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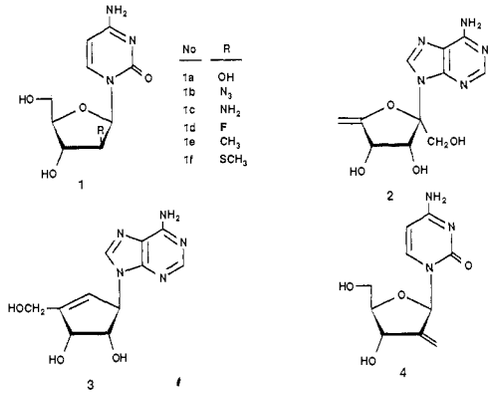
Communications to the Editor

Design, Synthesis, and Antineoplastic Activity of 2′-Deoxy-2′-methylidenecytidine[1]

Sir:

1-β-D-Arabinofuranosylcytosine (ara-C, 1**a**) is one of the most potent drugs for the treatment of acute human leukemia.[2] However, ara-C has several drawbacks; its half-life is very short because of deamination to chemotherapeutically inactive 1-β-D-arabinofuranosyluracil by cytidine deaminase, and it is not effective against solid tumors. In order to overcome these problems, efforts have been made to develop prodrugs[3] or introduce certain other substituents[4] into the 2′-*arabino* position in place of the hydrogen atom of 2′-deoxycytidine. As a result of the latter approach, 2′-azido- and 2′-amino-2′-deoxy-β-D-arabinofuranosylcytosines (1**b**,**c**)[4a] were found to be resistant to cytidine deaminase and still as potent as ara-C against mouse leukemic L1210 cells. It has also been reported that 2′-deoxy-2′-fluoro-β-D-arabinofuranosylcytosine (1**d**)[4b,c] is a potent cell-growth inhibitor of mouse leukemic L5178Y cells, although it was susceptible to deaminase.[5] As we reported quite recently, (2′*S*)-2′-deoxy-2′-methylcytidine (1**e**), the methyl group being introduced at the 2′-*arabino* position of 2′-deoxycytidine,[6] was also found to be a potent inhibitor of the growth of L1210 cells in vitro, whereas 2′-deoxy-2′-(methylthio)-β-D-arabinofuranosylcytosine (1**f**)[7] was not. Considering the nature of the substituents at the *arabino* position of 2′-deoxycytidine, both bulkiness and polarity seem to be significant factors in affecting biological activity. When such a nucleoside antimetabolite exhibits biological activity, it must be phosphorylated at the 5′-position by deoxycytidine kinase to some extent. Therefore, the overall shape of the nucleoside including sugar conformation and spatial position of the 5′-hydroxyl group is likely to be critical for enzyme recognition. Moreover, the electronegativity of the 2′-substituents may also influence sugar conformation and chemical reactivity of the nucleoside and its 5′-nucleotide. These considerations, together with the nature of the 3′-hydroxyl group which would mainly be affected by the 2′-substituent, should also be important when such a nucleoside 5′-triphosphate is to be incorporated into DNA molecules. If a double bond function can be introduced to the 2′-position of 2′-deoxycytidine, it would constitute an allylic alcohol system together with the 3′-secondary alcohol group. Furthermore, some chemical reactivity would be expected from such a structure when its nucleotide is incorporated into DNA molecules because the allylic alcohol system should constitute a more reactive allylic ester. The allylic alcohol system is found in a number of nucleoside antibiotics including angustmycin A (**2**)[8] and neplanocin A (**3**).[9] This structural feature may play an important role in the exhibited biological activity due to enhanced chemical reactivity and/or fixation of the sugar conformation. In order to examine this hypothesis, we have synthesized 2′-deoxy-2′-methylidenecytidine (**4**, DMDC) from uridine in eight steps. We also describe its antineoplastic activity in vitro using mouse and human tumor cell lines.



Chemistry. From uridine, 4-ethoxy-1-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-*erythro*-2-pentulofuranosyl]-2(1*H*)-pyrimidinone (**5**) was synthesized in five steps in good overall yields.[10] When compound **5** was treated with methylenetriphenylphosphorane (3 equiv

(1) This paper constitutes part 83 of Nucleosides and Nucleotides; part 82: Hayakawa, T.; Ono, A.; Ueda, T. *Nucleic Acids Res.*, in press.
(2) For a review on prodrugs, see: Creasy, W. A.; Papac, R. J.; Markin, M. E.; Calabresi, P.; Welch, A. D. *Biochem. Pharmacol.* **1966**, *15*, 1417.
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Scheme I

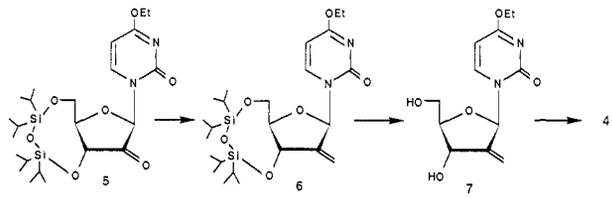

Table I. Inhibitory Effects of DMDC (4), ara-C, and 5-FU on the Growth of Various Mammalian Cell Lines in Vitro[a]

cell line	IC_{50},[b] μg/mL		
	DMDC (4)	ara-C	5-FU
L1210[c]	0.11	0.097	0.32
CCRFCEM[d]	0.047	0.065	40
MOLT 4[e]	0.025	0.056	3.8
K562[f]	1.2	3.2	38
PC10[g]	60.5	>100	>100
SW480[h]	3.8	>100	3.3
TE2[i]	2.9	>100	3.9
T24[j]	3.7	>100	6.1

