The Carbocyclic Analog of Cytidine, Synthesis and Antineoplastic Activity

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The carbocyclic analog (VI) of cytidine was prepared from the carbocyclic analog (I) of uridine. The intermediate stages were a tetrabenzoyl derivative of I, the tribenzoyl derivative of the uridine analog, and the tribenzoyl 4-chloropyrimidinone obtained from the latter derivative. The cytidine analog (VI) is active against KB cells in culture and against L1210 leukemia in mice. In the initial tests against L1210 leukemia, the highest dose (200 mg./kg./day, *q.d.* 1-9), of three active doses, increased lifespan by 82% and showed no evidence of toxicity.

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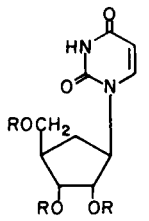
Sir:

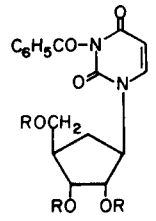
Carbocyclic analogs of purine nucleosides have demonstrated several types of biological activity (1, *cf.* references cited in reference 2). Several pyrimidine nucleosides, or heterocyclic ring-analogs of pyrimidine nucleosides, have clinical anticancer activity (3). For these two general reasons, syntheses of carbocyclic analogs of pyrimidine nucleosides were undertaken (2,4). In addition, all of the common pyrimidine nucleosides comprising nucleic acids are phosphorylated by mammalian cells (5), and it seemed reasonable to postulate that some of the carbocyclic analogs of pyrimidine nucleosides might also be activated by the same kinases. The resulting nucleotide analogs, the diphosphates, or the triphosphates might then inhibit steps in the synthesis of RNA or DNA from acyclic precursors or from preformed (salvaged) nucleosides. The carbocyclic analogs would not be subject to the catabolic action of phosphorylases. In this communication, we present a preliminary account of the synthesis and antineoplastic activity of the carbocyclic analog of cytidine.

The cytidine analog (VI, (±)-4-amino-1-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-2(1*H*)-pyrimidinone) was prepared by a four-step synthesis from the uridine analog (I, (±)-1-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1*H*,3*H*)pyrimidinedione) (2,6). Preparation of the tribenzoate (II) of I was effected in two steps because initial experiments on the benzoylation of a related compound, the carbocyclic analog (2) of 2′-deoxyuridine, indicated that benzoylation of the pyrimidine ring and the hydroxyl groups occurred concomitantly. It was assumed that, after complete benzoylation of I to a tetrabenzoyl derivative (III or IV), the benzoyl group attached to the pyrimidine moiety could be selectively removed under mildly acidic conditions (7). Treatment of I with an excess (4.9 equivalents) of benzoyl chloride in dry pyridine at 57-58° for 48 hours afforded

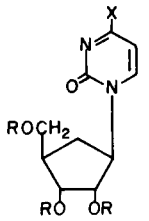


I: R = H

II: R = C_6H_5CO-

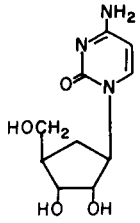


III: R = C_6H_5CO-



IV: X = $-OCOC_6H_5$; R = C_6H_5CO-

V: X = Cl; R = C_6H_5CO-



VI

a crude tetrabenzoyl derivative (III or IV) in 97-100% yields; field-desorption mass spectrum, 658 (M^+); tlc, major component and small amounts of impurities (including II). Refluxing the crude tetrabenzoyl derivative in dilute aqueous ethanolic hydrochloric acid (2:1 ethanol-water, 0.036*N*, 3.6 equivalents of hydrochloric acid) for 20 hours gave (after evaporation of ethanol) crude II, which was recrystallized from ethanol, yields (from I), 74-78%, m.p. 191-194°; field-desorption mass spectrum: 555 ($M^+ + 1$), 554 (M^+).

Anal. Calcd. for $C_{31}H_{26}N_2O_8$: C, 67.14; H, 4.73; N, 5.05. Found: C, 67.15; H, 5.10; N, 4.84.

The 4-chloro derivative (V) was obtained by refluxing a solution of II, thionyl chloride (10 equivalents), chloroform, and a small amount of dimethylformamide (8) for 6 hours, treating the solution with activated charcoal, and concentrating the filtrate to a cream-colored solid. A mixture of the crude V (m/e 573 ($M^+ + 1$), 572 (M^+)) and 50% methanolic ammonia was kept in a stainless steel (Parr) bomb at 100° for 18 hours and evaporated to a gummy residue. An aqueous solution of the residue was extracted with ethyl acetate to remove benzamide and chromatographed on a column of a cation-exchange resin (Amberlite CG-120, H^+). Elution of the column with water to remove neutral contaminants and then with 0.5*N* aqueous ammonia, lyophilization of the ammonia solution, and trituration of the residue with methanol-ether (1:1) afforded pure VI, yields (from II), 55-65%, m.p. 245-248° dec., inserted at 230°, 3°/min., progressive darkening; tlc, 1 spot (silica gel, 3:1 chloroform-methanol, detection by uv and by basic potassium permanganate); mass spectrum: m/e 242 ($M^+ + 1$), 241 (M^+); uv max 214 (ϵ, 10,200) and 284 (ϵ, 13,300) at *p*H 1, 225 (sh.) and 274 (ϵ, 9,300) at *p*H 7.

Anal. Calcd. for $C_{10}H_{15}N_3O_4 \cdot 0.5H_2O$: C, 47.99; H, 6.44; N, 16.79. Found: C, 48.29; H, 6.17; N, 16.75.

The cytidine analog (VI) inhibited the growth of KB cells in culture (ED_{50} = 6.5 mcg./ml.). Initial tests of VI against Leukemia L1210 in mice by standard protocols (9,10) showed that it is active at the following doses, *q.d.* 1-9: 200 mg./kg./day, T/C = 182% (6 mice, one 30-day survivor); 150 mg./kg./day, T/C = 150%; 100 mg./kg./day, T/C = 152% and 136% (2 tests). There was no evidence of toxicity in these tests. The clinical activity of the cytidine analogs arabinosylcytosine (Ara-C, 4-amino-1-β-D-arabinofuranosyl-2(1*H*)pyrimidinone) (11) and 5-azacytidine (12) emphasizes the potential importance of cytidine analogs that show antineoplastic activity.

Acknowledgments.

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(10) T = average survival time of treated mice; C = average survival time of control mice.

(11) *e.g.*, R. W. Talley, R. M. O'Bryan, W. G. Tucker, and R. V. Loo, *Cancer*, **20**, 809 (1967); E. Frei, III, J. N. Bickers, J. S. Hewlett, M. Lane, W. V. Leary, and R. W. Talley, *Cancer Res.*, **29**, 1325 (1969).

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