folic acid supplementation has no apparent effect on the pharmacokinetics of phenytoin in pregnant as well as in nonpregnant rats. Significantly, neither of these two studies involved prior or chronic administration of phenytoin.

The published clinical data concerning decreased phenytoin plasma concentrations during pregnancy were obtained, necessarily, from patients on a chronic oral regimen of the drug. While it would seem feasible to administer a single intravenous tracer dose of stable isotope labeled phenytoin to these individuals before, during, and after pregnancy, this has apparently not been done. In an acute (single dose) clinical study on nonpregnant individuals, pretreatment with 15- or 30-mg folic acid orally everyday for several weeks had no effect on the biological half-life of phenytoin (17). Plasma concentrations of phenytoin in male Wistar rats 1.5 and 5 h after a single intraperitoneal dose of phenytoin, were similar in animals given a single concomitant oral dose of folic acid, 5 mg/kg, and in controls (18).

One possibility not explored in the present investigation is that folic acid supplementation affects the *in vivo* clearance of phenytoin only in rats that have been treated chronically with phenytoin. If that possibility can be excluded, then the qualitative species difference between humans and rats, with respect to the effect of pregnancy on the pharmacokinetics of phenytoin, may be due, at least in part, to the difference in response to folate. If, however, increased dietary folic acid causes an increase in the metabolic clearance of phenytoin in both humans and rats on chronic phenytoin therapy, then the apparent species difference in the effect of pregnancy on phenytoin pharmacokinetics may be related to the common practice of supplementing the diet of pregnant women, but not of pregnant rats, with folate.

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NOTE ADDED IN PROOF

Subsequent to the submission of this manuscript, Carl and Smith (19) reported that oral folate supplementation (20 mg/kg/d for 6 d, then every 12 h for 4 d) had no apparent effect on phenytoin concentrations in the liver, brain, and plasma of male Sprague-Dawley rats who received oral phenytoin (100 mg/kg/d) for 6 d, then every 12 h for 4 d) concomitantly. In a prospective study of epileptic women during pregnancy, Hiilesmaa *et al.* (20) found a negative correlation between folate and phenytoin concentrations in serum ($r = -0.56$, $p = 0.002$, $n = 39$) but no association between serum folate concentrations and the number of seizures during pregnancy.

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Synthesis of Methyl 2,3-*Bis*(hydroxymethyl)-5-phenyl-7-oxabicyclo[2.2.1]hepta-2,5-diene-6-carboxylate *Bis*(*N*-methylcarbamate) Derivatives as Potential Antitumor Agents

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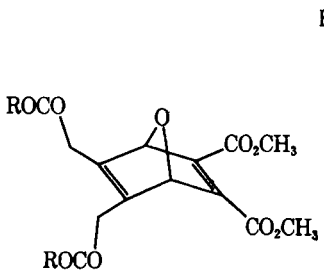
Received August 2, 1982, from the *Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14260.* Accepted for publication July 29, 1983.

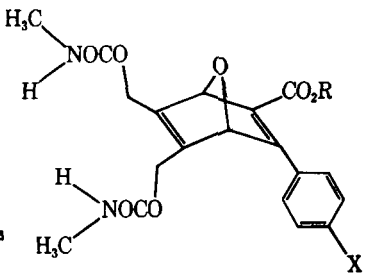
Abstract □ Methyl 2,3-*bis*(hydroxymethyl)-5-phenyl-7-oxabicyclo[2.2.1]-hepta-2,5-diene-6-carboxylate *bis*(*N*-methylcarbamate) along with the *p*-chlorophenyl and *p*-nitrophenyl analogues were synthesized using a Diels Alder reaction. The title compound and the *p*-chlorophenyl analogue were inactive against murine P388 lymphocytic leukemia.

Keyphrases □ *Bis*-carbamates—synthesis, antitumor activity □ 7-Oxabicyclo[2.2.1]hepta-2,5-diene derivatives—synthesis, antitumor activity

The discovery that certain derivatives of 7-oxabicyclo[2.2.1]heptane (1) showed cytotoxic activity in the 9KB tissue culture assay led to the synthesis and evaluation of related compounds. Dimethyl 2,3-*bis*(acetoxymethyl)-7-oxa-



IA: R=CH_3,
IB: R=NH-*N*-C_4H_9,



IIA: X=H; R=C_2H_5,
IIB: X=Cl; R=CH_3,
IIC: X=NO_2; R=CH_3,

0022-3549/84/0800-1182$01.00/0

bicyclo[2.2.1]hepta-2,5-diene-5,6-dicarboxylate (Ia) exhibited potent activity against L1210 leukemia in tissue culture assays, but failed to show any *in vivo* activity (2). The *bis*-carbamate (Ib) showed marginal activity against P388 lymphocytic leukemia *in vivo*, but this result was difficult to reproduce. This report describes an attempt to prepare derivatives of 2,3-*bis*(hydroxymethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene which show significant reproducible antineoplastic activity *in vivo*.

EXPERIMENTAL SECTION[1]

Ethyl 2,3-*bis*(hydroxymethyl)-5-phenyl-7-oxabicyclo[2.2.1]hepta-2,5-diene-6-carboxylate *bis*(*N*-methylcarbamate) (IIa) and its *p*-chloro (IIb) and *p*-nitro (IIc) analogues were prepared as follows. A mixture of 3,4-*bis*-(trimethylsilyloxymethyl)furan (2) (0.0147 mol), ethyl phenylpropiolate (0.0206 mol), and toluene (20 mL) was heated under reflux for 40 h. (Note, methyl *p*-methoxyphenylpropiolate failed to react under these conditions.) The mixture was concentrated under reduced pressure and the residue was treated with ethanol–water (1:1, 80 mL) at 60°C for 30 min. The hot mixture was filtered to remove the insoluble phenylpropiolate ester, and the filtrate was concentrated under reduced pressure. The residue was dried under high vacuum (P_2O_5) to give the unstable diol in 90–95% yield.

