# Iodination of 2′-Deoxycytidine and Related Substances[1]

PAULINE K. CHANG AND ARNOLD D. WELCH

*Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut*

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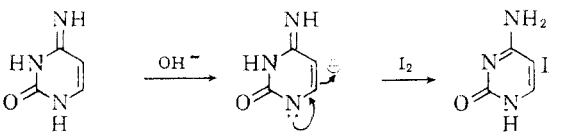
2′-Deoxycytidine has been iodinated in the presence of iodic acid to yield 5-iodo-2′-deoxycytidine and 5,6-dihydro-5,6-diiododeoxyuridine: the latter, on treatment with base, produced 6-iododeoxyuridine, which should be an extremely useful precursor of deoxyuridine-6-H$^3$. The same conditions have been applied to deoxycytidine 5′-phosphate and cytidine to yield 5-iodo-2′-deoxycytidine 5′-phosphate and 5-iodocytidine, respectively. The preparation of 5-iododeoxycytidine-I$^{125}$ and the acetylation of the deoxyribonucleosides are described.

In an earlier brief communication,[2] we have reported the preparation of 5-iododeoxycytidine (II), a compound that exhibits greater cytotoxicity than its bromo analog[3,4] and, in some mammalian systems, compares favorably to 5-iododeoxyuridine[5] as either a chemotherapeutic agent or one to reduce the threshold of cells to radiation injury.[6,7] Biological studies of 5-iododeoxycytidine with cells in culture and in animals,[7,8] as well as in man,[9,10] have demonstrated that II is not readily converted to 5-iododeoxyuridine 5′-phosphate via the intermediation of 5-iododeoxycytidine 5′-phosphate; however, II *per se* is rapidly deaminated by an enzyme variably distributed in tissues (particularly kidney, liver and intestine), with the release of 5-iododeoxyuridine; this, in turn, is converted intracellularly to phosphorylated forms of 5-iododeoxuridine.[11] It is clear, however, that as predicted,[12] II is not cleaved biologically to 5-iodocytosine, in contrast to 5-iododeoxyuridine, from which 5-iodouracil is formed very rapidly, particularly in the liver.[13] Although the biological activity of II is still under investigation, its potential utility in man has been influenced adversely by the finding that some individuals convert II into IUDR too slowly to permit II to be used effectively as a reservoir-form of 5-iododeoxyuridine.[9]

It has been established that 5-iododeoxyuridine can suppress profoundly the development of corneal lesions caused by herpes simplex virus[14–16] when applied topically, and the dermal lesions of vaccinia virus infections, in both rabbit and man, when the drug is administered parenterally.[17] Recent studies with human adenovirus, type 12, highly oncogenic in newborn hamsters, have demonstrated that 5-iododeoxyuridine, injected in the same region as the virus, almost completely prevents the development of neoplasms.[18] The volume of solution (0.1 ml.) that can be given to these newborn hamsters has restricted severely the maximal amount of 5-iododeoxyuridine that can be administered (0.5 mg.); however, the greater solubility of II and its rapid deamination by newborn hamster tissue[9] permit larger doses of II than of 5-iododeoxyuridine and comparative studies of the effects of these agents are now in progress.

Although deoxycytidine (I) is readily chlorinated or brominated,[3,4] it cannot be iodinated by standard procedures. Iodination in dilute nitric acid, under conditions similar to those used in the preparation of 5-iododeoxyuridine,[5] produced only 5-iodocytosine. The standard basic iodination (in the presence of potassium hydroxide and potassium iodide), similar to that used by Johnson and Johns[19] for the iodination of cytosine, led to the production of deaminated materials. It may be suggested that cytosine, in which the $N^1$ position is unsubstituted, is easily iodinated in the basic condition in the following manner




Obviously, this cannot occur in the case of deoxycytidine (I); furthermore, the amino group in I should be more labile because of the decreased aromaticity of the pyrimidine moiety. By carrying out the iodination of deoxycytidine in the presence of iodic acid, under conditions similar to those used by Wirth, *et al.*,[20] for the iodination of aromatic compounds, we obtained 5-iododeoxycytidine (II) in 68% yield, together with a 9–10% yield of 5,6-dihydro-5,6-diiodo-2′-deoxyuridine (IV). It appears that two modes of iodination are taking place simultaneously, *i.e.*, substitution and addition, with the former predominating.

