Iodination of 2′-Deoxycytidine and Related Substances. A Reinvestigation of the Structures of the By-product, $C_9H_{10}I_2N_2O_5$, and Its Derivatives[1]

PAULINE K. CHANG

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

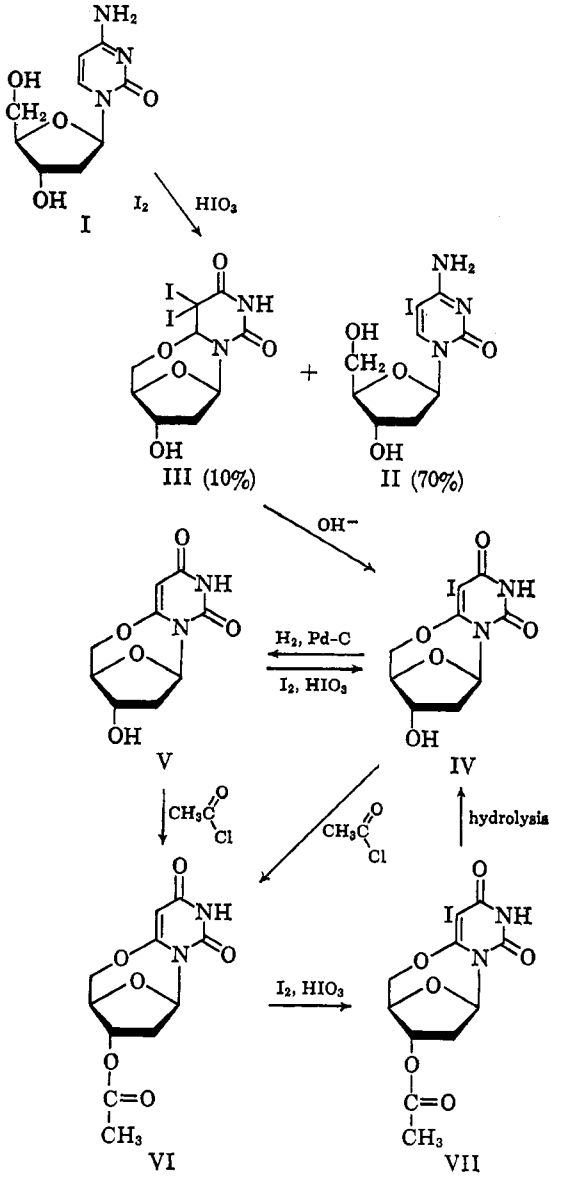
Received May 28, 1965

The by-product, $C_9H_{10}I_2N_2O_5$, isolated following the iodination of deoxycytidine to 5-iododeoxycytidine, was found to be 5,5-diiodo-6-hydro-O⁶,5′,6-hydroxycyclodeoxyuridine (III). Treatment of III with base yielded 5-iodo-O⁶,5′-6-hydroxycyclodeoxyuridine (IV). The structures of III and IV were assigned on the basis of their chemical reactions as well as their n.m.r. and infrared spectra.

In an earlier communication,[2] it was shown that 2′-deoxycytidine (I), when iodinated in the presence of iodic acid, yielded 5-iododeoxycytidine (II) and a minor by-product (III). The latter was initially assigned the structure 5,6-dihydro-5,6-diiododeoxy uridine, $C_9H_{12}I_2N_2O_5$, on the basis of its elementary analysis, lack of ultraviolet absorption, and dehydroiodination to an iododeoxyuridine-like material (IV). It was concluded that the data were compatible with the structure for IV of 6-iododeoxyuridine; thus, its elementary analysis, nonidentity with 5-iododeoxyuridine, ultraviolet spectra, and its deiodination to a uracil derivative (V), having identical chromatographic characteristics in several systems with those of an authentic sample of 2′-deoxyuridine, were in agreement with this view. In the course of attempting to apply the above findings to the production of deoxyuridine-6-H³ from IV, however, we found that the reduced product of IV, namely V, is not deoxyuridine but possesses two hydrogen atoms less. Accordingly, a reinvestigation of the structures of compounds III, IV, and V was undertaken.

Elementary analyses were repeated for several different samples of IV. The data indicate that IV also possesses two hydrogen atoms less than that of a normal iododeoxyuridine. In the case of compound III, the analytical data agree with the theoretical values for $C_9H_{10}I_2N_2O_6$, an empirical formula having two hydrogen atoms less than that of 5,6-dihydro-5,6-diiododeoxyuridine. Thus, it appears that compounds III, IV, and V may be of a new type of deoxyribonucleoside with two hydrogen atoms less than normal deoxyribonucleosides. This is reminiscent of the O⁶,5′-6-hydroxycyclouridine[3] discovered recently by Lipkin among the alkaline hydrolysis products of 5-iodouridine.

