Allylic Esters as Potential Antitumor Agents. Derivatives and Analogues of 2,3-Bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-2-ene

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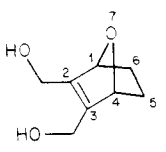
Derivatives and analogues of 2,3-bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-2-ene were synthesized. Of the compounds prepared, dimethyl 2,3-bis(acetoxymethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-5,6-dicarboxylate (**5**) was the most potent in a leukemia L1210 tissue culture assay (93% inhibition at 10^{-6} M). None of the compounds showed significant reproducible in vivo antileukemic activity.

The past several years have witnessed the discovery of a variety of new and interesting natural products with significant antitumor activity.[1,2] Several of these new compounds are currently being employed in the treatment of cancer, others are in various stages of development leading ultimately to use in man, and still others have proved too toxic for drug use. Nevertheless, all of these new naturally occurring tumor inhibitors present new structural classes into the growing array of antineoplastic agents and may be regarded as "chemical templates" for new synthetic approaches to cancer chemotherapy.

Polyfunctionality is a feature commonly identified in the structures of these new naturally occurring tumor inhibitors. Many possess at least one and often two or more reactive electrophilic sites; the more active compounds usually display additional structural features such as aromatic rings, hydroxyl groups, amine functions, and so on, which may enhance antitumor activity. These nonelectrophilic groups may increase activity through intramolecular interactions which augment the reactivity of a particular electrophilic center and/or through increased affinity for a particular site of action.[1,2]

One reactive functional group encountered in certain naturally occurring tumor inhibitors is the allylic ester function. The mitomycins[3] and the pyrrolizidine alkaloids[4] represent two widely studied groups of compounds which possess this functionality. These compounds are converted in vivo into mitosenes and pyrroles, respectively. The reactivity of the allylic ester moieties is increased by intramolecular participation of the ring nitrogen atom and possibly by neighboring hydroxyl group(s). The reactivity of the acylated vinylogous carbinolamine moiety can be controlled in certain systems, and this has provided a basis for the design and synthesis of a series of substituted pyrrolizines and pyrroles which possess significant antileukemic activity, as well as activity in the more intractable solid tumors.[5]

Other methods may be available to control the reactivity of allylic ester moieties. In an effort to study potential intramolecular interactions that control the reactivity of allylic esters, we chose to examine derivatives of 2,3-bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-2-ene (**1**).



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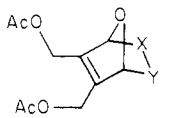
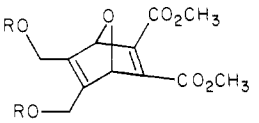
This molecule possesses three potential reactive sites, the two allylic alcohol moieties (which are presented in the same spatial array as the reactive portions of the mitosenes and the pyrrole metabolites of pyrrolizidine alkaloids) and the oxygen bridge of the strained oxabicyclic ring system (which can undergo nucleophilic ring-opening reactions[6] similar to epoxides). In addition, **1** may be further substituted to provide additional binding sites, to introduce new reactive sites, to increase steric shielding of one or more reactive sites, and to introduce substituents which may enhance the reactivity of the molecule through neighboring group participation.

The bridge oxygen, O-7, is not likely to enhance the reactivity of the allylic esters, since photoelectron spectral studies with 7-oxabicyclo[2.2.1]hept-2-ene have shown that there is very little interaction between the nonbonded electrons of O-7 and the π electrons of the 2,3 double bond.[7] This is supported by rate data in the solvolysis of 2-halo-7-oxabicyclo[2.2.1]heptanes.[8] Appropriate substituents at C-5 and C-6 in **1** should offer the possibility of rate enhancing neighboring group reactions. For example, the exo/endo rate ratio for the acetolysis of 6-brosyl-2-oxabicyclo[2.2.1]heptane was 7×10^7 at 25 °C.[9]

Four compounds were selected for preliminary examination. 2,3-Bis(acetoxymethyl)-5,6-bis(*endo*-hydroxy)-7-oxabicyclo[2.2.1]hept-2-ene (**2a**) and the tetraacetate **2b**



2a, X-Y = *endo*-CD(OH)-CH(OH)
b, X-Y = *endo*-CH(OAc)-CH(OAc)
3, X-Y = $C(CO_2CH_3)=C(CO_2CH_3)$
4, X-Y = $N(CO_2C_2H_5)$-$N(CO_2C_2H_5)$



5, R = H
6, R = C(=O)NH-alkyl

were selected on the basis of potential neighboring group reactions involving either the free hydroxyl or the acetoxy groups. Dimethyl 2,3-bis(acetoxymethyl)-7-oxabicyclo-[2.2.1]hepta-2,5-diene-5,6-dicarboxylate (**3**) presents a transannular double bond which may enhance the reactivity of the allylic esters and, in addition, provides another reactive center in the molecule. Diethyl 4,6-bis(acetoxymethyl)-2,3-diaza-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate (**4**) provides a heteroatom in a transannular location to the double bond and again incorporates an additional reactive center in the molecule.