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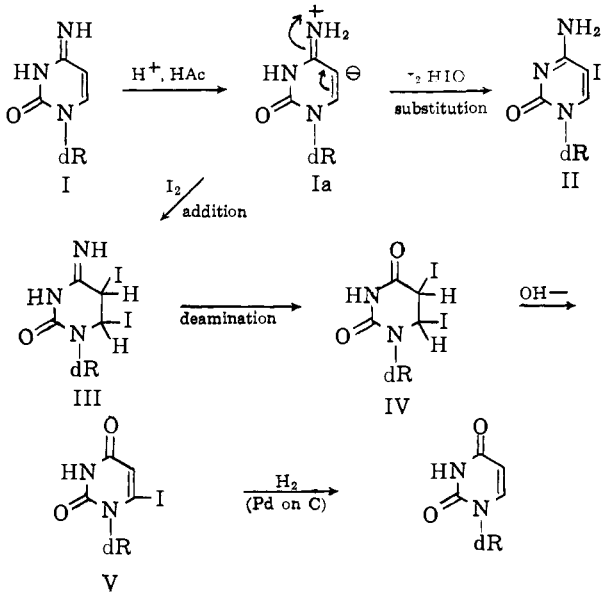
TABLE I

ULTRAVIOLET ABSORPTION SPECTRA[a]

| Pyrimidine | 0.1 *N* HCl | | 0.1 *N* NaOH | | $H_2O$ | | 95% EtOH | |
|---|---|---|---|---|---|---|---|---|
| | $\lambda_{max}$ | ($\epsilon_{max}$) | $\lambda_{max}$ | ($\epsilon_{max}$) | $\lambda_{max}$ | ($\epsilon_{max}$) | $\lambda_{max}$ | ($\epsilon_{max}$) |
| 5-Iodocytosine[b] | 305 | (7000) | 297 | (5770) | 289 | (3980) | 292 | (3160) |
| 5-Iodo-2′-deoxycytidine | 309 | (8150) | 294 | (5930) | 293 | (6020) | 294 | (5750) |
| 5-Iodo-3′,5′-di-*O*-acetyl-2′-deoxycytidine ·HCl | 309 | (9700) | 294 | (9430) | 294 | (8440) | 297 | (6110) |
| 5-Iodo-3′,5′-di-*O*-acetyl-2′-deoxycytidine | 309 | (9510) | 294 | (6790) | 294 | (6530) | 297 | (6010) |
| 5-Iodo-2′-deoxycytidine 5′-phosphate | 309 | (7060) | 293 | (5130) | 291 | (5160) | 297 | (4080) |
| 5-Iodocytidine | 309 | (8280) | 294 | (5960) | 293 | (5660) | 295 | (5500) |
| $N^4$-Acetyl-5-iodo-2′-deoxy-5′-*O*-acetyl-cytidine[c] | 329 | (5560) | 316 | (9960) | 324 | (5950) | 323 | (5720) |
| 5-Iodouracil[b,d] | 284 | (6170) | 296 | (6950) | 284 | (6050) | 283 | (5750) |
| 5-Iodo-2′-deoxyuridine | 289 | (7830) | 280 | (5790) | 289 | (7690) | 287 | (8050) |
| 5-Iodo-3′,5′-di-*O*-acetyl-2′-deoxyuridine | 288 | (7976) | 280 | (5980) | 287 | (7690) | 289 | (6820) |
| 5-Iodouridine | 289 | (8020) | 280 | (6170) | 288 | (7960) | 289 | (6660) |
| 6-Iodo-2′-deoxyuridine[c] | 285 | (9190) | 278 | (7250) | 285 | (9030) | 283 | (7310) |

