 (br s, 1, H-1), 5.93 [s, 2, $CH_2(O)_2$], 6.20 (s, 2, H-2′,6′), 6.50 (s, 1, H-8), 6.68 ppm (s, 1, H-5). Anal. Calcd for $C_{23}H_{26}O_6$: C, 72.23; H, 6.85. Found: C, 72.01; H, 6.92.

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3-Fluoro-1-hydroxypropan-2-one (Fluorohydroxyacetone) and Some Esters. Syntheses and Effects in BDF_1 Mice[1]

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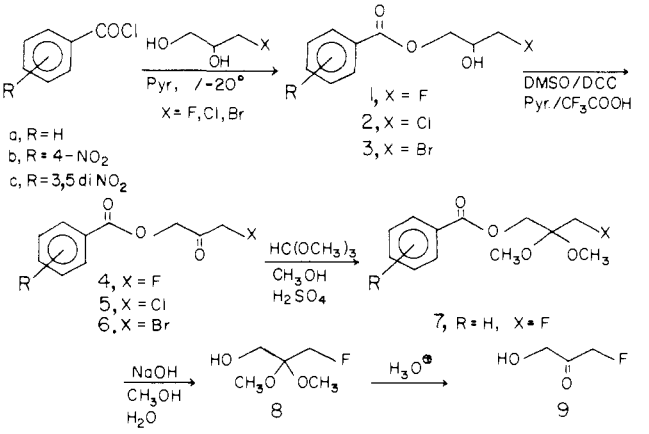
1-(Benzoyloxy), 1-(4-nitrobenzoyloxy), and 1-(3,5-dinitrobenzoyloxy) derivatives of 3-fluoro-, 3-chloro-, and 3-bromopropan-2-one were prepared by oxidation of the 1-benzoyloxy-3-halopropan-2-ols in turn prepared from the appropriate benzoyl chloride and 3-halo-1,2-propanediols. 1-Benzoyloxy-3-fluoropropan-2-one was allowed to react with acidic trimethyl orthoformate to yield 1-benzoyloxy-2,2-dimethoxy-3-fluoropropane which upon basic hydrolysis afforded 2,2-dimethoxy-3-fluoropropan-1-ol (fluorohydroxyacetone dimethyl ketal). This was deketalized with aqueous HCl to afford 3-fluoro-1-hydroxypropan-2-one (fluorohydroxyacetone), the title compound. By reacting 1-chloro-3-fluoropropan-2-one and 1,3-dichloropropan-2-one with potassium acetate, 1-acetoxy-3-fluoropropan-2-one and 1-acetoxy-3-chloropropan-2-one (fluoro- and chlorohydroxyacetone acetate, respectively) were obtained. Similarly, sodium benzoate and 1-chloropropan-2-one produced 1-benzoyloxypropan-2-one. Structure–activity relationships are discussed which relate chemical structure, alkylating ability, toxicity, and antitumor effects. Comparative toxicities in mice showed decreasing toxicity, on a molar basis, in the 1-benzoyloxy-3-halopropan-2-one series of bromo > fluoro > chloro. Ketones were much more toxic than the corresponding alcohols. In general the phosphate and benzoyloxy derivatives are more toxic than acetoxy compounds, with nitro-substituted benzoyloxy derivatives being much less toxic.

Differences in glycerolipid metabolism between neoplastic and host normal cells may be exploitable for cancer chemotherapy. In one such chemotherapeutic approach, we have prepared 1-halo analogues of DL-,[3a] D-,[3b] and L-glycerol 3-phosphate[3c] and the corresponding glycerols as agents designed to exploit differences in levels of cytosolic NAD-linked glycerol-3-phosphate dehydrogenase in neoplastic compared to normal cells. We have also prepared 1-halo analogues of dihydroxyacetone 3-phosphate potentially to affect glycerol-3-phosphate dehydrogenase or the acyldihydroxyacetone phosphate alternative pathway to phosphatidic acid and ether lipids.[4]

The use of phosphorylated analogues as chemotherapeutic agents is likely to be limited by transport restrictions in vivo, suggesting the use of deoxyfluoroketohexoses[5] as transportable precursors of the fluorotriose phosphates, or suggesting the use of nonphosphorylated derivatives of the fluorotrioses. Moreover, LaBelle and Hajra[6] have observed that monobenzoate esters of dihydroxyacetone inhibit the acyldihydroxyacetone phosphate pathway but that inhibition in vivo is limited by the activity of a kinase generating the phosphoryl ester of 1,3-dihydroxyacetone monobenzoate, which is noninhibitory.

Scheme I



It is apparent that fluorohydroxyacetone and nonphosphorylated derivatives of it might serve both to avoid transport limitations and, in the case of the esters, to function as potential inhibitors of the acyldihydroxyacetone phosphate pathway incapable of deactivation by phosphorylation in vivo.