tallized from glacial acetic acid: mp 305 °C.

3-Carboxybenzaldehyde *O*-methyloxime was recrystallized from ethanol: mp 149–150 °C.

4-Carboxybenzaldehyde *O*-methyloxime was recrystallized from ethanol–water: mp 177–178 °C.

3-Carboxybenzylidenemalonitrile. 3-Carboxybenzaldehyde (0.1 M) and malonitrile (0.1 M) were dissolved in 150 ml of 95% ethanol. A few drops of triethylamine were added and, on standing for several hours, a white solid separated which was recrystallized from ethanol: mp 228–229 °C.

4-Carboxybenzylidenemalonitrile was prepared and purified in the same way as the 3 isomer: mp 179–180 °C.

3-Carboxyphenylpyrrole. 3-Aminobenzoic acid (0.04 M) and 4-picoline (0.08 M) were dissolved in a small amount of hot DMF. With stirring, 0.04 M mucic acid was added slowly after which the mixture was refluxed for 3 h. After standing at room temperature overnight, the picoline was removed on a rotary evaporator and the resulting syrup dissolved in ethyl acetate and extracted with 5% sodium hydroxide. The alkaline extract was acidified to pH 5.0 and the white precipitate removed by filtration, dissolved in chloroform, applied to a silica gel column, and eluted with chloroform. The fractions which showed a single TLC spot were combined and the chloroform was removed by rotary evaporation: mp 268–270 °C.

4-Carboxyphenylpyrrole was prepared and purified in the same way as the 3 isomer: mp 288–290 °C.

3-(2,5-Dimethylpyrrolyl)benzoic Acid. 3-Aminobenzoic acid (0.02 M) and acetonylacetone (0.02 M) were dissolved in 30 ml of 95% ethanol and 2.4 ml of glacial acetic acid. The solution was refluxed with stirring for 4 h. The crystals which separated on cooling were recrystallized from ethanol: mp 144 °C.

4-(2,5-Dimethylpyrrolyl)benzoic acid was prepared and purified in the same way as the 3 isomer: mp 177–179 °C.

3-(2-Pyridyl)benzoic Acid. 3-Methyl-1,2′-pyridylcyclohexanol was prepared according to Abramovitch and Saha.[11] It was dehydrated in glacial acetic acid with sulfuric acid, according to their directions. The acetic acid was removed under vacuum and the residue poured into water. After making the aqueous phase alkaline, the product was extracted with ether which was dried over $MgSO_4$ and evaporated, and the residue was dehydrogenated by refluxing overnight in dry decalin with 5% palladium on charcoal (200 mg of compound/50 mg of 5% Pd/carbon). Abramovitch and Saha did not mention[11] the necessity of this step which we found to be necessary. The carbon was filtered from the hot solution and the filtrate extracted with 5% HCl. Neutralization yielded 3-(2-pyridyl)toluene which was not purified but oxidized with aqueous $KMnO_4$ to yield the desired acid: mp 213 °C after recrystallization from ethanol.

4-(2-Pyridyl)benzoic acid was prepared and purified in the same way as the 3 isomer: mp 235–237 °C.

pK_a Determinations. In determining the pK_a values of the various benzoic acids, the guidelines of Albert and Serjeant[12] were followed. A Corning digital 112 research model pH meter was used to determine the hydrogen ion concentration in the potentiometric titrations which were carried out in a 200-ml jacketed beaker. The beaker was fitted with a specially designed Plexiglas cover with holes fitted to Corning glass calomel electrodes, a Corning automatic temperature compensator, a nitrogen inlet tube, and buret tip. Since the determinations were made in 50% ethanol–water (by volume), the electrodes were allowed to soak in this solution for several days before use. The pH meter was standardized against NBS potassium hydrogen phthalate buffer (pH 4) and potassium dihydrogen phosphate–disodium hydrogen phosphate (pH 7) buffer. The temperature was maintained at 25 ± 0.01 °C.

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Synthesis and Structure–Activity Relationship Studies of Cytotoxic Epoxide Derivatives of 7-Oxabicyclo[2.2.1]heptane

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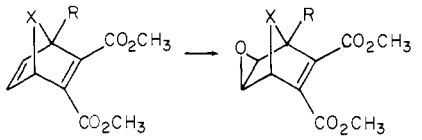
Dimethyl *exo*-5,6-oxido-7-oxabicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate (1) and the 1-methyl homologue 2 were shown to exhibit significant cytotoxicity in the 9KB tissue culture assay. Several analogues of 1 were prepared and it was found that removal of the epoxide, or the oxygen bridge, or the 2,3 double bond from 1 resulted in loss of significant cytotoxic activity. One compound which lacked the epoxide moiety, dimethyl 1-methyl-7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate (5), also exhibited marginal cytotoxic activity.

