Table 1. Phenylthiourea taste in glaucoma.

Sex	No. tested	Nontasters (%)
*Primary open-angle glaucoma**		
Male	104	53
Female	107	52
Angle-closure glaucoma		
Male	44	16
Female	111	18
"Normal"		
Male	195	29
Female	251	27

* With loss of visual field.

coma has been demonstrated repeatedly (*1*).

In studies of the general population, taste sensitivity for phenylthiourea is distributed in bimodal fashion, about 30 percent of Caucasians falling into the nontaster group (*2*, *3*). The ability to taste this and related compounds is genetically determined and provides a convenient genetic marker (*2*). In the course of genetic studies on glaucoma patients, taste sensitivity to phenylthiourea was determined. This preliminary report presents an association, found in Caucasian patients over the age of 40 years, between taste sensitivity for phenylthiourea and glaucoma.

We studied patients attending the eye clinic of the Washington University Clinics, as well as those hospitalized on the eye service of the Barnes Hospital Group. A serial-dilution technique was used for evaluating the taste threshold of each patient, the test starting with the most dilute solution (No. 11, containing 1.27 mg of phenylthiourea per liter of tap water) and proceeding by twofold increases of concentration as described by Harris and Kalmus (*2*). We performed the taste test without knowing of the patient's glaucoma classification, and we made our clinical glaucoma diagnoses without knowledge of the results of the taste test.

Patients were classified as having primary open-angle glaucoma when elevated intraocular pressures, decreased outflow facilities, and charasteristic cupping of the optic nerve head and loss of visual field could be documented. All patients included in this category had open angles (grade II or more) and no evidence of inflammatory, traumatic, or neoplastic eye disease. Sixty-seven percent of these patients were over the age of 60 years.

The diagnosis of angle-closure glaucoma was made when elevated intraocular pressure was associated with occluded anterior chamber angles. Sixty-six percent of the patients in this category were over the age of 60 years.

Patients were classified as "normal" when they had no family history of glaucoma, intraocular pressure measurements less than 20 mm-Hg, and no evidence of cupping of their optic nerves or visual field loss. Sixty-six percent of these patients were over the age of 60 years.

We also studied patients with various secondary glaucomas, as well as those whose intraocular pressures, provocative tests, and family histories fell into intermediate categories.

The distribution of taste sensitivities in each of the three groups was bimodal, with the antimode between solutions 4 and 5. With "nontasters" defined as all individuals who failed to experience a bitter taste to solutions up to No. 5 (81.25 mg/liter), the percentage of nontasters for each group is shown in Table 1. It was of interest that the "normal" eye clinic population had a 28-percent incidence of nontasters. This differed very little from the 30 percent nontasters found by other observers in Caucasian populations (*3*). Of particular interest was the high percentage (53 percent) of nontasters found in patients with primary open-angle glaucoma, and the remarkably low incidence (17 percent) of nontasters in individuals with angle-closure glaucoma. Each of the glaucoma groups differed significantly from the "normal" (for open-angle glaucoma compared to normal, $p < .001$; for angle-closure glaucoma compared to normal, $p < .01$), and most significantly from each other ($p < .001$).

In each of the three groups no significant differences were noted between individuals 41 to 60 years of age and those over 60 years. As in other glaucoma studies, angle-closure glaucoma was more prevalent among females, but in none of the three categories did the taste testing results differ in males and females. All glaucoma patients were using topical miotic therapy and some also received systemic carbonic anhydrase inhibitors. Conceivably, the long-term use of these agents might alter taste sensitivity, but there were no differences in therapy between the group of glaucoma patients classified as tasters and the nontasters. Furthermore, similar medications were used by the open-angle glaucoma group (53 percent nontasters) and the angle-closure glaucoma patients (17 percent nontasters).

The findings raise questions concerning the nature of the association between phenylthiourea taste sensitivity and glaucoma.

