Preparation and Antitumor Activities of Some Derivatives of 5-Methoxysterigmatocystin

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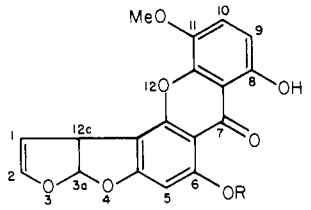
Received February 27, 1976

A series of derivatives of 5-methoxysterigmatocystin (3a,12c-dihydro-8-hydroxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one) has been prepared and evaluated for antitumor activity. The potency of the parent compound has been associated with the intact bisfurano ring system and with the double bond in the terminal furan ring. It has been shown that new substituents can be introduced in the xanthone portion of the molecule and that the antitumor activity is in some cases preserved.

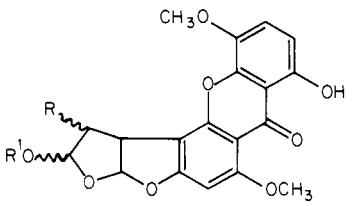
In 1968, Holker and Kagal[1a] reported the isolation from a strain of *Aspergillus versicolor* of 5-methoxysterigmatocystin[1b] (5-MS, **1a**) which is a derivative of the



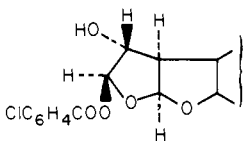
1a, R = CH_3
b, R = H

mycotoxin sterigmatocystin.[2] Recently, these compounds were shown to cause significant inhibition of the transplanted mouse leukemias P-388 and L1210.[3] The antitumor activities for some derivatives were reported at the same time and tentative conclusions were drawn as to structural features in the molecule necessary for the observed biological activity. We have subsequently prepared derivatives of 5-MS in which (a) some functional groups have been modified and (b) new functional groups have been introduced, with the goals of further defining the important functional groups and increasing the biopotency.

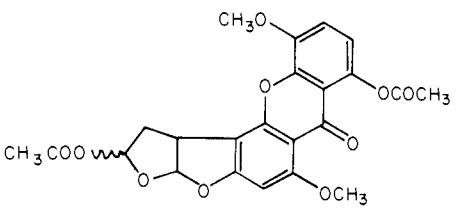
On epoxidation of **1a** with *m*-chloroperbenzoic acid in chloroform, the only product isolated was **2a** which



2a, R = OH; R′ = COC_6H_4Cl
b, R = R′ = H



2c



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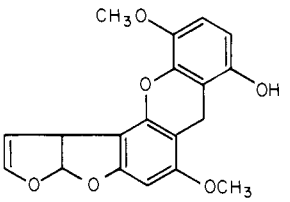
presumably arose by addition of *m*-chlorobenzoic acid to the initially formed epoxide. The same product was obtained using Anderson's procedure for acid-sensitive olefins[4] in which epoxidation was carried out in a two-phase system of methylene chloride–aqueous sodium bicarbonate. The NMR spectrum of this product showed zero coupling between the protons attached at C_1 and C_2 and also between the protons at C_1 and C_{12c}. This observation can be accounted for by the partial structure **2c** in which molecular models indicate that the bond angles between each of these pairs of adjacent protons approach 90°. Acid-catalyzed addition of water to the 1,2 double bond provided **2b** and the action of acetic anhydride and acetic acid gave **3**.

Previously it was shown that acetylation of the phenol led to retention of biological activity (presumably because of deacetylation in vivo) whereas methylation of the phenol resulted in a loss of antitumor activity.[3] Since NMR data showed that there is strong hydrogen bonding between the phenolic hydrogen and the carbonyl oxygen, it was of interest to see if the carbonyl function was necessary for bioactivity. The carbonyl group was reduced with lithium aluminum hydride to provide the xanthene **4**.