A number of naturally occurring and synthetic epoxy compounds have been shown to possess significant antitumor and/or cytotoxic activity. Within this category one finds the complex triepoxides, triptolide[1] and tripdiolide,[1] and diepoxides such as crotepoxide,[2] fumagillin,[3] mikanolide,[4] 1,2,3,4-diepoxybutane,[5] and "Mannitol-Myleran".[6] In addition, a variety of polyfunctional natural products which possess an epoxide moiety as one potentially reactive site in the molecule has been shown to possess significant antitumor and/or cytotoxic activity.[7-12]

During the course of our continuing effort to prepare simple polyfunctional analogues of naturally occurring tumor inhibitors, we synthesized dimethyl *exo*-5,6-oxido-7-oxabicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate (1). The significant reproducible cytotoxic activity of 1 in the 9KB tissue culture assay prompted us to undertake a more

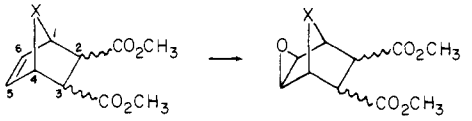
extensive study of this system to evaluate the structural requirements for biological activity. The ideal simplicity of this system facilitates structural modification through which one can study the influence of stereochemistry, ring strain, polyfunctionality, and lipid–water solubility on the biological activity. Similar studies on the more complex natural products are markedly hindered by the synthetic inaccessibility of these compounds. We now report the results of our preliminary studies with 1 which involve definition of the gross structural features which are required for cytotoxic activity (9KB).



4, R = H; X = O
5, R = CH_3; X = O
6, R = H; X = CH_2

1, R = H; X = O
2, R = CH_3; X = O
3, R = H; X = CH_2

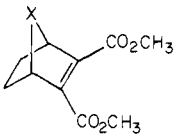
Chemistry. The epoxides **1**, **2**, and **3** were prepared in two steps. Dimethyl acetylenedicarboxylate was treated with furan,[13] 2-methylfuran, and cyclopentadiene[14] to give the Diels–Alder adducts **4**, **5**, and **6**, respectively. Subsequent treatment of the olefins with peracetic acid afforded the desired epoxides (Table II). Similarly, epoxidation of **7**[15] and **8** gave **9** and **10**. The latter compounds serve as 2,3-dihydro analogues of **1** and **3**. The commercially available norbornene epoxide (**11**) serves as a very simple analogue of **3**. Catalytic hydrogenation of **4** and **6** gave **12**[16] and **13**, deepoxy analogues of **1** and **3**.



7, X = O; 2,3-exo
8, X = CH_2; 2,3-endo

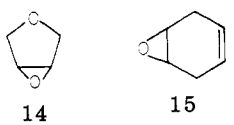
9, X = O; 2,3-exo
10, X = CH_2; 2,3-endo

11

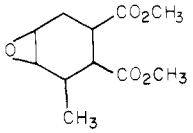


12, X = O
13, X = CH_2

The mono epoxides **14**,[17] **15**,[18] and **16** were prepared from 2,5-dihydrofuran (**17**), cyclohexa-1,4-diene (**18**), and dimethyl 3-methylcyclohexene-4,5-dicarboxylate (**19**),[19] respectively.



14 **15**



16

The exo configuration of the epoxide moiety in **1**, **2**, **3**, **9**, and **10** was assigned on the basis of NMR spectral analyses. It has been shown in NMR studies with 7-oxabicyclo[2.2.1]hept-5-ene and norbornene derivatives that the bridgehead protons, H_1 and H_4, are not coupled to any significant extent to the endo protons at C_2 and C_3; in contrast, the C_2 and C_3 exo protons have been shown to be coupled to H_1 and H_4 to the extent of ca. 4 Hz.[20] The NMR spectra of **1**, **2**, **3**, **9**, and **10** showed the epoxide protons at C_2 and C_3 unsplit by the adjacent bridgehead protons.

