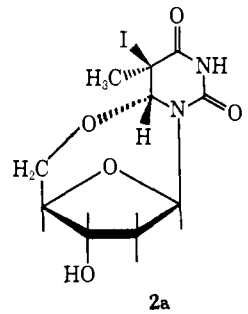
The Iodination of Thymidine. A Synthesis of O^6,5′-Cyclothymidine[1]

Sir:

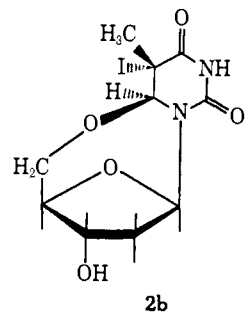
Recent work has led to the synthesis of a number of O^6,5′-cyclonucleosides. The syntheses were achieved by treatment of 5-halogenonucleosides with base[2-4] or by a more complex procedure.[5]

Two general types of mechanisms have been suggested for the conversion of 5-halogenonucleosides to O^6,5′-cyclonucleosides in basic media. One of these hypothesizes the formation of a pyrimidyne intermediate.[2,6,7] The other type of mechanism[2,4] is based upon the concept of the intramolecular addition of the 5′-hydroxyl group to the 5,6-double bond of the pyrimidine ring to give a 5,6-saturated cyclonucleoside intermediate. Presumably this is converted to O^6,5′-cyclonucleoside by the loss of hydrogen halide. Although indirect evidence for this intermediate has been obtained,[4] attempts at direct detection of it in reaction mixtures thus far have been unsuccessful.[8] On the other hand, Chang[5] and Honjo, *et al.*,[9] each have isolated a diiodo-5,6-dihydropyrimidine cyclonucleoside which then was converted to a 5-iodocyclonucleoside by treatment with base.

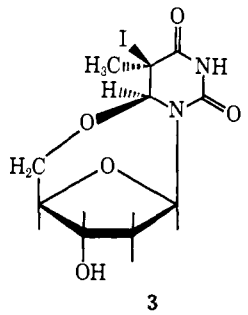
Thymidine (**1**) was treated with *N*-iodosuccinimide (NIS) in anhydrous dimethyl sulfoxide (DMSO) containing trifluoroacetic acid (TFA).[10] After *ca.* 15 hr at room temperature in the dark, **1** was no longer present as shown by the absence of its H-6 absorption in the pmr spectrum (δ 7.67) and the disappearance of its characteristic absorption in the region 250–290 nm. In its place, a quantitative yield of two products appeared (**2a** and **2b**) which have pmr absorptions due to their H-6 protons at δ 4.80 and 5.28 (*vide infra*). The participation of the 5′-hydroxyl group in the saturation of the double bond to form **2a** and **2b** is indicated by the fact that 3′,5′-di-*O*-acetylthymidine[11] did not react under identical conditions and by the decrease by one proton of the combined pmr absorption of TFA plus hydroxyl groups after the reaction was complete. The product distribution (pmr spectrum) was 25% of **2a** and 75% of **2b**: pmr[12] **2a** (DMSO-d_6) δ 2.04 (s, 3, 5-methyl), 4.80 (s, 1, H-6), 6.41 (m, 1, H-1′), 10.73 (broad s, 1, N*H*); **2b** δ 1.98 (s, 3, 5-methyl), 5.28 (s, 1, H-6), 5.90 (m, 1, H-1′), 10.61 (broad s, 1, N*H*). These values are consistent with the assigned structures and, furthermore, they are in good agreement with the corresponding values obtained for 5-iodo-6-methoxy-5,6-dihydrothymine,[13] 5-iodo-6-methoxy-5,6-dihydrothymidine,[13] and other related compounds.[14] Since **2a** and **2b** are converted quantitatively to **1** by means of base, it is reasonable to assume that they are formed by the trans addition of iodonium cation at C-5 and 5′-OH at C-6.