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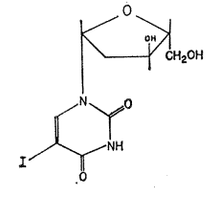
5-Iodo-2′-Deoxyuridine: Relation of Structure to Its Antiviral Activity

Abstract. *The crystal and molecular structure of 5-iodo-2′-deoxyuridine has been determined by x-ray diffraction methods. The most interesting feature of the structure is an unusually short intermolecular distance between the iodine and oxygen of a carbonyl group; this type of bonding may be the molecular basis for the antiviral activity.*

5-Iodo-2′-deoxyuridine (IDU)



has been used in the treatment and cure of *herpes simplex* keratitis (*1*) and it is the first antiviral agent to have proven clinical chemotherapeutic value. The blocking of the metabolic pathways of viral synthesis by IDU has been attributed either to the selective action of IDU on a virus-specific enzyme system which may take part in the synthesis of viral DNA, or to the incorporation of IDU itself in place of thymidine into an aberrant DNA which presum-

ably cannot be utilized to form infective virus particles (*2*). Iododeoxyuridine and the bromine analog (BrDU) can certainly be incorporated in place of thymidine into DNA of bacteria, bacteriophages, and even human and other mammalian cells (*3*); heavily substituted DNA can sometimes still undergo full biological functioning but with retarded growth rate, enhanced genetic instability, and increased radiation sensitivity. Incorporation of IDU and BrDU into DNA results in significantly higher melting temperatures than the melting temperature of normal DNA. Of the several explanations put forward to explain this enhanced stability, the most probable has been that the substitution of iodine or bromine for CH_3 in thymidine alters the electron density in the pyrimidine ring with concomitant strengthening of the hydrogen bonding to the purine base in the complementary DNA chain (*4*). It thus would appear that the incorporation of IDU and BrDU instead of thymidine into DNA hinders further DNA synthesis by increasing the interchain attraction and thereby impairing the capacity of the complementary chains to separate and serve as templates for more DNA.

We have investigated the crystal and molecular structures of 5-iododeoxyuridine and 5-bromodeoxyuridine in the hope that the detailed structures might yield some direct information on the possible role of these compounds in combatting viral diseases.

5-Iodo-2′-deoxyuridine and 5-bromo-2′-deoxyuridine are isomorphous; cell constants and crystal data for both are given in Table 1. A detailed investigation of the structure of IDU was performed. The intensities of 1445 reflections were measured with a scintillation counter and Mo$K\alpha$ radiation. All of the atomic positions were obtained from two successive three-dimensional electron-density distributions, and the structure was refined by least squares; the final discrepancy factor was $R = .054$. Superimposed sections of the electron density calculated at the atomic centers are shown in Fig. 1, along with a perspective drawing of the molecule. The base is in the di-keto form, and all bond lengths and angles are normal (*5*).

Figure 2 shows the packing of the molecules. The most significant intermolecular contact is an iodine to oxygen (carbonyl) distance of 2.96 Å; this is very considerably shorter than the sum of the iodine and oxygen van der Waals radii (3.55 Å) and suggests fairly strong charge-transfer bonding involving donation of oxygen lone-pair electrons to vacant 5*d* orbitals of the iodine atom. Such charge-transfer bonds between halogens and oxygen-, nitrogen-, and even sulfur- and selenium-containing compounds have been reported previously (*6*), the stability of the bond being greater for the heavier halogens, and in some instances, the

