Synthesis and biological activities of iododeoxyuridine, an analog of thymidine

Iodinated derivatives of uracil and of uracil-containing compounds have been synthesized previously and some of their biological activities investigated[1,2]. Whereas 5-iodouracil has been shown to be an effective inhibitor of microbial growth[2] and to be readily incorporated into the deoxyribonucleic acids of several microbial species[3,4], 5-iodouridine and 5-iodoorotic acid were biologically inert in the systems which were investigated[1]. These studies have been extended by the present report to the corresponding iodinated deoxyribonucleoside.

5-Iododeoxyuridine was synthesized by a modification of the method described previously for the synthesis of 5-iodouridine[1]. Uracildeoxyriboside (400 mg), iodine (400 mg), chloroform (2 ml) and HNO_3 (1 *N*, 4 ml) were refluxed gently for 2 h during which time white needle crystals of iododeoxyuridine formed. The reaction mixture was decanted into a sintered-glass funnel and the product was washed with ether until the unreacted iodine had been extracted. After recrystallization from hot water the yield was 350 mg or 56 % of theory.

Analysis. Found: C, 30.46; H, 2.95; N, 8.05; I, 35.45; Calc. for $C_9H_{11}O_5N_2I$: C, 30.51; H, 3.11; N, 7.91; I, 35.88. Decomposition occurred at 160° and fumes of iodine appeared at 180°.

The u.v.-absorption characteristics of several iodinated pyrimidines and their parent compounds are shown in Table I.

TABLE I

ULTRAVIOLET ABSORPTION MAXIMA AND MINIMA OF SOME DERIVATIVES OF URACIL AND IODOURACIL

Compound	*NaOH (0.01 N)*		*HCl (0.01 N)*	
	max. mμ	*min.* mμ	*max.* mμ	*min.* mμ
Iodouracil	304	256	283	245
Uracil	284	241	259	227
Iodouridine	278	253	289	249
Uridine	262	236	262	230
Iododeoxyuridine	278	253	288	248
Deoxyuridine	262	242	262	231

The u.v.-absorption spectrum of the synthetic iododeoxyuridine agrees with that of material isolated from microbial DNA by ZAMENHOF *et al.*[3] and by DUNN AND SMITH[4]. FRIEDKIN AND ROBERTS[5] presented evidence for the formation of iododeoxyuridine by a mammalian enzyme, but no characterisation was given. The insertion of the iodine atom into each pyrimidine derivative results in a bathochromic effect; however, in alkaline solution the u.v. maximum for iodouracil shifts to a longer wavelength, whereas the corresponding riboside and deoxyriboside shift to a lower wavelength. The non-iodinated nucleosides in alkali show no shift in their absorption maxima.

The iodinated derivatives are readily separated from the parent compound and

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid,

from each other by paper chromatography employing the ethyl acetate–phosphate buffer system[6] (Table II).

Insertion of the iodine atom into the molecule results in a marked increase in the mobility of each of the pyrimidine derivatives; however, there is no alteration in the order of migration.

Iododeoxyuridine may also be synthesized by a modification of the method of JOHNSON AND JOHNS[7] for iodouracil. Deoxyuridine (228 mg), iodine (250 mg), and NaOH (3 *N*, 2 ml) were heated on a steam bath for 15 min; after dilution with water (50 ml) the solution was passed through a Dowex-1-formate column. After washing the column with NaOH (0.01 *N*) until no iodide appeared in the effluent, as indicated by reaction with $AgNO_3$, elution was continued with formic acid (0.1 *N*). Immediately after the elution of unreacted deoxyuridine, iododeoxyuridine appeared.

TABLE II

R_F VALUES OF SOME DERIVATIVES OF URACIL AND IODOURACIL

Solvent: Ethyl acetate saturated with phosphate buffer (0.05 *M*, pH 6.0).

Compound	R_F
Iodouracil	0.75
Uracil	0.21
Iododeoxyuridine	0.67
Deoxyuridine	0.12
Iodouridine	0.44
Uridine	0.06

In contrast to iodouridine, iododeoxyuridine is almost as effective as iodouracil as an inhibitor of the growth of *Streptococcus faecalis* (ATCC 8043), when grown in media supplemented with thymine, thymidine or pteroylglutamic acid. With mouse Ehrlich ascites carcinoma cells *in vitro*, iododeoxyuridine but not iodouracil or iodouridine reversibly inhibited the utilization of ^{14}C-labeled thymidine for the biosynthesis of DNA-thymine. Iododeoxyuridine inhibited markedly the utilization of [^{14}C]orotic acid or [^{14}C]formate for the biosynthesis of DNA-thymine, but not of [^{14}C]orotic acid for the biosynthesis of DNA-cytosine or RNA pyrimidines. Hence the mechanism of action of iododeoxyuridine would appear to be an inhibition of the utilization of a thymine-containing precursor of DNA-thymine. Details of the biological studies will appear elsewhere.

This investigation was supported by a grant (CY-3076) from the National Institutes of Health, U.S. Public Health Service.

Department of Pharmacology Yale University, School of Medicine, New Haven, Conn. (U.S.A.) WILLIAM H. PRUSOFF

[1] W. H. PRUSOFF, W. L. HOLMES AND A. D. WELCH, *Cancer Research*, 13 (1953) 221.
[2] G. H. HITCHINGS, E. A. FALCO AND M. B. SHERWOOD, *Science*, 102 (1945) 251.
[3] S. ZAMENHOF, B. REINER, R. DEGIOVANNI AND K. RICH, *J. Biol. Chem.*, 219 (1956) 165.
[4] D. B. DUNN AND J. D. SMITH, *Biochem. J.*, 67 (1957) 494; *Nature*, 174 (1954) 305.
[5] M. FRIEDKIN AND D. ROBERTS, *J. Biol. Chem.*, 207 (1954) 257.
[6] W. H. PRUSOFF, *J. Biol. Chem.*, 215 (1955) 809.
[7] T. B. JOHNSON AND C. O. JOHNS, *J. Biol. Chem.*, 1 (1905–06) 305.

Received November 20th, 1958