[a] Drug sensitivity assays were performed according to the method of Carmichael et al.[13] Each tumor cell line (1×10^4/well) was incubated in the presence or absence of compounds for 72 h. Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added and OD($^{550-660}$nm) was measured. Percent inhibition was determined as follows: % inhibition = [1 − (OD-($^{550-660}$nm) of sample well/OD($^{550-660}$nm) of control well)] × 100. [b] IC_{50} (μg/mL) was given as the concentration at 50% inhibition of cell growth. [c] Mouse leukemia. [d] Human T-cell acute lymphoblastoid leukemia. [e] Human T-cell acute lymphoblastic leukemia. [f] Human chronic myelogenous leukemia. [g] Human lung squamous cell carcinoma. [h] Human colon adenocarcinoma. [i] Human esophagus adenocarcinoma. [j] Human bladder transitional-cell carcinoma.

prepared by reaction of potassium hydride and methyltriphenylphosphonium bromide in dimethyl sulfoxide), the desired 2′-methylidene nucleoside (6) was obtained in 41% yield as a foam [MS m/z 510 (M^+); ^1H NMR ($CDCl_3$) δ 5.39 (dd, 1 H, 2′-vinyl proton, J = 2.9 Hz, J = 1.5 Hz), 5.69 (dd, 1 H, 2′-vinyl proton, J = 2.9 Hz, J = 1.2 Hz), 6.64 (d, 1 H, 1′-H, J = 1.5 Hz)]. Deprotection of 6 with tetra-*n*-butylammonium fluoride in tetrahydrofuran afforded 7 in 91% yield. Compound 7 was then converted to the target nucleoside, 2′-deoxy-2′-methylidenecytidine (4, DMDC), by treatment with methanolic ammonia in a sealed tube at 100 °C for 2 days and isolated in 81% yield as an HCl salt [mp >300 °C; ^1H NMR (D_2O) δ 5.51 (br s, 1 H, 2′-methylidene proton), 5.68 (br s, 1 H, 2′-methylidene proton), 6.64 (br d, 1 H, 1′-H, J = 1.8 Hz). Anal. ($C_{10}H_{14}ClN_3O_4$) C, H, N.] (Scheme I).

Biological Activity. The nucleoside, DMDC (4), ara-C, and 5-fluorouracil (5-FU) were tested for their ability to inhibit the growth of various tumor cells including human tumor cells in vitro. The IC_{50} values for these compounds are summarized in Table I. Ara-C showed inhibitory activity against mouse leukemic, human T-cell acute leukemic, and chronic leukemic cells, but not against human carcinoma and adenocarcinoma cells. By contrast, 5-FU exhibited a broad spectrum of activity to this range of cells. Although DMDC is an analogue of 2′-deoxycytidine, its spectrum of activity against tumor cells is quite different from that of ara-C. DMDC was active at rather low concentrations against not only mouse leukemic and human leukemic cell lines but also human carcinoma cell lines. Furthermore, DMDC is more active than 5-FU in T24 human bladder transitional-cell carcinoma cells and comparably active to 5-FU in SW480 human colon adenocarcinoma and human esophagus adenocarcinoma cells.

The effect of DMDC and ara-C on the synthesis of DNA, RNA, and proteins was also examined with L1210 cells. At 10 μg/mL, both DMDC and ara-C inhibited incorporation of [^{3}H]thymidine into DNA by 98%, while no inhibition of RNA synthesis (incorporation of [^{3}H]uridine) and protein synthesis (incorporation of [^{3}H]Leu) was observed. It is noteworthy that no significant deamination of DMDC was detected in 2 h by partially purified cytidine deaminase from mouse kidney.[11] Under similar conditions, cytidine and ara-C were deaminated (100% and 88%, respectively).

As this unique and broad spectrum of inhibitory activity of DMDC may be related to the allylic alcohol system in its structure, detailed studies on its mechanism of action in vitro as well as its activity in vivo[12] are being undertaken.

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Registry No. 4, 113648-25-2; 5, 113648-22-9; 6, 113648-23-0; 7, 113648-24-1; $H_2C{=}PPh_3$, 3487-44-3; uridine, 58-96-8.

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(±)-4-*tert*-Butyl-3-cyano-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octane: Synthesis of a Remarkably Potent $GABA_A$ Receptor Antagonist

Sir:

A great variety of potent convulsants including 1,4-disubstituted 2,6,7-trioxabicyclo[2.2.2]octanes, bicyclophosphorus esters, polychlorocycloalkanes, and picrotoxinin analogues act as noncompetitive $GABA_A$ receptor antagonists.[1-3] These toxicants and insecticides are considered to bind to a specific site(s) within the GABA receptor–ionophore complex and thereby to block the GABA-regulated chloride channel.[4-6] This specific site

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