Acetylation of IV with acetyl chloride (acetic anhydride failed to react) gave a monoacetylated, although deiodinated, product (VI) that was identical with the acetylation product of V. This indicates the presence of only one hydroxyl group in the glycosidic portion of IV, as well as of V. On the other hand, V failed to react with trityl chloride; this indicates that a hydroxyl group is not present in the 5′- position, and compound VI could be only the 3′-acetate of V. Both V and VI can be reiodinated to give IV and the 3′-acetate of IV, respectively, although the latter (VII) is easily hydrolyzed to IV during purification.



The ultraviolet spectra of these compounds (IV–VII) are reasonably similar to those of the normal deoxyribonucleosides, a finding indicating that no drastic changes in the pyrimidine ring have occurred. The n.m.r. spectra of IV, V, and VI, however, all show the absence of the 6-H peak of the pyrimidine ring in the 7.5–8.5-p.p.m. region, whereas the 6-H peak in 5-

(1) This work was supported by a grant (CA-02817) from the National Cancer Institute, U. S. Public Health Service.

(2) P. K. Chang and A. D. Welch, *J. Med. Chem.*, **6**, 428 (1963).

(3) D. Lipkin, F. B. Howard, D. Noworthy, and M. Sano, 6th International Congress of Biochemistry, New York, N. Y., 1964; Abstracts I, 70, Paper No. I-117.

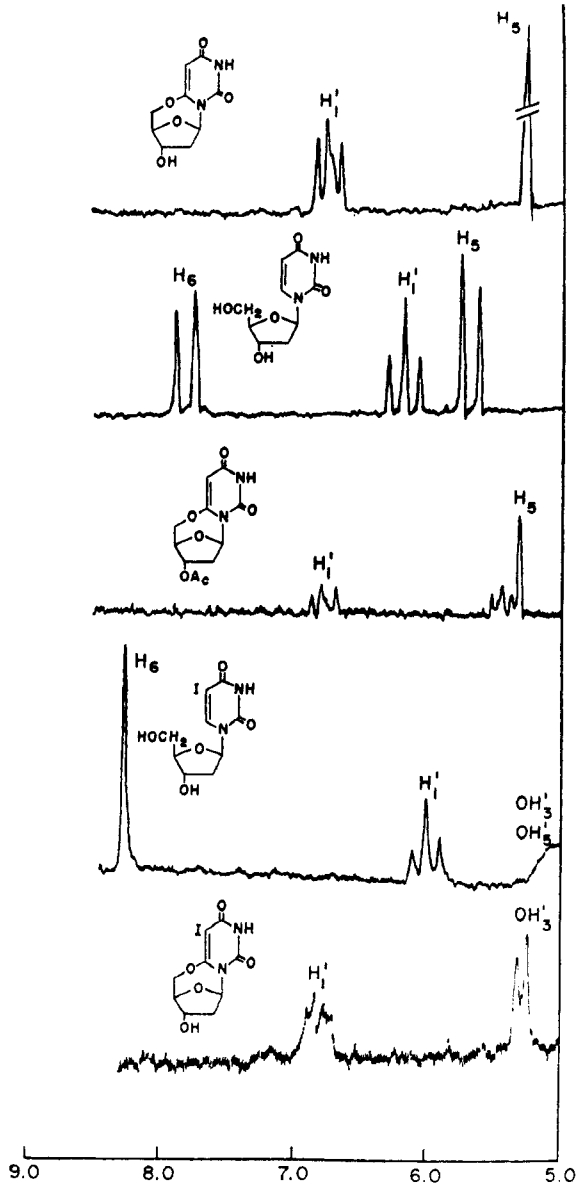


Figure 1.—The 60-Mc. n.m.r. spectra of deoxyribosides and cyclodeoxyribosides in $(CD_3)_2SO$; the field increases from left to right. Primed protons refer to those of the deoxyribose ring, and unprimed ones to those of the pyrimidine ring. The spectra are by D. Vea of Varian Associates, Palo Alto, Calif., and M. Vogel of Rutgers University, New Brunswick, N. J.