[a] Spectra were determined with a Model 505 Bausch & Lomb Spectronic recording spectrophotometer. [b] Commercial material. [c] Unstable on standing in solution for several days. [d] $\lambda_{max}^{304}$ in 0.01 *N* NaOH.[5]




In this case, iodic acid is the fortunate choice as the oxidizing agent, since, unlike nitric acid, it does not attack the glycosidic linkage, which apparently is chemically more labile in deoxycytidine than in deoxyuridine. It is of interest to note that the addition of iodine to the polarized double bond must occur before the deamination, since (a) the imino group in III is now more labile than before addition of iodine and (b) deoxyuridine, under the same conditions, produces only the substitution product, with no trace of IV. This circumstance implies that in an acidic medium, the 5,6-double bond in Ia is more reactive than its counterpart in deoxyuridine. It is also observed that deoxycytidine, as the base, gives a higher yield of II and lower yield of IV, but requires a longer reaction time than does its hydrochloride; this finding is in accordance with the vital role of the protonation of the imino group. The structure of IV is established by its lack of absorption in the region of 250–320 mμ and its dehydroiodination by base to form 6-iododeoxyuridine (V), a compound less stable than its 5-iodo isomer.[5] The elimination from IV of the iodine in position-5, rather than that in position-6, is contrary to the known examples of dehydrohalogenation of 5,6-dihydro-5,6-dihalouracils, in which the $N^1$ position is unsubstituted.

Catalytic hydrogenation of V produced deoxyuridine in excellent yield, a procedure that provides a useful route to deoxyuridine-6-$H^3$, from which 5-iodo-deoxyuridine-6-$H^3$ of very high specific activity can be prepared. A slight modification of the iodination procedure afforded 5-iododeoxycytidine-$I^{125}$ from sodium iodide-$I^{125}$ and deoxycytidine.

Deoxycytidine 5′-phosphate and cytidine also can be iodinated in the presence of iodic acid, but with lower yield and longer reaction time. Because of their greater solubility than the deoxyribonucleosides in polar solvents, the isolation of these products is best accomplished by chromatography. 5-Iododeoxycytidine 5′-phosphate and 5-iodocytidine were required in studies of the mechanism of action of the nucleosides of 5-iodopyrimidines and of the enzymatic deamination of a large series of substituted cytosine derivatives.[11]

In order to increase the lipid solubility of these iodinated compounds in biological systems, the preparation of the acetyl derivatives was investigated. The acetylation of 5-iododeoxyuridine proceeds smoothly to give the 3′,5′-di-*O*-acetyl derivative in excellent yield; on the other hand, 5-iododeoxycytidine, depending on the conditions, gives different products. When acetyl chloride is used, the generation of hydrogen chloride apparently prevents the acetylation of the amino group and the 3′,5′-di-*O*-acetyl derivative is first isolated as the salt. However, when the acetylating agent is acetic anhydride, acetylation occurs on the amino group and on only the 5′-position of the sugar. The product, 2 - oxo - 4 - acetamido - 5 - iodo - 1 - (2 - deoxy - 3,5 - di-*O*-acetyl-β-D-ribofuranosyl) pyrimidine,[21] separates out at this stage with no further acetylation of the 3′-hydroxyl, because of its insolubility in the highly polar medium.

## Experimental[22]

**5-Iodo-2′-deoxycytidine (II) and 5,6-Dihydro-5,6-diiodo-2′-deoxyuridine (IV).**—A mixture of deoxycytidine[23] (3.4 g., 0.015

(21) Shortened to $N^4$-acetyl-5-iodo-2′-deoxy-5,-*O*-acetylcytidine in subsequent usage.

(22) Melting points were determined in a capillary tube in a copper block and are corrected. Analyses by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y., and by Midwest Microlab, Inc., Indianapolis, Ind.