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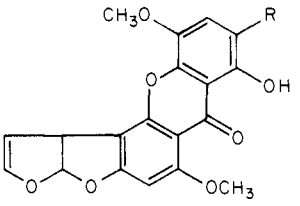
Degradation of the parent molecule **1a** resulted on attempted demethylation with electrophilic reagents such as aluminum chloride, hydrogen bromide in acetic acid, or boron tribromide. On heating with sodium ethyl mercaptide in DMF,[5] however, a monodemethyl derivative was obtained which was assigned structure **1b** on the basis of NMR spectral data. The two phenolic protons absorbed at 12.10 and 11.20 ppm, whereas in **1a** the phenolic proton absorbed at 12.64 ppm in the same solvent. This upfield shift is consistent with changing from a strongly hydrogen-bonded 1-hydroxyxanthone to a 1,8-dihydroxyxanthone in which each hydrogen bond is weaker, as was previously observed by Markham.[6] Further, while all the aromatic protons in **1b** were shifted from their positions in **1a**, the largest shift (0.21 ppm) was noted for the C_5 proton. A diacetate was formed from the diphenol with acetic anhydride and on comparison of the NMR spectrum with that of the monoacetate of **1a**, the most notable chemical shift difference was in the C_5 proton.

New functional groups were introduced in the hydroxylated aromatic ring. Reaction of **1a** with allyl bromide gave the allyl ether which underwent a Claisen rearrangement in diphenyl ether to provide the 9-allyl derivative **5a**. Nitration of **1a** with either tetranitromethane or nitric acid in acetic anhydride gave the 9- (or 10-) mononitro derivative of 5-methoxysterigmatocystin. When the *O*-acetyl derivative of **1a** was allowed to react under the same conditions with nitric acid in acetic anhydride, no nitro compound was obtained suggesting that in the nitration of the phenol the nitro group was introduced ortho to the phenol to give **5b**. The nitro group

Table I. Effect (T/C) of Derivatives of 5-MS on P-388 Mouse Lymphatic Leukemia[a]

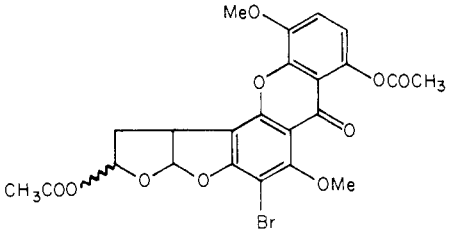
No.	Dose, mg/kg/day								
	256	128	64	32	16	8	4	2	1
1a (5-MS)				175	210	175	145	150	125
1b	125	120	105	100	90	85	95	90	90
2a	109	136	109	109					
2b	Tox	Tox	Tox	Tox	100	95	90	90	95
3	Tox	Tox	Tox	110	120	105	115	100	100
4	122	133	148	128	128	122	122	117	100
5a	Tox	155	210	150	125	125	115	100	110
5b	Tox	Tox	195	200	150	155	145	130	150
5c		145	105	160	130	120	95	90	90

[a] Inoculum: 10^6 leukemia cells per mouse. Treatment: once daily for 9 days starting on day 1, administered ip as suspension. Evaluation: median survival time (MST); results expressed as percent T/C = (MST treated)/(MST control) × 100. Criteria: T/C ≥ 125 considered significant tumor inhibition. Tox denotes toxicity as shown by weight loss and/or death.



5a, R = $CH_2CH{=}CH_2$
b, R = NO_2
c, R = NH_2

could not be selectively reduced to the amine by catalytic hydrogenation because of the facile reduction of the 1,2 double bond. The low solubility of **5b** in a wide variety of solvents complicated the reduction by chemical means. The *O*-acetyl derivative of **5b** was reduced with hydrazine hydrate and Raney nickel to provide the amine **5c**, the acetate being hydrolyzed during the course of the reaction. Halogenation at the aromatic positions of **1a** was attempted using Br_2, SO_2Cl_2, and NBS but was complicated by the facile reaction with the double bond of the vinyl ether system. When this double bond is not present, aromatic halogenation can be effected. Thus, **3**, when treated with bromine in chloroform at room temperature, gave the monobromo derivative **6** whose structure was



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assigned from NMR spectral data. This compound did not eliminate acetic acid to regenerate the double bond under pyrolytic or hydrolytic conditions and was not tested for antitumor activity.

To ensure that the derivatives tested gave meaningful results, it was important to demonstrate the absence of 5-MS in the samples. This was achieved by either liquid chromatography, on a phenyl Corasil column (Waters Associates) with 3:7 CH_3CN–H_2O as the mobile phase, or thin-layer chromatography on silica gel (Brinkmann) using either 4:1 $PhCH_3$–MeOH or 99:1 $CHCl_3$–MeOH. All the compounds tested were shown to contain less than 1% of 5-MS.