Ross and co-workers have successfully correlated the toxicity and tumor inhibitory activity of simple epoxides with the rate of nucleophilic epoxide opening by thiosulfate ion in refluxing aqueous acetone; the rate of the reactions was determined by titration with 0.2 N acetic acid.[21] In our study, simple epoxides such as 7-oxabicyclo[4.1.0]heptane gave rate data consistent with those of Ross et al. but epoxides **3**, **9**, **11**, and **16** failed to react with thiosulfate at any detectable rate (although **3** decomposed slightly over a period of 24 h) and the epoxides were recovered unchanged. In the case of **1** and **2** the reaction rapidly consumed the epoxides *without* generation of any titratable base. The nature of the very polar product(s) formed in the reaction is not known. We have demonstrated that **1** and **2** are stable in the solution in the absence of thiosulfate and that the reaction does not occur with only catalytic amounts of thiosulfate. We have also shown that **1** and **2** do not react with thiocyanate under similar conditions. It should be noted that 7-oxabicyclo[2.2.1]heptane and dimethyl 7-oxabicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate (**12**) failed to react with thiosulfate at any detectable rate (i.e., $k_{thio} < 1.0 \times 10^{-5}$ min^{-1}). Work is in progress to characterize the product(s) of the reaction in an effort to relate the reactivity of the compounds to the observed cytotoxicity.

Table I. In Vitro 9KB Tissue Culture Assay Data[a]

Compd	ED_{50}, μg/ml	Compd	ED_{50}, μg/ml
1	1.5-3.5	10	100
2	3-5.5	11	100
3	22	12	22
5[b]	3-12	13	100
6	100	14	100
7	100	15	130
8	100	16	100
9	100	19	48

[a] 9KB tissue culture assays were performed under the auspices of the National Cancer Institute, National Institutes of Health; ED_{50} values ≤4 μg/ml are considered significant. The compounds were tested in DMF solution; the procedures were those described in *Cancer Chemother. Rep., Part 3*, 3 (2), 1 (1972). [b] The diene **4** was not submitted for assay because it was quite unstable.

Biological Results and Discussion. The 9KB tissue culture assay data are given in Table I. It is immediately apparent that all of the structural features of **1** are quite essential for cytotoxic activity; removal of the epoxide (cf. **12**), the bridge oxygen (cf. **3**), or the unsaturation (cf. **9**) resulted in loss of significant cytotoxic activity. It is also of interest to note that activity is only observed in those epoxides (i.e., **1** and **2**) which reacted "abnormally" with thiosulfate ion. The cytotoxicity of the oxabicyclic diene **5** is perhaps surprising since this compound lacks the 5,6-epoxide moiety. Further studies with **5** and analogues of **5** merit attention and this work is in progress.

Preliminary in vivo screening of **1**, **2**, and **5** against L1210 lymphoid leukemia in BDF_1 mice failed to show any significant activity. Tests will be continued in other in vivo tumor systems. Doses of 50 mg/kg of **1**, **2**, and **5** were lethal to mice while doses of 12.5 mg/kg were not lethal. None of the other compounds given in Table I showed any antileukemic activity in the L1210 lymphoid leukemia screen. With the exception of **1**, **2**, and **5**, none of the compounds in Table I were toxic at doses of 200 mg/kg and, with the further exception of **3** and **15**, none showed toxicity at 400 mg/kg.

Experimental Section

Melting points were determined in an open capillary on a Hoover-Thomas apparatus and are uncorrected. Analytical results were obtained for C and H on all new compounds reported and were with ±0.4% of the theoretical values. Infrared and NMR spectra were consistent for all compounds reported; reported

Table II. Epoxides Prepared Using the General Epoxidation Procedure

Olefin	Epoxide	% yield	Bp, °C (mm)
4[d]	1	71	*a*
5	2	74	112-113 (0.4)
6[e]	3	48	*c*
7	9[f]	73	*b*
8	10	80	*c*
17	14[g]	47	53-54 (20) [lit. 45 (14)][g]
18	15[h]	48	65 (30) [lit. 41-43 (14)][h]
19[i]	16	72	99 (0.4)

[a] Mp 91-91.5 °C (benzene-cyclohexane). [b] Mp 141 °C (benzene; lit.[15] mp 146 °C). [c] Liquids could not be distilled and were purified using silica gel dry column chromatography. [d] See ref 13. [e] See ref 14. [f] See ref 15. [g] See ref 17. [h] See ref 18. [i] See ref 19.

spectra for **2** and **5** are typical. Compounds **4**, **6**, **7**, **12**, **13**, and **19** were prepared according to reported literature procedures; compounds 8, 11, 17, and 18 were available commercially.