A solution of the diol (0.0059 mol) and triethylamine (0.2 mL) in dichloromethane (10 mL) was treated with methylisocyanate (2 mL), and the mixture was heated at reflux for 36 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue was crystallized from ethyl acetate–isopropyl ether to give IIa in 60% yield, mp 140–143°C. IR: 3350, 2950, 1700, 1550, 1250, 1150, 1000, and 850 cm^{-1}; ^{1}H-NMR: δ 1.30 (t, 3, J = 8 Hz), 2.60 (d, 3, J = 6 Hz), 2.80 (d, 3, J = 6 Hz), 4.40 (q, 2, J = 8 Hz), 4.90 (s, 4), 5.70 (s, 2), and 7.20–7.80 ppm (m, 5).

Anal.—Calc. for $C_{20}H_{23}N_2O_7$: C, 60.56; H, 5.80; N, 6.72. Found: C, 60.45; H, 5.88; N, 6.69.

The chloro and nitro derivatives (IIb and IIc), as ethyl esters, were prepared in the same manner. Compound IIb, obtained in 72% yield, had mp 176–178°C (ethyl acetate–isopropyl ether). IR: 3350, 2950, 1700, 1540, and 1260 cm^{-1}; ^{1}H-NMR: δ 2.60 (d, 3, J = 6 Hz), 2.80 (d, 3, J = 6 Hz), 3.75 (s, 3), 4.85 (s, 4), 5.65 (s, 2), and 7.2–7.8 ppm (m, 4).

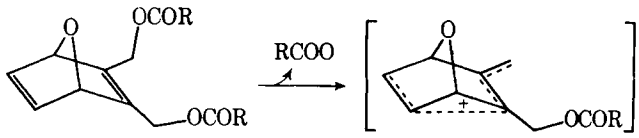
Anal.—Calc. for $C_{20}H_{22}ClN_2O_7$: C, 54.98; H, 4.84; N, 6.41; Cl, 8.11. Found: C, 54.92; H, 4.91; N, 6.35; Cl, 8.14.

Compound IIc (68%) had mp 172–176°C (ethyl acetate–isopropyl ether). IR: 3550, 2950, 1700, 1550, 1250, 975, and 850 cm^{-1}; ^{1}H-NMR: δ 2.60 (d, 3, J = 6 Hz), 2.80 (d, 3, J = 6 Hz), 3.75 (s, 3), 4.85 (s, 4), 5.65 (s, 2), and 7.2–7.8 ppm (m, 4).

Anal.—Calc. for $C_{20}H_{22}N_3O_9$: C, 53.69; H, 4.73; N, 9.39. Found: C, 53.74; H, 4.76; N, 9.34.

RESULTS AND DISCUSSION

The rationale for the design of I was based on two factors. These were, first, the electrophilicity of the allylic esters, which can react by an alkyl–oxygen cleavage mechanism (3, 4) and, second, the neighboring group participation



Scheme I

of C=C π-bonds in nucleophilic substitution reactions. These two factors are summarized in Scheme I.

The 5,6-double bond in I is substituted with two electron-withdrawing groups [for $-CO_2CH_3$ the Swain–Lupton resonance (R) and field (F) contributions are 0.552 and 0.140, respectively]. Compounds of the general structure II were designed to have a 5,6-double bond which was less electron-deficient than I (the phenyl substituent has F = 0.139 and R = −0.088). Thus, compounds of the general structure II should be more reactive than I.

These *bis*-carbamates were prepared in a reaction sequence which began with a Diels Alder reaction between 3,4-*bis*(trimethylsilyloxymethyl)furan (2) and the appropriate phenylpropiolic acid derivative. The trimethylsilyl-protected diol was used because it could be removed from the reaction mixture by distillation at a sufficiently low temperature to avoid decomposition of the product. The phenylpropiolic acid derivatives were prepared from the appropriate cinnamic acid by a bromination–dehydrobromination sequence (5–9).

The silyl blocking groups were removed from the Diels Alder adducts (ethanol–water), and the diols were converted to the *bis*-carbamates by treatment with methylisocyanate in the presence of triethylamine. The intermediate diols were unstable and attempts to purify these compounds led to extensive decomposition; thus, the diols were used directly in the next step.

Two of the compounds, selected as "lead" structures, were tested against P388 lymphocytic leukemia in mice under the auspices of the National Cancer Institute (10). The *p*-chloro compound (IIb) was inactive over the dose range of 1.56–200 mg/kg, and IIa was inactive over the dose range of 12.5–400 mg/kg. Compound IIa was toxic at the 400-mg/kg dose; three of the six test animals died before the fifth day of the test (the animals were given once daily intraperitoneal injections). Only one of six animals died before day 5 at the 200-mg/kg level with both IIa and IIb; however, IIb appeared to be more toxic as judged by the weight losses of the treated animals.

The reactivity of II was greater than I, as evidenced by the greater instability of II. Nevertheless, the reactivity is still not sufficient to confer *in vivo* antitumor activity to *bis*-carbamate derivatives in the oxabicyclic series.

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[1] Melting points (uncorrected) were determined in open capillary tubes with a Thomas-Hoover Unimelt apparatus. IR spectra were determined for KBr wafers with a Perkin-Elmer 727 spectrophotometer. ^{1}H-NMR spectra were determined for deuterochloroform solutions containing ~1% (v/v) tetramethylsilane as the internal standard with a Varian T-60A spectrometer. Microanalyses were performed by Atlantic Microlabs, Inc., Atlanta, Ga.