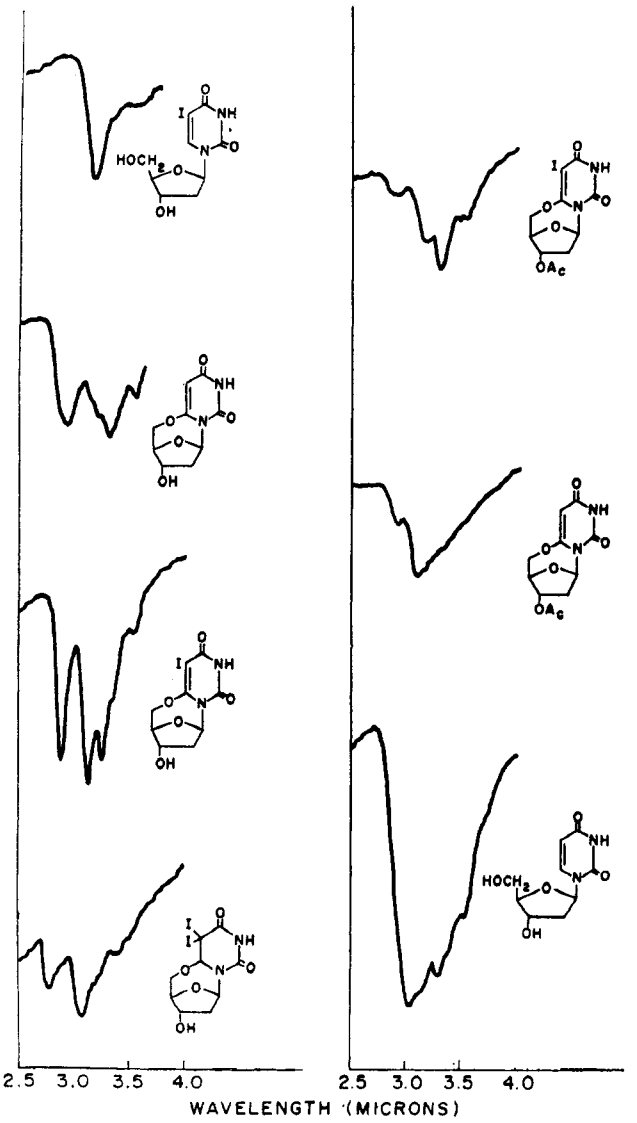


Figure 2.—The infrared spectra of deoxyribosides and cyclodeoxyribosides, measured in pressed potassium bromide disks on a Perkin-Elmer double-beam instrument, Model 21.

iododeoxyuridine or deoxyuridine[4] was measured as a singlet at 8.3 p.p.m. or a doublet at 7.55 and 7.88 p.p.m., respectively. The 5-H peak, absent in IV or 5-iododeoxyuridine, was measured as a singlet in V (5.38 p.p.m.) and VI (5.32 p.p.m.) and as a doublet in deoxyuridine (5.62 and 5.75 p.p.m.) (Figure 1). A comparison of the infrared spectra of these compounds has shown that III, IV, and V possess two major peaks in the 2.75–3.25-μ region, characteristic of a secondary hydroxyl group and a N–H group, whereas 5-iododeoxyuridine and deoxyuridine both show only one broad peak due to the primary hydroxyl, secondary hydroxyl, and the N–H groups in the 2.75–3.5-μ region. On the other hand, VI and VII, the acetylated derivatives, show only the N–H peak in the 3.07–3.3-μ region (Figure 2). It is concluded, therefore, that compounds IV–VII, lacking 6-H and 5′-OH, are O^6,5′-6-hydroxycyclodeoxynucleosides. A comparison of V and the alkaline hydrolysis product[5] of 5-iododeoxyuridine, prepared in a manner analogous to the preparation of O^6,5′-6-hydroxycyclouridine,[3] showed identical chromatographic characteristics and very similar infrared spectra.

We wish to note that the by-product III is more likely to be 5,5-diiodo-6-hydro-O^6,5′-6-hydroxycyclodeoxyuridine, rather than the formerly assigned 5,6-diiodo-5,6-dihydrodeoxyuridine. This conclusion is in agreement with the findings of Wang,[6] who reported the formation of 5,5-dibromo-6-hydroxylhydrouracil during the bromination of uracil in an aqueous medium.

Experimental Section[7]

5-Iodo-O^6,5′-6-hydroxycyclodeoxyuridine (IV).—A suspension of III (4.8 g., 0.01 mole) in water (50 ml.) was adjusted to pH 11

(4) C. D. Jardetzky, *J. Am. Chem. Soc.*, **83**, 2919 (1961).

(5) D. Lipkin, private communication. The author thanks Professor Lipkin for a sample of the alkaline hydrolysis product of 5-iododeoxyuridine.

(6) S. Y. Wang, *J. Org. Chem.*, **24**, 11 (1959).