Biological Results. The results of in vivo tests against the transplanted mouse leukemia P-388 are reported in Table I. The methods used are those of Geran et al.[7] The results for compounds **2b** and **3** confirm the previous finding[3] that the double bond of the vinyl ether system is necessary for the antitumor activity of **1a**. These compounds not only did not show significant tumor inhibition but were more toxic than 5-MS. Loss of antitumor activity was also noted with **2a**. It is of interest that antitumor effects have been recorded for sterigmatocystin[3] and aflatoxin B_1[8] whose structures also incorporate a vinyl ether group as part of a dihydrofurobenzofuran ring system. Reduction of the carbonyl to a methylene group (4) lowered but did not abolish the bioactivity. The demethylated product **1b** was, however, inactive. Thus, full activity has been associated with the carbonyl and the 8-hydroxy group together with loss of potency when two phenolic groups are peri to the carbonyl. Of the ring-substituted compounds prepared, the 9-nitro derivative **5b** was the most active and was, in fact, comparable to the parent compound although the maximum effect was shown at higher dose levels. This activity was considerably diminished on reduction of the nitro to the amino group. The 9-allyl compound **5a** also was active in the 32–128 mg/kg/day dose range. These results, together with those obtained previously,[3] suggest that the bisfurano ring system and the vinyl ether system of 5-methoxysterigmatocystin are essential for the observed antitumor effects and that it may be possible to improve on the activity of 5-MS by changing or adding substituents at the aromatic positions.

Furthermore, the structural relationship between the compounds described here and aflatoxin B_1 and sterigmatocystin is to be emphasized because of the carcinogenicity and liver toxicity of the latter compounds. This has been discussed by Bradner et al.[3] (and references cited therein), who also reported cytotoxicity data for **1a**, and suggests that the compounds here described, and closely related ones, should be handled and used with extreme caution.

Experimental Section

Melting points were determined in capillary tubes in a Mel-Temp apparatus and are uncorrected. Ir spectra were recorded on a Beckman IR-9 spectrophotometer as KBr disks and are reported in cm^{-1} units. NMR spectra were run either at 60 MHz with a Perkin-Elmer R12B spectrometer or at 100 MHz with a Varian HA-100 instrument with Me_4Si as internal standard. Chemical shift values are reported in ppm units and coupling constant (J) values are in hertz. Elemental analyses were performed by the Analytical Department of Bristol Laboratories and where the analyses are indicated only by the symbols of the elements, the analytical values were within ±0.4% of the theoretical values.

1,2,3a,12c-Tetrahydro-2-(3-chlorobenzoyloxy)-1,8-dihydroxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one (2a). To a solution of 5.31 g (0.015 mol) of 5-methoxysterigmatocystin (**1a**) in 45 ml of $CHCl_3$ at 5° was added with stirring a solution of 2.85 g (0.0165 mol) of *m*-chloroperbenzoic acid in 30 ml of $CHCl_3$. After the addition, the mixture was stirred

for 4 h at room temperature and was then cooled to 5° and washed successively with 3 × 45 ml portions of saturated aqueous $NaHCO_3$ and 2 × 50 ml portions of H_2O. The organic phase was dried ($MgSO_4$) and the solvent removed under reduced pressure. The residual solid was recrystallized from MeOH and then from MeOH–acetone (5:1) to provide 1.25 g of yellow crystals having mp 240–242°: ir 1740, 1655, 1630, 1590, 1255 cm^{-1}; NMR (100 MHz in $CDCl_3$–Me_2SO-d_6) 7.49–7.09 (5 H, m includes *m*-chlorobenzoyl protons and H_9 or H_{10}), 6.75 (1 H, d, J = 6 Hz, H_{3a}), 6.65 (1 H, d, J = 9 Hz, H_9 or H_{10}), 6.54 (1 H, s, H_2), 6.38 (1 H, s, H_5), 5.81 (1 H, d, J = 4 Hz OH), 4.84 (1 H, d, J = 4 Hz, H_1), 4.30 (1 H, d, J = 6 Hz, H_{12c}), 3.96 (6 H, s, OCH_3). On addition of MeOD, OH exchanged and H_1 signal became a singlet. Anal. ($C_{29}H_{19}ClO_6$·0.5H_2O) C, H, Cl.