Chemistry. The di- and tetraacetates **2a** and **2b** were synthesized from the corresponding tetrol, which in turn was prepared by alkaline hydrolysis of the endo adduct obtained in the Diels–Alder reaction of vinylene carbonate with 3,4-bis(acetoxymethyl)furan. Compounds **3** and **4** were prepared in a Diels–Alder reaction with 3,4-bis-

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Table I. Leukemia L1210 Tissue Culture Data[a]

no.	concn, M	no. of cells ($\times 10^5$)[b]	% inhibn	ID_{50}, M
2a	1×10^{-4}	5.5	41	$>10^{-4}$
	1×10^{-5}	8.1	3	
2b	1×10^{-4}	6.0	34	$>10^{-4}$
	1×10^{-5}	7.0	19	
	1×10^{-6}	8.2	1	
3	1×10^{-4}	2.0	93	
	1×10^{-5}	2.0	93	$<10^{-6}$
	1×10^{-6}	2.0	93	
4	1×10^{-4}	3.0	78	
	1×10^{-5}	5.5	41	$\sim 3 \times 10^{-5}$
	1×10^{-6}	7.4	13	

[a] Results were evaluated after incubation at 37 °C for 40 h; 1.5×10^5 cells were inoculated and the control contained 8.3×10^5 cells after 40 h of incubation (based upon two determinations). [b] Based upon two determinations.

(acetoxymethyl)furan and dimethyl acetylenedicarboxylate (DMAD) and diethyl azodicarboxylate, respectively.

The endo stereochemistry of **2a** and **2b** was assigned on the basis of NMR spectral data. Comparison of the spectra (Me_2SO-d_6/D_2O) of the exo and endo isomers of 2,3-bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-2-ene-*cis*-5,6-diol revealed that the exo protons (in the endo isomer) at C-5 and C-6 were at lower field (δ 4.04) than the endo protons (in the exo isomer; δ 3.70). Furthermore the H_5 and H_6 exo protons appeared as multiplets, coupled to the bridgehead protons, while the H_5 and H_6 endo protons were singlets. The bridgehead protons H_1 and H_4 appeared at lower field in the endo isomer (δ 4.80) compared to the exo isomer (δ 4.51); this latter effect is presumably due to the shielding effect of the C-5 and C-6 exo substituents.[10] All of these data are in accord with literature reports of the NMR spectra of 7-oxabicyclo[2.2.1]hept-2-enes.[11]

After the preliminary leukemia L1210 tissue culture data were determined for **2a**, **2b**, **3**, and **4**, additional analogues of **3** were prepared in which the acetate moieties were replaced by more stable carbamate esters. The carbamates were synthesized from the diol **5** by treatment with the appropriate isocyanate; the diol **5** was prepared in a Diels–Alder reaction between DMAD and 3,4-bis[[(trimethylsilyl)oxy]methyl]furan and hydrolysis of the silyl groups. The silyl groups were employed so that the product from the initial cycloaddition reaction could be purified by distillation.

Biological Results and Discussion. The four initial compounds prepared in this study were evaluated for cytotoxicity against a leukemia L1210 tissue culture;[12] the data are summarized in Table I. The diacetate **3** was the most potent in this in vitro assay, but none of the compounds showed significant in vivo activity in the mouse L1210 assay.[13]

One of the five carbamates tested, **6d** (RCONH-*n*-C_4H_9), was active in the mouse P388 leukemia assay (% T/C = 123 at 25 mg/kg) but this was not reproducible.[13] The *N*-methylcarbamate **6a** was the most toxic compound in the carbamate series, and toxicity decreased as lipophilic character increased. Further studies are in progress with the allylic ester as a pharmacophoric group in an effort to define the nature of activation required in order to maximize antineoplastic activity and minimize host toxicity.

Experimental Section

Melting points were determined in open capillary tubes in a Thomas-Hoover melting point apparatus and are uncorrected. NMR spectra were determined on a Varian T-60 spectrometer, for $CDCl_3$ solutions containing ca. 1% Me_4Si as internal standard (unless otherwise specified). IR spectra were determined for KBr pellets (unless otherwise specified) on a Perkin-Elmer Model 237 or 727B spectrophotometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn., and by Atlantic Microlab, Inc., Atlanta, Ga. Where analyses are indicated by molecular formulas, analytical results were within ±0.4% of theory.