(23) Purchased from California Corp. for Biochemical Research, Los Angeles, Cal., and from Nutritional Biochemicals Corp., Cleveland, Ohio; deoxycytidine hydrochloride may be used in place of the free base, but a slightly lower yield of the iodo compound is obtained.[2]

mole), glacial acetic acid (12 ml.), iodic acid (1.35 g.), iodine (2.25 g.), carbon tetrachloride (3 ml.), and water (4.5 ml.) was stirred at 40° for 95 min. To the now clear solution, water (45 ml.) was added to yield 5,6-dihydro-5,6-diiodo-2′-deoxyuridine (IV) (0.66 g., 9.3%). The analytical sample was prepared by dissolving the crude material (IV) in tetrahydrofuran and reprecipitating by addition of water, m.p. 130–134° dec.

*Anal.* Calcd. for $C_9H_{12}I_2N_2O_5$: C, 22.42; H, 2.50; N, 5.81; I, 52.65. Found: C, 22.69; H, 2.37; N, 5.37; I, 52.21.

The filtrate from IV was extracted several times with carbon tetrachloride (600 ml.). The aqueous layer, after concentration to dryness *in vacuo* at 40°, was dissolved in methanol (60 ml.) and again concentrated to dryness *in vacuo* at 40°; this operation was repeated 5 times in order to remove most of the acetic acid. The crystalline residue obtained was taken up in water (45 ml.) and the solution was adjusted to pH 10 with sodium hydroxide (10 *N*); 5-iododeoxycytidine (II) began to precipitate and refrigeration of the mixture produced the crude product (3.6 g., 68%). After one recrystallization from water, II (3.2 g.) melted at 176–182° dec.; the analytical sample was recrystallized once more from water, m.p. 179–182° dec.

*Anal.* Calcd. for $C_9H_{12}IN_3O_4$: C, 30.61; H, 3.43; N, 11.90; I, 35.94. Found: C, 30.68; H, 3.52; N, 11.95; I, 35.82.

Additional amounts of II may be isolated from the mother liquors by use of a charcoal column.[2]

**5-Iodo-2′-deoxycytidine-I$^{125}$.**—To the shipping vial containing approximately 10 mc. of sodium iodide-I$^{125}$ in 0.7 ml. of sodium bicarbonate solution, 15.76 mc./ml.[24] ($\gamma$-emitter: half-life, 60 days) and sodium iodide-I$^{126}$, 0.738 mc./ml. ($\beta$- and $\gamma$-emitter: half-life, 13 days), total solids 0.3 mg./ml., was added deoxycytidine (454 mg., 2 mmoles), iodine (300 mg.), iodic acid (180 mg.), carbon tetrachloride (0.4 ml.), and glacial acetic acid (1.6 ml.) and the mixture was stirred (stoppered) at 45° for 105 min. A small amount of IV was removed by filtration and washed thoroughly with water. The filtrate and washings were collected in a #24/40 125-ml. erlenmeyer flask and shaken several times with carbon tetrachloride, which was removed by means of a pipet each time. The aqueous layer, after concentration to dryness *in vacuo* at 40°, was dissolved in methanol (5 ml.) and again concentrated *in vacuo* at 40° to dryness (4 times). The crystalline residue was taken up in water (5 × 1-ml. portions for complete transference to a 10-ml. beaker), the solution adjusted to pH 10 with sodium hydroxide (12.5 *N*, 2 drops) and refrigerated overnight to yield the crude product (380 mg., 54%); this was recrystallized once more from water to yield 5-iododeoxycytidine-I$^{125}$ (372 mg.; sp. act. 4.24 mc./mmole; containing a small amount of I$^{126}$).

**6-Iodo-2′-deoxyuridine (V).**—A suspension of 5,6-dihydro-5,6-diiododeoxyuridine (IV, 4.8 g., 0.001 mole) in water (50 ml.) was adjusted to pH 11 with sodium hydroxide (12.5 *N*) dropwise until a clear solution resulted. The solution was stirred for 90 min. and the pH now adjusted to 7 with concd. hydrochloric acid. Crude 6-iododeoxyuridine separated as a colorless solid (2.4 g.). The analytical sample was recrystallized carefully from ethanol, m.p. 155–160° dec.; the compound decomposes on standing in aqueous solution for several days.