1,2,3a,12c-Tetrahydro-2,8-dihydroxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one (2b). A slurry of 3.0 g (8.5 mmol) of **1a** in 150 ml of H_2O and 90 ml of trifluoroacetic acid was stirred for 2 h at room temperature. The mixture was filtered and the filtrate was added to 300 ml of ice–water and extracted with 2 × 300 ml portions of $CHCl_3$. The combined extracts were washed with 2 × 150 ml portions of H_2O, dried over $MgSO_4$, and evaporated to dryness. The solid was recrystallized from a mixture of $CHCl_3$ and MeOH to give 1.87 g of solid which was purified by chromatography over 137 g of silica gel (Mallinckrodt Silicar CC-7) using chloroform as the solvent. The first 1300 ml of eluent was discarded and the next 2800 ml provided 1.03 g of product. An analytical sample was obtained by recrystallization from $CHCl_3$–Et_2O and gave yellow crystals of mp 228–229°: ir 3460, 1660, 1630, 1590, 1495 cm^{-1}; NMR ($CDCl_3$–Me_2SO-d_6) poorly resolved. Liquid chromatography showed no contamination by **1a**. Anal. ($C_{19}H_{16}O_8$·0.5H_2O) C, H.

1,2,3a,12c-Tetrahydro-2,8-diacetoxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one (3). A slurry of 5.0 g (0.014 mol) of **1a** in 100 ml of glacial acetic acid and 100 ml of acetic anhydride was treated with 200 mg of *p*-toluenesulfonic acid and stirred 18 h at room temperature to give a clear solution. The mixture was stirred for a total of 3 days and was then evaporated to dryness under reduced pressure. The residue was dissolved in 400 ml of $CHCl_3$ and washed with 4 × 400 ml portions of H_2O. The organic phase was dried over $MgSO_4$ and evaporated to give a solid which was slurried in 300 ml of hot MeOH and taken into solution with the minimum amount of $CHCl_3$. The solution was evaporated to about 250-ml volume under reduced pressure and cooled to 0° to provide a crystalline solid which was recrystallized from acetone to give 2.93 g of **3** as colorless needles of mp 226–227°: ir 1760, 1660, 1640, 1600, 1490 cm^{-1}; NMR ($CDCl_3$) AB quartet centered at 7.05 (2 H, H_9 and H_{10}), 6.59–6.36 (3 H, m, includes H_5, H_{3a}, H_2), 4.46–4.20 (1 H, m, H_{12c}), 3.97 (6 H, s, OCH_3), 2.76–2.46 (5 H, m, includes H_1, acetate protons), 1.66 (3 H, s, $OCOCH_3$). Anal. ($C_{23}H_{20}O_{10}$) C, H.

The chemical shift of the proton at 12c in these derivatives of 5-MS (i.e., **2a**, **3**, **6**) is apparently influenced by substitution at the 2 position. This has been noted previously in the sterigmatocystin series; e.g., values of 3.8 ppm for this proton in (±)-2,3-dihydro-*O*-methylsterigmatocystin and 4.23 ppm in (−)-2-acetoxy-2,3-dihydro-*O*-methylsterigmatocystin were reported by Rance and Roberts.[9]

3a,12c-Dihydro-8-hydroxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthene (4). A mixture of 1.77 g (5 mmol) of 5-methoxysterigmatocystin (**1a**) and 8.75 g of lithium aluminum hydride in 750 ml of anhydrous Et_2O was stirred and heated under reflux for 18 h. The mixture was cooled and to it was added dropwise with stirring 100 ml of 20% H_2SO_4 followed by 100 ml of H_2O. The aqueous phase was separated and extracted with 50 ml of fresh Et_2O. The Et_2O phases were combined, washed (2 × 100 ml of H_2O), dried ($MgSO_4$), and concentrated to give a brown solid which was recrystallized twice from 100% EtOH to afford 750 mg of pale tan crystals of mp 226–227°: ir 1650, 1635, 1500, 1115 cm^{-1}; NMR ($CDCl_3$–Me_2SO-d_6, 2:1) 8.61 (1 H, s, OH), 6.35–6.70 (4 H, m, includes H_2, H_{3a}, H_9, H_{10}), 6.15 (1 H, s, H_5), 5.40 (1 H, t, H_1), 4.65 (1 H, m, H_{12c}), 3.80 (3 H, s, OCH_3), 3.77 (3 H, s, OCH_3), 3.64 (2 H, s, ring CH_2). Anal. ($C_{19}H_{16}O_6$) C, H.