2,3-Bis(acetoxymethyl)-7-oxabicyclo[2.2.1]hept-2-ene-*cis*-5,6-diol Cyclic Carbonates. A mixture of 3,4-bis(acetoxymethyl)furan (2.2 g, 0.01 mol) and vinylene carbonate (4.3 g, 0.05 mol) was heated in an oil bath at 125 °C for 24 h. Unreacted vinylene carbonate (3.2 g) and 3,4-bis(acetoxymethyl)furan (0.9 g) were removed by distillation in vacuo, and the residue was purified by silica gel chromatography ($CHCl_3$). The various fractions were pooled according to the TLC behavior and crystallized from chloroform–ether to yield 0.57 g of the endo isomer (mp 111–111.5 °C. Anal. $C_{13}H_{14}O_8$), 0.39 g of mixed endo and exo isomers and 0.45 g of the exo isomer (mp 118.5–119 °C. Anal. $C_{13}H_{14}O_8$). The total yield of the mixed isomers was 66% [based on recovered 3,4-bis(acetoxymethyl)furan].

2,3-Bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-2-ene-*exo*- and -*endo*-5,6-diol. A suspension of 2,3-bis(acetoxymethyl)-7-oxabicyclo[2.2.1]hept-2-ene-*endo*-5,6-diol cyclic carbonate (1.0 g, 0.333 mol) in 2 N aqueous NaOH was heated at 60 °C for 1 h. HCl, 12 N, was added dropwise until the pH of the mixture was ca. 6, and the water was removed by refluxing with benzene under a Dean–Stark trap. The mixture was concentrated in vacuo, and the resulting solid residue was extracted continuously with EtOAc for 24 h in a Sohxlet apparatus; crystallization from EtOAc yielded 0.59 g (95%) of the *endo*-tetrol: mp 130–130.5 °C. Anal. ($C_8H_{12}O_5$) C, H, O. The *exo*-tetrol was obtained in an identical fashion: mp 95.5–97 °C. Anal. ($C_8H_{12}O_5$) C, H.

***endo*-2,3-Bis(acetoxymethyl)-5,6-dihydroxy-7-oxabicyclo[2.2.1]hept-2-ene (2a).** Acetic anhydride (6.5 g, 0.045 mol) was slowly added to a solution of the *endo*-tetrol (1.02 g, 0.054 mol) in pyridine 50 mL) at −78 °C. The mixture was allowed to stand at −20 °C for 24 h. Acetic anhydride (6.5 g, 0.054 mol) was added, and the mixture was allowed to stand for an additional 24 h at −20 °C. Volatiles were removed under high vacuum at 20 °C and the residue was purified by silica gel chromatography. Crystallization of the major product (benzene–EtOAc, 3:1) yielded 0.30 g (20%) of **2a**: mp 106 °C. Anal. ($C_{12}H_{16}O_7$) C, H.

***endo*-2,3-Bis(acetoxymethyl)-5,6-diacetoxy-7-oxabicyclo[2.2.1]hept-2-ene (2b).** A solution of *endo*-tetrol (0.62 g, 0.0033 mol) and acetic anhydride (5.0 g, 0.05 mol) in pyridine (5 mL) was allowed to stand at 25 °C for 24 h. Water (100 mL) was added and the mixture was extracted with benzene (2×25 mL). The organic phase was dried (azeotropic distillation with benzene) and concentrated in vacuo to yield a yellow syrup, which was crystallized from benzene–cyclohexane to yield 1.05 g (90%) of **2b**: mp 98.7–99.2 °C. Anal. ($C_{16}H_{20}O_9$) C, H.

Dimethyl 2,3-Bis(acetoxymethyl)-7-oxabicyclo[2.2.1]-hepta-2,5-diene-5,6-dicarboxylate (3). Dimethyl acetylenedicarboxylate (1.42 g, 0.01 mol) was allowed to react with 3,4-bis(acetoxymethyl)furan (2.12 g, 0.01 mol) for 24 h at 40 °C. The

Table II. Preparation of Dimethyl 2,3-Bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-5,6-dicarboxylate Bis(alkylcarbamate) 6a–e

no.	R	yield, %	mp, °C	recrystn solv	formula (anal.)
6a	-C(=O)NHCH$_3$	71	155–158	EtOAc-(*i*-Pr)$_2$O	$C_{16}H_{20}N_2O_9$
6b	-C(=O)NHC$_2$H$_5$	70	149–151	EtOAc	$C_{18}H_{24}N_2O_9$
6c	-C(=O)NH-*n*-C$_3$H$_7$	68	129–132	EtOAc-(*i*-Pr)$_2$O	$C_{20}H_{28}N_2O_9$
6d	-C(=O)NH-*n*-C$_4$H$_9$	69	128–130	EtOAc-(*i*-Pr)$_2$O	$C_{22}H_{32}N_2O_9$
6e	-C(=O)NH-c-C$_6$H$_{11}$	73	168–172	EtOAc	$C_{26}H_{36}N_2O_9$

product was isolated by dry column chromatography (silica gel–$CHCl_3$) and crystallized (four times, benzene–cyclohexane) to yield 0.65 g (18%) of **3**: mp 65.66 °C. Anal. ($C_{16}H_{18}O_9$) C, H.