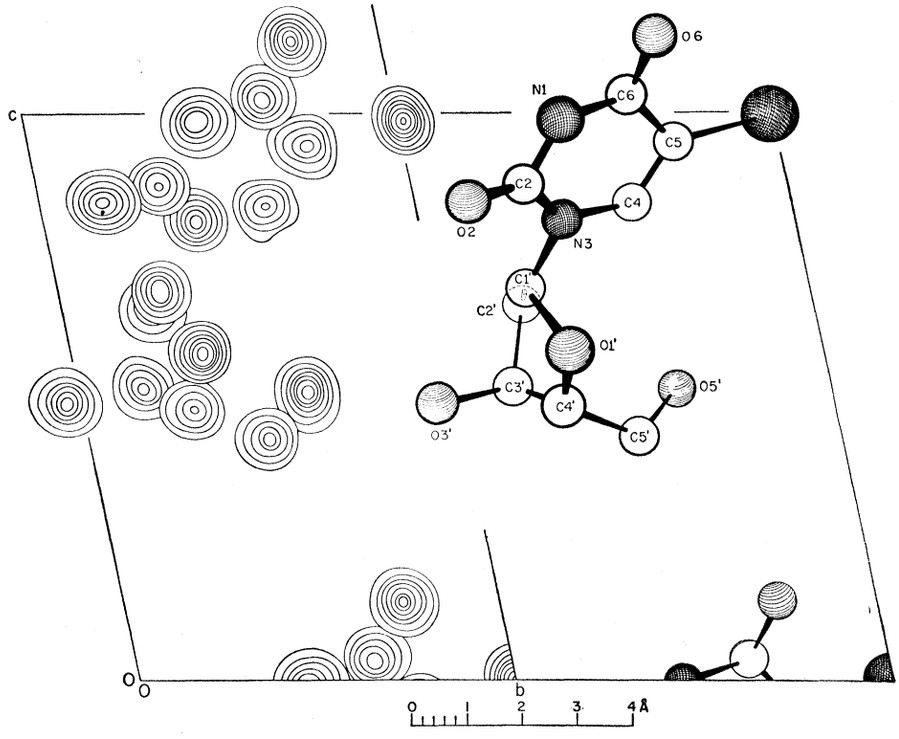


Fig. 1. Sections of the three-dimensional electron-density distribution of IDU through the atomic centers, and a perspective drawing of the molecule.

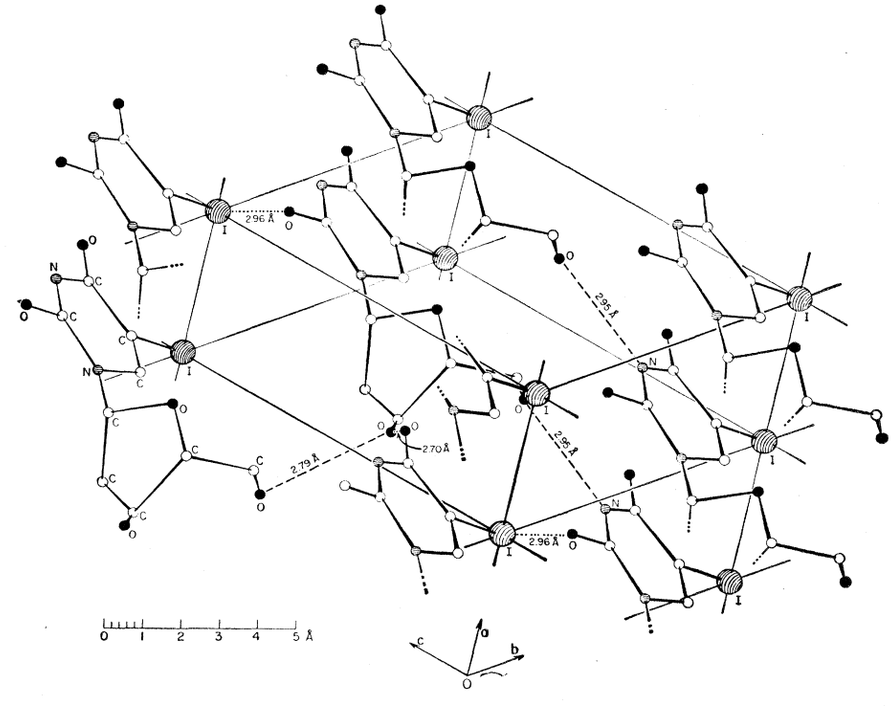


Fig. 2. Isometric projection showing the packing of the IDU molecules in the crystal.

Table 1. Comparison of crystal data of IDU and BrDU.

Physical property	IDU	BrDU
Crystal system	Triclinic	Triclinic
a(Å)	4.98	4.87
b(Å)	6.83	6.72
c(Å)	9.60	9.56
α	101°40′	100°10′
β	109°18′	107°24′
γ	98°20′	98°31′
Volume of cell (Å^3)	292.9	285.1
Measured density (g cm^{-3})	2.014	
Molecules per cell	1	1
Calculated density (g cm^{-3})	2.008	1.789
Space group	$P1$	$P1$

iodine to oxygen distance being even shorter than that reported here.

On the basis of this information it does not seem unreasonable to suggest that the strong intermolecular attraction may be the explanation for the role played by IDU in the treatment of *herpes simplex* keratitis. The ability of the iodine to form intermolecular charge-transfer bonds with atoms having lone pairs of electrons, such as oxygen or nitrogen, may cause the increased interchain attraction which is observed when IDU is substituted for thymidine in DNA, with subsequent retardation of the reproductive ability of the DNA. In the case of IDU incorporation by *herpes simplex*, this could prevent the synthesis of viral DNA or retard it to the extent that the body could combat the virus successfully.