3a,12c-Dihydro-9-allyl-8-hydroxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one (5a). A slurry of 3.54 g (0.01 mol) of 5-methoxysterigmatocystin (**1a**), 1.38 g (0.01 mol) of anhydrous K_2CO_3, 1.33 g (0.011 mol) of allyl bromide, 50 ml of acetone, and 20 ml of DMF was stirred and heated under reflux for 17 h. The slurry was cooled and filtered and the solid was air-dried. The solid was stirred for 30 min with 60 ml of H_2O and refiltered to provide 2.6 g of an off-white solid of mp 252–253°. Recrystallization from $CHCl_3$–MeOH (2:1) gave 1.8 g of pale yellow crystals of mp 252–253°. Anal. ($C_{22}H_{18}O_7$) C, H.

A solution of 1.3 g of this allyl ether in 65 ml of diphenyl ether was heated at 200° for 6 h under an N_2 atmosphere. The solvent was removed at 80–85° (0.5 mm) and the residual solid was recrystallized twice from acetonitrile to provide 0.71 g of **5a** as yellow crystals of mp 190–191°: ir 1650, 1630, 1590, 1235 cm^{-1}; NMR ($CDCl_3$) 12.90 (1 H, s, OH), 7.11 (1 H, s, H_{10}), 6.83 (1 H, d, H_{3a}), 6.51 (1 H, t, H_2), 6.42 (1 H, s, H_2), 6.30–4.74 (5 H, m, includes H_1, H_{12c}, CH=CH_2), 4.02 (3 H, s, OCH_3), 3.94 (3 H, s, OCH_3), 3.60–3.36 (2 H, m, allyl CH_2). Anal. ($C_{22}H_{18}O_7$) C, H.

3a,12c-Dihydro-9- (or 10-) nitro-8-hydroxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one (5b). Method A. A mixture of 1.77 g (5 mmol) of 5-methoxysterigmatocystin (**1a**), 0.98 g (5 mmol) of tetranitromethane, 0.40 g (5 mmol) of pyridine, and 100 ml of acetone was stirred and heated under reflux for 16 h. The precipitated product was collected by filtration, washed with acetone, and dried in vacuo to give 0.77 g of beige solid which was recrystallized from acetonitrile to provide an analytical sample of mp 293–294°: ir 1660, 1635, 1580, 1525, 1345, 1230 cm^{-1}; NMR (Me_2SO-d_6–pyridine-d_5, 1:1) 7.97 (1 H, s, $H_{9\text{ or }10}$), 7.10 (1 H, d, H_{3a}), 6.82–6.65 (2 H, m, includes H_2 and H_5), 5.47 (1 H, t, H_1), 4.77 (1 H, m, H_{12c}), 3.82 (3 H, s, OCH_3), 4.19 (3 H, s, OCH_3). Anal. ($C_{19}H_{13}NO_9$) C, H, N.

Method B. To a stirred slurry of 1.77 g (5 mmol) of **1a** in 20 ml of acetic anhydride was added dropwise 0.22 ml (5 mmol) of nitric acid (*d* 1.42). The mixture was stirred for 1.5 h and was filtered. The solid was recrystallized from dioxane–acetonitrile (1:9) to give 0.5 g of yellow needles with an identical ir spectrum with the product from method A. TLC on silica gel with a mixture of toluene and MeOH (4:1) confirmed the identity of the product.