General Procedure for the Synthesis of Epoxides. The reagent was prepared as follows. A stock solution was prepared consisting of 33.25 g of acetic anhydride and 1.0 g of trifluoroacetic acid diluted to 25 ml with dichloromethane. The reagent was prepared by the addition of 5.0 ml of the stock solution to 1.0 g of 90% hydrogen peroxide. The solution was allowed to stand in an ice bath for 1 h and was diluted to 25 ml with dichloromethane; this provided a solution which was ca. 1 M in the peracetic acid.

The olefin was dissolved in the peracetic acid solution containing ca. 1.1 equiv of peracid. The mixture was cooled to control the initial exothermic reaction and then was allowed to stand for 24 h at 25 °C (the epoxidation of **8** required a 48-h reaction time).

The reaction mixture was treated with a saturated solution of sodium carbonate until a pH of 8 was reached. Solid sodium bisulfate was added slowly with stirring (with cooling if necessary) until the mixture gave a negative reaction for peracid with starch-iodide paper. The aqueous phase was separated and extracted with dichloromethane. The dichloromethane solutions were combined, dried (Na_2CO_3), and concentrated in vacuo. The epoxides were purified by crystallization, distillation, and/or column chromatography and are listed in Table II.

Dimethyl 1-Methyl-7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate (5). A solution of freshly distilled 2-methylfuran (23.8 g, 0.29 mol), and dimethyl acetylenedicarboxylate (10.0 g, 0.076 mol) in ether (50 ml) was heated at 30 °C for 48 h. The ether and excess 2-methylfuran were removed in vacuo and the residue was distilled to yield 13.0 g (77%) of **5**: bp 89 °C (0.4 mm); IR (neat) 3.32, 3.38, 5.80, 6.10, 6.95, 7.20, 7.40, and 8.30 μ; NMR (CCl_4–Me_4Si) δ 7.20 (q, 1 H, J = 2 Hz), 7.00 (d, 1 H, J = 5 Hz), 5.55 (d, 1 H, J = 2 Hz), 3.80 (s, 3 H), 3.75 (s, 3 H), and 1.75 (s, 3 H).

***exo*-5,6-Epoxy-1-methyl-2,3-bis(carbomethoxy)-7-oxabicyclo[2.2.1]hept-2-ene (2).** The epoxide **2** had IR (neat) 3.34, 3.38, 5.80, 6.10, 6.95, 7.50–8.10, and 11.45 μ; NMR (CCl_4–Me_4Si) δ 5.00 (s, 1 H), 3.84 and 3.85 (2 s, 6 H), 3.80–3.30 (m, 2 H), and 1.60 (s, 3 H).

Determination of Rates of Reaction of Epoxides with Thiosulfate Ion. Rate constants were determined by means of the procedure of Ross.[21] A 1.58-g (0.01 mol) quantity of reagent grade sodium thiosulfate was dissolved in water and diluted to 25 ml. This solution was further diluted to 50 ml with reagent grade acetone to give an approximately 0.2 N thiosulfate solution. The thiosulfate solution was transferred to a 100-ml two-necked round-bottom flask equipped with a reflux condenser and a 10-ml buret. Two drops of 1% phenolphthalein in acetone were added and the solution was adjusted to a pale pink color by dropwise addition of 0.2 N sodium hydroxide solution. A boiling stone was added and the solution was brought to a gentle reflux. A small, carefully measured volume of accurately standardized 0.2 N acetic acid was introduced into the flask by means of the buret and approximately 1 mmol of epoxide weighed to the nearest milligram was introduced and a stopwatch was started. Liquid epoxides were introduced by means of a small syringe which was weighed before and after discharge of the sample in the manner of a weight buret. Solids were introduced by means of a small glass cup which was dropped into the flask. Whenever a pink end point was noted, the elapsed time was recorded together with the buret reading. Additional acid was then run in and the procedure was repeated. As many as 15 readings could be made on 1 mmol of epoxide before the decreasing speed of the reaction rendered the end point uncertain. The rate of determinations was all halted prior to consumption of half of the epoxide. Under the conditions of this reaction first-order kinetics are followed. Treatment of the data was accomplished by means of a Hewlett-Packard Model 9820 programmable calculator which was programmed to accept stopwatch readings in minutes and seconds, buret readings, weights, and normalities. The first-order treatment described by Ross was then applied to calculate rate constants for each observation as well as average values for the entire experiment. The results were analyzed by means of a standard deviation program and average values were reported to within one standard deviation unit.

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