Diethyl 5,6-Bis(acetoxymethyl)-2,3-diaza-7-oxabicyclo-[2.2.1]hept-5-ene-2,3-dicarboxylate (4). A mixture of 3,4-bis(acetoxymethyl)furan (1.06 g, 0.005 mol) and diethyl azodicarboxylate (0.87 g, 0.005 mol) was heated in a water bath at 50 °C for 3 h. Dry column chromatography of the crude reaction (silica gel–Et_2O) gave 0.60 g (31%) of **4** as a colorless oil: IR (neat) 1745, 1233, 1030 cm^{-1}.

Dimethyl 2,3-Bis(hydroxymethyl)-7-oxabicyclo[2.2.1]-hepta-2,5-diene-5,6-dicarboxylate (5). A solution of freshly distilled chlorotrimethylsilane (21.6 g, 0.20 mol) in anhydrous ether (60 mL) was added dropwise to a vigorously stirred cold mixture (cooled in an ice bath) of 3,4-bis(hydroxymethyl)furan (10 g, 0.073 mol) and triethylamine (25 mL) in anhydrous ether (60 mL). The addition was conducted under a nitrogen atmosphere over a 20-min period; when the addition was complete, the mixture was allowed to warm to room temperature and was stirred for an additional 12 h. The mixture was filtered, the solid residue was washed with anhydrous ether, and the filtrate was concentrated in vacuo; the residue was distilled to yield 18 g (85%) of 3,4-bis[[(trimethylsilyl)oxy]methyl]furan as an unstable liquid: bp 66–68 °C (0.4 Torr); NMR (CCl_4 with no added Me_4Si) δ 0.00 (s, 18 H), 4.40 (s, 1 H), 7.15 (s, 2 H).

A mixture of 3,4-bis[[(trimethylsilyl)oxy]methyl]furan (2.72 g, 0.01 mol) and dimethyl acetylenedicarboxylate (1.98 g, 0.014 mol) was heated at 110 °C for 30 h. Fractional distillation of the reaction mixture gave 2.4 g (57%) of dimethyl 2,3-bis[[(trimethylsilyl)oxy]methyl]-7-oxabicyclo[2.2.1]hepta-2,5-diene-5,6-dicarboxylate: bp 118–120 °C (0.4 Torr); NMR (CCl_4 with no added Me_4Si) δ 0.0 (s, 18 H), 3.62 (s, 6 H), 4.28 (s, 4 H), 5.22 (s, 2 H).

A solution of dimethyl 2,3-bis[[(trimethylsilyl)oxy]methyl]-7-oxabicyclo[2.2.1]hepta-2,5-diene-5,6-dicarboxylate (4 g, 0.01 mol) in ethanol–water (1:1, 25 mL) was heated at 50 °C for 5 min and then concentrated to dryness in vacuo; the crude residue (2.8 g) was dried under high vacuum (over P_2O_5) and crystallized from dichloromethane to yield 2.4 g (92%) of dimethyl 2,3-bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-5,6-dicarboxylate: mp 164–167 °C; NMR δ 3.60 (s, 2 H), 3.80 (s, 6 H), 4.45 (s, 4 H), 5.60 (s, 2 H); IR 1700–1720 cm^{-1} ($\nu_{C=O}$).

Dimethyl 2,3-Bis(hydroxymethyl)-7-oxabicyclo[2.2.1]-hepta-2,5-diene-5,6-dicarboxylate Bis(alkylcarbamate) 6a–e. A cooled solution (0 °C) of dimethyl 2,3-bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-5,6-dicarboxylate (6.0 g, 0.01 mol) and triethylamine (0.6 mL) in dichloromethane (60 mL) was treated with the appropriate alkyl isocyanate (0.05 mol), and the mixture was heated under reflux for 36 h. The solution was cooled and concentrated to dryness in vacuo; the residue was dissolved in hot ethyl acetate and filtered. The yields, melting points, and recrystallization solvents for the compounds prepared are given in Table II.

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