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

with sodium hydroxide (12.5 *N*) dropwise until a clear solution resulted. The solution was stirred for 90 min. and the pH was then adjusted to 7 with concentrated hydrochloric acid. Crude IV separated as a colorless solid (2.4 g.). The analytical sample was recrystallized from methanol: m.p. 160° dec., $\lambda_{max}^{pH_2}$ 285 mμ (*E* 9.2 × 10³), $\lambda_{max}^{pH_{11}}$ 278 mμ (*E* 7.3 × 10³), $\lambda_{max}^{H_2O}$ 285 mμ (*E* 9.0 × 10³), $\lambda_{max}^{90\%\ EtOH}$ 283 mμ (*E* 7.3 × 10³).

Anal. Calcd. for $C_9H_9IN_2O_5$: C, 30.70; H, 2.58; I, 36.04; N, 7.96. Found: C, 30.81; H, 2.59; I, 35.76; N, 8.13.

O⁶,5′-6-Hydroxycyclodeoxyuridine (V).—To 1.8 g. (5 mmoles) of IV in 3% methanolic potassium hydroxide solution (100 ml.) was added 10% palladium on charcoal (3.6 g.), and the mixture was hydrogenated in a Parr apparatus for 1 hr. at 1.5 atm. pressure. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was dissolved in water (200 ml.) and put on a column (2.5 × 30 cm.) of Dowex-1 (formate form). This was eluted with formic acid (0.025 *N*) and the eluate was collected in 15-ml. fractions. The product (V) (1.1 g., 95%), obtained after the removal of the formic acid *in vacuo*, was recrystallized twice from methanol to give the analytical sample: m.p. 200° dec., R_f (relative to deoxyuridine) 0.98 (70% isopropyl alcohol–ammonia) and 1.85 [ethyl acetate–water–formic acid (60:35:5)], $\lambda_{max}^{pH_2}$ 262 mμ (*E* 11.9 × 10³), $\lambda_{max}^{pH_{11}}$ 263 mμ (*E* 9.7 × 10³), $\lambda_{max}^{H_2O}$ 262 mμ (*E* 12.2 × 10³); $\lambda_{max}^{90\%\ EtOH}$ 261 mμ (*E* 11.8 × 10³).

Anal. Calcd. for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.46; N, 12.39. Found: C, 47.95; H, 4.34; N, 12.37.

3′-O-Acetyl-O⁶,5′-6-hydroxycyclodeoxyuridine (VI).—(This compound may be prepared from either IV or V by an essentially identical procedure; however, the product from the latter is more easily purified.) A mixture of V (678 mg., 3 mmoles), glacial acetic acid (15 ml.), acetyl chloride (20 ml.), and acetic anhydride (20 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*. This operation was repeated several times until a crystalline residue remained. It was recrystallized from ethanol to yield the pure acetyl derivative (624 mg., 77%). The analytical sample was recrystallized once more from ethanol: m.p. 246° dec., $\lambda_{max}^{pH_2}$ 262 mμ (*E* 13.2 × 10³), $\lambda_{max}^{pH_{11}}$ 262.5 mμ (*E* 9.6 × 10³), $\lambda_{max}^{H_2O}$ 262 mμ (*E* 13.2 × 10³), $\lambda_{max}^{90\%\ EtOH}$ 260 mμ (*E* 13.1 × 10³).

Anal. Calcd. for $C_{11}H_{12}N_2O_6$: C, 49.25; H, 4.51; N, 10.44. Found: C, 49.47; H, 4.75; N, 10.66.

5-Iodo-3′-O-acetyl-O⁶,5′-6-hydroxycyclodeoxyuridine (VII).—A mixture of VI (536 mg., 2 mmoles), glacial acetic acid (8 ml.), iodic acid (180 mg.), iodine (300 mg.), carbon tetrachloride (2 ml.), and water (3 ml.) was stirred for 3 hr. Water (25 ml.) was added to the mixture. The aqueous layer, after one extraction with carbon tetrachloride, was concentrated *in vacuo* at 40°. The residue was dissolved in methanol (20 ml.) and the solution was again concentrated *in vacuo* at 40°; this operation was repeated several times to remove most of the acetic acid. The yellow crystalline residue (480 mg.) was recrystallized from hot water to yield the pure product (111 mg., 14%). The mother liquor, when concentrated to dryness *in vacuo*, gave IV (180 mg., 26%). The 3′-O-acetyl derivative was recrystallized once more from hot water to give the analytical sample: m.p. 142° dec., $\lambda_{max}^{pH_2}$ 282 mμ (*E* 8.9 × 10³), $\lambda_{max}^{pH_{11}}$ 275 mμ (*E* 6.9 × 10³), $\lambda_{max}^{H_2O}$ 280 mμ (*E* 9.0 × 10³), $\lambda_{max}^{90\%\ EtOH}$ 277 mμ (*E* 9.6 × 10³).