*Anal.* Calcd. for $C_9H_{11}IN_2O_5$: C, 30.51; H, 3.11; N, 7.91; I, 35.88. Found: C, 31.08; H, 2.73; N, 8.05; I, 35.34.

**Hydrogenation of 6-Iodo-2′-deoxyuridine (V).**—To 360 mg. (0.001 mole) of 6-iododeoxyuridine in 50% ethanol (100 ml.) were added 10% palladium-on-charcoal (0.2 g.), and magnesium oxide (0.3 g.), and the mixture was hydrogenated in a Parr apparatus for 21 hr. at 3 atm. pressure. The catalyst and some magnesium oxide were removed by filtration and the filtrate, shown by ultraviolet absorption to contain essentially deoxyuridine, was first treated with a saturated solution of sodium carbonate to remove the magnesium ion and then with a little Dowex-50 (hydrogen form) to remove the sodium ion. The solution (now pH 6) was concentrated *in vacuo* to give deoxyuridine (206.2 mg., 90%) which behaved chromatographically identically with an authentic sample.

**5-Iodo-3′,5′-di-*O*-acetyl-2′-deoxycytidine.**—To a solution of 5-iododeoxycytidine (5 g., 0.014 mole) in glacial acetic acid (100 ml.) was added acetyl chloride (150 ml.); some solid material appeared, but this redissolved on stirring. The mixture was stirred at room temperature for 17 hr. Some of the crude diacetyl product (2.47 g., hydrochloride salt, m.p. 140–150° dec.) was removed by filtration. The filtrate was concentrated *in vacuo* at 40° to yield more of the hydrochloride (3.75 g., m.p. 130–140° dec., total yield, 94%). The analytical sample was twice recrystallized carefully from ethanol, m.p. 148–150° dec.

*Anal.* Calcd. for $C_{13}H_{17}ClIN_3O_6$: C, 32.96; H, 3.62; N, 8.87; I, 26.79. Found: C, 33.30; H, 3.68; N, 8.57; I, 26.95.

From a solution of the crude hydrochloride (0.5 g., 1.06 mmoles) in water (6.5 ml.), neutralized with sodium hydroxide (12.5 *N*), 5-iodo-3′,5′-di-*O*-acetyl-2′-deoxycytidine separated (0.31 g., 67%); the analytical sample was recrystallized from ethanol, m.p. 164.5–166.5°.

*Anal.* Calcd. for $C_{13}H_{16}IN_3O_6$: C, 35.71; H, 3.69; N, 9.84; I, 29.03. Found: C, 36.06, H, 3.64; N, 9.60; I, 29.15.

**$N^4$-Acetyl-5-iodo-2′-deoxy-5′-*O*-acetyl-cytidine.**[18]—A solution of 5-iododeoxycytidine (2 g., 0.0057 mole) in glacial acetic acid (20 ml.) and acetic anhydride (40 ml.) was stirred at room temperature for 17 hr.; the crude product separated as a colorless solid (2.02 g., 80%). The analytical sample was recrystallized from absolute ethanol, m.p. 152–154°.

*Anal.* Calcd. for $C_{13}H_{16}IN_3O_6$: C, 35.71; H, 3.69; N, 9.84; I, 29.03. Found: C, 35.44; H, 3.97; N, 9.64; I, 29.04.

**5-Iodo-2′-deoxyuridine.**—Deoxyuridine (1.3 g., 0.005 mole) was substituted for deoxycytidine and the procedure for 5-iododeoxycytidine was followed to give 5-iododeoxyuridine (0.6 g., 34%). After one recrystallization from water, m.p. 160° dec., a mixture m.p. with an authentic sample[5] did not show any depression and the ultraviolet absorption data were identical with those obtained using an authentic sample. During this preparation there was no formation of 5,6-dihydro-5,6-diiododeoxyuridine (IV).