There are a number of oxygen and nitrogen atoms on the complementary purine base (adenine), and on the complementary chain skeleton, with which the iodine might be able to form charge-transfer bonds; such a bond need not be with carbonyl oxygen as is the case in the IDU crystal. Hence a large number of possibilities exist as to the actual intermolecular bonding scheme which could be effected by the electron-accepting property of the iodine, some possibly involving disruption of the normal hydrogen-bonding system, and some not. Whether such intermolecular attraction would actually take place in addition to the normal hydrogen-bonding scheme between base pairs, or would replace this scheme with an alternate one, is a matter of speculation. More direct information would require structural investigation of the aberrant DNA.

The other short intermolecular distances in the IDU crystal correspond to normal hydrogen bonds. Crystals of thymidine are orthorhombic and the cell dimensions bear no resemblance to those of IDU and BrDU, so that it has a different crystal structure.

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Nucleotides: Separation from an Alkaline Hydrolysate of RNA by Thin-Layer Electrophoresis

Abstract. *Mixtures of the 2′, 3′ nucleoside monophosphates obtained from an alkaline hydrolysis of RNA can be resolved into the four main RNA mononucleotides by electrophoresis on thin layers of cellulose. The separated nucleotides are recovered by removing the cellulose layer from the supporting glass plate and suspending the cellulose layer in* tris *hydrochloride buffer. The extracted cellulose is removed by low-speed centrifugation.*

The separation of low molecular weight derivatives of nucleic acid by thin-layer chromatography on cellulose or on ion-exchange cellulose layers has been reported (*1–4*). The systems developed by Randerath (*1, 2*), are most suitable for the separation of components of nucleotide mixtures of differing phosphate content, such as monophosphates and diphosphates. The separation of 10^{-3} μmole amounts of the four 5′ mononucleotides of RNA has been described by Randerath (*5*) for polyethylenimine-cellulose layers with a stepwise elution technique; but this method does not give good resolution of the 2′, 3′ phosphates obtained from an alkaline hydrolysis of RNA.

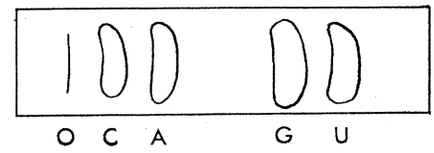

Fig. 1. The separation obtained with sodium formate buffer, *p*H 3.4, ionic strength 0.1, with a 75-minute run. *O*, origin; *C*, cytidylic acid; *A*, adenylic acid; *G*, guanylic acid; and *U*, uridylic acid.

This lack of resolution increases very markedly when the sample applied to the thin layer is in the 10^{-1}-μmole range, which is the amount of material necessary for adequate identification by spectroscopy in the ultraviolet region. The isobutyric acid, ammonia (specific gravity 0.90), and water solvent used by Coffey and Newburgh (*2*) to separate the four RNA mononucleotides works well with a 10^{-1}-μmole sample, but 5 hours or more are necessary for development and separation. The propanol, ammonia (specific gravity 0.88), and water solvent used by Dyer (*3*) on DEAE-cellulose (diethylaminoethyl) layers to separate guanylic acid from the remaining three mononucleotides operates at such a high *p*H that the ion-exchange process is not possible. This method produced a great deal of "tailing" when 10^{-1} μmole samples were applied to DEAE-cellulose layers. These difficulties indicated that thin-layer electrophoresis rather than chromatography might be a more suitable method for the separation of the four RNA mononucleotides with samples in the micromole range.

The ribonucleotides tested were obtained from Nutritional Biochemicals Corporation and from an RNA sample, purified by phenol extraction of HeLa cells, which was degraded with 0.3*N* NaOH at 35°C for 18 hours. The hydrolysate was neutralized by shaking with a small quantity of Dowex 50 in the H form, and then dialyzed against distilled water for 1 hour at 25°C.

MN-cellulose powder 300 (*6*) was washed by centrifugation in 1*N* NaOH, water, 0.1*N* HCl, water, ethanol, and water. Forty grams of the washed powder was brought to 300 ml with distilled water and then stored.

Plates were prepared by vigorously shaking the suspended cellulose powder and pouring 100 ml into a spreader (*7*) which was then passed over 20 (200 × 50 mm) plates set in position on the Desaga mounting board.