3a,12c-Dihydro-6,8-dihydroxy-11-methoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one (1b). To an ice-cold stirred slurry of 6.0 g (0.0125 mol) of 50% sodium hydride in mineral oil in 75 ml of *N,N*-dimethylformamide (dried over 4A molecular sieves) under a nitrogen atmosphere was added dropwise a solution of 25 ml of ethyl mercaptan in 45 ml of DMF. When the evolution of hydrogen was complete, a solution of 3.54 g (0.01 mol) of **1a** in 100 ml of DMF was added and the dark brown mixture was stirred for 20 h at room temperature and then for 1.5 h at 100–120°. The mixture was cooled and the solvent was removed under reduced pressure. The residue was dissolved in 350 ml of H_2O and acidified to pH 3 with 6 N HCl and extracted with 2 × 200 ml portions of CH_2Cl_2. The extracts were washed (2 × 200 ml portions of H_2O), dried ($MgSO_4$), and evaporated to dryness. The residue was dissolved in 550 ml of CH_3CN and washed with 3 × 300 ml portions of *n*-pentane. On cooling the CH_3CN phase to 0°, crystalline product was obtained and was recrystallized from acetonitrile to afford 1.88 g of the title compound of mp 244–246°: ir 1665, 1630, 1590, 1490 cm^{-1}; NMR (Me_2SO-d_6) 12.10 (1 H, s, OH), 11.20 (1 H, s, OH), 7.50 (1 H, d, J = 9 Hz, H_9 or H_{10}), 6.97 (1 H, d, J = 7 Hz, H_{3a}), 6.84–6.67 (2 H, m, includes H_9 or H_{10}, H_2), 6.47 (1 H, s, H_5), 5.40 (1 H, m, H_1), 4.88 (1 H, m, H_{12c}), 3.93 (3 H, s, OCH_3). Anal. ($C_{18}H_{12}O_7$) C, H.

A 300-mg sample of **1b** was dissolved in 15 ml of pyridine by heating on a steam bath. Acetic anhydride (6 ml) was added and the solution was stored at room temperature for 28 h. The solution was evaporated to dryness under reduced pressure and the residue recrystallized from 25 ml of absolute ethanol to give 264 mg of the diacetate of mp 211–212°: ir 1770, 1660, 1640, 1615, 1210, 1195 cm^{-1}; NMR ($CDCl_3$) 7.19 (1 H, d, J = 9 Hz, H_9 or H_{10}), 6.88 (1 H, d, J = 9 Hz, H_9 or H_{10}), 6.85 (1 H, d, J = 7 Hz, H_{3a}), 6.62 (1 H, s, H_5), 6.54 (1 H, m, H_2), 5.54 (1 H, m, H_1), 5.02–4.82 (1 H, m, H_{12c}), 3.98 (3 H, s, OCH_3), 2.41 (6 H, s, $OCOCH_3$). Anal. ($C_{22}H_{16}O_9$) C, H.

3a,12c-Dihydro-9-amino-8-hydroxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one (5c). To a solution of 2.7 g of **5b** in 190 ml of warm pyridine was added 30 ml of acetic anhydride. The solution was stored for 24 h and was then

evaporated to dryness under reduced pressure. The residue was recrystallized from a mixture of $CHCl_3$ and EtOH to provide 2.64 g (6 mmol) of the acetate. This was dissolved in 250 ml of hot dioxane and diluted with 250 ml of absolute EtOH. To the hot, stirred solution was added a small amount of W-2 Raney nickel followed by 4.0 g of 95% hydrazine added in portions over 20 min. The mixture was heated on the steam bath for 1 h and more Raney nickel was added to destroy the excess of hydrazine. The mixture was filtered through diatomaceous earth which was washed with hot EtOH. The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was chromatographed on 100 g of silica gel (Mallinckrodt Silicar CC 7) with $CHCl_3$ to give as a first fraction 1.25 g of **5b** and as a second fraction 0.73 g of the title compound. This fraction was rechromatographed on 80 g of the silica gel with CH_2Cl_2 as the solvent and the first 1950 ml of eluent was discarded. The next 350 ml of eluent was evaporated to about 20 ml and cooled to 0° to give 0.46 g of crystalline product of mp 238–239° dec: ir 3460, 3380, 1650, 1630, 1590, 1490, 1240 cm^{-1}; NMR (Me_2SO-d_6) 12.54 (1 H, s, OH), 6.97 (1 H, d, J = 7 Hz, H_{3a}), 6.97 (1 H, s, H_{10}), 6.76 (1 H, t, H_2), 6.64 (1 H, s, H_5), 5.44 (1 H, t, H_1), 4.89–4.70 (3 H, m, H_{12c} and NH_2), 3.91 (3 H, s, OCH_3), 3.88 (3 H, s, OCH_3). Anal. ($C_{19}H_{15}NO_7$) H, N; C: calcd, 61.79; found, 62.34.