**5-Iodo-2′-deoxy-3′,5′-di-*O*-acetyluridine.**—A mixture of 5-iododeoxyuridine (5 g., 0.014 mole), glacial acetic acid (100 ml.), acetic anhydride (150 ml.), and acetyl chloride (150 ml.) was stirred at room temperature for 17 hr. The clear solution was concentrated *in vacuo* at 40° to remove the excess acetyl chloride; the residue was dissolved cautiously in methanol (20 ml.) and the solution again concentrated *in vacuo* at 40°. This operation was repeated several times until a crystalline residue remained. It was recrystallized from ethanol (100 ml.) to yield the pure diacetyl derivative (5.46 g., 89%). The analytical sample was recrystallized once more from ethanol, m.p. 158–160°.

*Anal.* Calcd. for $C_{15}H_{15}IN_2O_7$: C, 35.63; H, 3.45; N, 6.39; I, 28.96; Found: C, 35.79; H, 3.77; N, 6.28; I, 29.10.

**5-Iodo-2′-deoxycytidine 5′-Phosphate.**—A mixture of deoxycytidine 5′-phosphate (307.2 mg., 1 mmole), iodic acid (90 mg.), iodine (150 mg.), water (1.5 ml.), glacial acetic acid (4 ml.), and carbon tetrachloride (1 ml.) was stirred at 45° for 5 hr. The mixture was dissolved in water (70 ml.) and the aqueous solution was extracted several times with carbon tetrachloride. The aqueous phase, after concentration *in vacuo* at 45°, was dissolved in water (10 ml.). The solution was adjusted to pH 11 with sodium hydroxide (12.5 *N*) and put on a column (2.5 × 30 cm.) of Dowex-1 (formate-form). This was eluted with formic acid (0.5 *N*) and the eluate collected in 10-ml. fractions. The hygroscopic, pinkish residue, obtained after removal of the formic acid *in vacuo*, was dissolved in water (7 ml.) and the impurities were removed by filtration; the product was reprecipitated by the addition of absolute ethanol (200 mg., 46%). This operation was repeated to give the analytical sample, m.p. 150–153°.

*Anal.* Calcd. for $C_9H_{13}IN_2O_7P$: C, 24.97; H, 3.03; N, 9.70; P, 7.17. Found: C, 25.02; H, 3.25; N, 9.61; P, 7.16.

**5-Iodocytidine.**—A mixture of cytidine hemisulfate (1.46 g., 0.005 mole), acetic acid (4 ml.), iodic acid (0.45 g.), iodine (0.75 g.), water (1.5 ml.), and carbon tetrachloride (1 ml.) was stirred at 40° for 7 hr. Water (15 ml.) was added to the clear solution, which was extracted several times with carbon tetrachloride. The aqueous phase, after concentration to dryness *in vacuo* at 40°, was dissolved in methanol (10 ml.) and again concentrated to dryness *in vacuo* at 40°; this operation was repeated once more and the colorless residue was dissolved in water (50 ml.). The solution was adjusted to pH 11 with sodium hydroxide (12.5 *N*) and put on a column of charcoal,[25] 2.5 × 10 cm.; this was washed with distilled water until free of halide; the 5-iodocytidine (0.65 g., 36%) was eluted from the column with 95% ethanol. The analytical sample was prepared by dissolving the crude material in a minimum amount of methanol and reprecipitating by addition of ethyl acetate, m.p. 152–155°.

*Anal.* Calcd. for $C_9H_{12}IN_3O_5$: C, 29.29; H, 3.28; N, 11.38. Found: C, 29.83; H, 3.94; N, 11.09.

(24) Purchased from Oak Ridge National Laboratory, Oak Ridge, Tenn.

(25) Grade 20 × 40, Atlas Powder Co., Wilmington, Delaware.