Anal. Calcd. for $C_{11}H_{11}IN_2O_6$: C, 33.52; H, 2.79; I, 32.20; N, 7.11. Found: C, 33.36; H, 3.08; I, 32.41; N, 6.87.

Iodination of O⁶,5′-6-hydroxycyclodeoxyuridine (V).—A mixture of V (349 mg., 1.5 mmoles), iodine (225 mg.), iodic acid (135 mg.), glacial acetic acid (6 ml.), carbon tetrachloride (1.5 ml.), and water (2.5 ml.) was stirred at room temperature for 5 hr. IV (85 mg., 16%) precipitated from the solution. Its infrared spectrum was identical with that of an authentic sample.

Tritylation of O⁶,5′-6-Hydroxycyclodeoxyuridine (V).—A solution of V (226 mg., 1 mmole) and trityl chloride (433 mg., 1.5 mmoles) in dry pyridine (10 ml.) was allowed to stand in a stoppered flask at room temperature for 7 days. Ice–water (25 ml.) was poured into the reaction mixture with stirring. Trityl alcohol (380 mg., 94%) was removed by filtration and the filtrate was concentrated *in vacuo* at 45°. The residue was dissolved in water (20 ml.) and the solution was adjusted to pH 11 with sodium hydroxide. It was put on a column of Dowex-1 (formate form) and eluted with formic acid (0.025 *N*). The eluate was concentrated *in vacuo* to give unreacted starting material (196 mg., 87%).

Acknowledgment.—The author is greatly indebted to Professor A. D. Welch of this department, Professor D. Lipkin of Washington University, St. Louis, Missouri, and Professor M. Saunders of Yale University for valuable discussions.

C-23 Acylation of Pseudodiosgenin Diacetate

FREDERICK C. UHLE

Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

Received May 18, 1965

Prolonged treatment of diosgenin acetate with acetic anhydride in the presence of pyridine hydrochloride affords an enol acetate of 23-acetylpseudodiosgenin diacetate. Gentle hydrolysis of the ester with potassium bicarbonate during brief reaction periods gives 23-acetylpseudodiosgenin. Extended exposure under the same conditions results in migration of the olefinic linkage to the exocyclic position to supply the Δ^{22}-furostene isomer. More vigorous alkaline treatment promotes conjugate addition of the terminal hydroxyl group to the α,β-unsaturated carbonyl system, furnishing 23-acetyldiosgenin.

Discovery by Marker and Rohrmann[1] of the acetolysis of spiroketal sapogenins (1) affording open-chain dihydrofuranoid pseudo derivatives (2) immeasurably enriched steroid chemistry.[2] Evidence[3] from oxidative cleavage affirms generally excellent conversion with the original procedure employing acetic anhydride at 200°. Convenience of isolation and yields of crystalline products realized in practice vary, however, among members of the natural group. Much effort expended in study of the fundamental process has culminated in the recommendation of numerous expedients for dispensing with closed-system conditions. Thus, *n*-butyric anhydride[4] and *n*-octanoic anhydride,[5] as well as *n*-octanoic acid,[5] at reflux temperature have proved applicable. In still another refinement, addition of acid salts such as pyridine hydrochloride[6] or ammonium chloride[3] has permitted the reaction to proceed in refluxing acetic anhydride.

(1) R. E. Marker and E. Rohrmann, *J. Am. Chem. Soc.*, **61**, 3592 (1939).

(2) L. F. Fieser and M. Fieser, "Steroids," Rheinhold Publishing Corp., New York, N. Y., 1959, p. 547.

(3) M. E. Wall, H. E. Kenney, and E. S. Rothman, *J. Am. Chem. Soc.* **77**, 5665 (1955).

(4) R. E. Marker and E. Rohrmann, *ibid.*, **62**, 518 (1940); G. P. Mueller, R. E. Stobaugh, and R. S. Winniford, *ibid.*, **75**, 4888 (1954); F. C. Uhle, *ibid.*, **76**, 4245 (1954), **83**, 1469 (1961).

(5) A. B. F. Cameron, R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones, and A. G. Long, *J. Chem. Soc.*, 2807 (1955).

(6) W. G. Dauben and G. J. Fonken, *J. Am. Chem. Soc.*, **76**, 4618 (1954).