1,2,3a,12c-Tetrahydro-5-bromo-2,8-diacetoxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one (6). A solution of 456 mg (1 mmol) of the diacetoxy compound **3** in 10 ml of $CHCl_3$ was cooled and 3.0 ml of 0.5 M $NaHCO_3$ solution was added. The two-phase system was cooled to 5° and with stirring was treated dropwise over 2 h with a solution of 0.055 ml (1 mmol) of bromine in 5 ml of $CHCl_3$. The cooling bath was removed and, after the mixture had attained room temperature, stirring was continued for 3 h. The $CHCl_3$ layer was separated and washed with 4 × 25 ml portions of H_2O, then dried over $MgSO_4$, and evaporated to dryness under reduced pressure to give 546 mg of a solid. This was chromatographed on 100 g of silica gel (Mallinckrodt Silicar CC-4) using $CHCl_3$–MeOH (99:1) as the solvent. The first 368 ml of eluate was discarded and the next 42 ml gave the product which was recrystallized from isopropyl alcohol to afford 77 mg of pale yellow needles of mp 214–215°: ir 1760, 1660, 1490, 1210 cm^{-1}; NMR ($CDCl_3$) AB quartet centered at 7.04 (2 H, J = 9 Hz, H_9 and H_{10}), 6.64 (1 H, d, J = 6 Hz, H_{3a}), 6.52 (1 H, m, H_2), 4.58–4.10 (1 H, m, H_{12c}), 3.96, 3.93 (6 H, 2 s, OCH_3), 2.65–2.45 (5 H, m, H_1 and $OCOCH_3$), 1.65 (3 H, s, $OCOCH_3$). Anal. ($C_{23}H_{19}O_{10}Br$) C, H, Br.

References and Notes

(1) (a) J. S. E. Holker and S. A. Kagal, *Chem. Commun.*, 1574 (1968). (b) In this paper, **1a** will be referred to as both 5-methoxysterigmatocystin (5-MS) to preserve its relationship with previous publications on this compound and 3a,12c-dihydro-8-hydroxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one to facilitate naming of its derivatives.
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Synthesis and Biological Activity of 3,5-Dinitro-4- and -2-(1*H*-purin-6-ylthio)benzoates, Prodrugs of 6-Mercaptopurine

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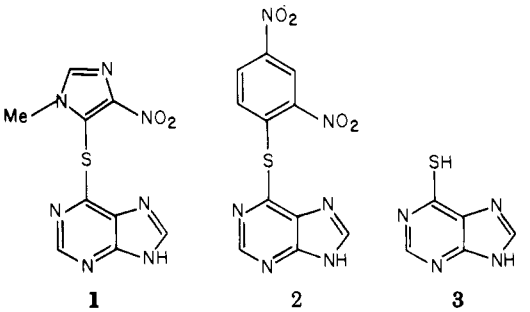
Received March 19, 1976

A series of prodrug modifications of 6-mercaptopurine (6-MP) containing dinitrobenzoate ester moieties with varying chain length has been prepared. These compounds were shown to be cytotoxic in several cell culture screens and also exhibited significant activity against L1210 lymphoid leukemia in vivo. The possibility exists that the transport and distribution of these compounds in vivo will be determined, in part, by increased lipophilic character, with a consequent selective localization in lymphatic and CNS tissue.

Lipid–water partition coefficients have been correlated with central nervous system (CNS) drug uptake in a study designed to assess the importance of lipophilicity in drugs designed for blood–brain barrier penetration and central nervous system effects.[1] A study of methotrexate diesters of increasing chain length and thus increasing lipophilicity has been reported.[2] The antitumor activity of these esters in vivo was the same as methotrexate in tests against both intracerebrally and ip inoculated L1210 lymphoid leukemia. This was because of rapid in vivo hydrolysis of the esters to parent drug.

We report here the preparation of prodrug derivatives of 6-mercaptopurine (6-MP) with increased lipophilicity. These were synthesized with the objective of favorably modifying transport and distribution of active purine in the lymphatic system and CNS. The desirability of having a clinically useful 6-MP derivative which readily reaches the CNS without the necessity for intrathecal injection is clear when the problems of treating CNS leukemias are considered.

6-MP has previously been modified with the objective of altering its transport, distribution, and metabolism. Two important modifications involve bonding of sulfur to nitroaromatic or nitroheteroaromatic rings, **1**[3] and **2**.[4] 6-MP is extensively metabolized in vivo, the main degradative pathways being oxidative. Both **1** and **2** were prepared with the hope that the protective group would



1 **2** **3**

be able to shield the sulfur atom from oxidative attack.