2a



2b



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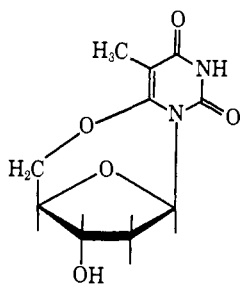

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Attempts to isolate **2a** or **2b** failed. When the mixture containing these was illuminated for a few minutes at 24° with light from an iodine quartz lamp filtered through a solution of copper sulfate, and the pmr spectrum of the resulting mixture was taken, it was observed that **2b** had isomerized to **3** (>95%), while the amount of **2a** remained constant. Further illumination did not change the composition of this mixture. Concentration of the reaction mixture *in vacuo*, followed by addition of water, brought about precipitation of **3**. Recrystallization from water gave pure **3** (84% based on **2b**): mp 151–152° dec; pmr (DMSO-d_6) δ 1.94 (s, 3, 5-methyl), 3.79 (s, 1, H-6), 6.54 (m, 1, H-1′), 10.55 (broad s, 1, N*H*). *Anal.* Calcd for $C_{10}H_{13}IN_2O_5$: C, 32.62; H, 3.56; I, 34.47; N, 7.60. Found: C, 32.66; H, 3.57; I, 34.43; N, 7.55. It gives a positive cysteine test for 2′-deoxyribonucleosides,[15] with 60% hydrofluoric acid it yields thymine (98%),[16] the uv spectrum in neutral or acid solution shows only end absorption, the pmr spectrum in DMSO-d_6 containing TFA shows only one exchangeable hydroxyl proton,[10] hydrogenation (palladium on charcoal) gives only **1**,[17]

(1) This investigation was supported by Grant No. CA-03870 from the National Cancer Institute and by Grant No. GB-2980 from the National Science Foundation.

(2) D. Lipkin, F. Howard, D. Nowotny, and M. Sano, Abstracts, Sixth International Congress on Biochemistry, New York, N. Y., 1964, paper no. I-117; D. Lipkin, C. T. Cori, and M. Sano, *Tetrahedron Lett.*, 5993 (1968).

(3) B. A. Otter, E. A. Falco, and J. J. Fox, *ibid.*, 2967 (1968).

(4) B. A. Otter, E. A. Falco, and J. J. Fox, *J. Org. Chem.*, 34, 1390 (1969).

(5) P. K. Chang, *ibid.*, **30**, 3913 (1965).

(6) G. M. Blackburn, *Annu. Rep. Progr. Chem.*, **65**, 535 (1968).

(7) T. Kauffmann and R. Wirthwein, *Angew. Chem.*, **83**, 21 (1971).

(8) C. T. Cori, Ph.D. Thesis, Washington University, St. Louis, Mo., 1970.

(9) M. Honjo, Y. Furukawa, M. Nishikawa, K. Kamiya, and Y. Yoshioka, *Chem. Pharm. Bull. (Tokyo)*, **15**, 1076 (1967).

(10) When TFA was omitted, the half-time for the reaction was *ca.* 4 days. The presence of TFA in the reaction mixture is advantageous in other respects too. The hydroxyl protons of the sugar moiety of the nucleoside exchange rapidly with that of the TFA to give a single absorption at low field (*ca.* 8–10 ppm) [D. Gagnaire and D. Robert, *Bull. Soc. Chem. Fr.*, 781 (1968)]. This makes it possible to study the high-field region of the pmr spectrum without the usual interference produced by the broad absorption of the hydroxyl protons and it provides one more measure of the extent of the reaction. The proton on N-3 does not exchange.

(11) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 816 (1955).

(12) 60 MHz. Only pertinent absorptions are listed.

(13) These were made by the reaction of thymine or thymidine with NIS in a mixture of methanol and DMSO.

(14) P. Rouillier, J. Delmau, and C. Nofre, *Bull. Soc. Chem. Fr.*, 3515 (1966).

(15) J. G. Buchanan, *Nature (London)*, **168**, 1091 (1951).

(16) D. Lipkin, B. E. Phillips, and J. W. Abrell, *J. Org. Chem.*, **34**, 1539 (1969).

(17) 5-Bromo-6-methoxy-5,6-dihydrothymidine has been converted

and it can be converted to O^6,5′-cyclothymidine (**4**). Based on these data and on an X-ray diffraction study of crystals of **3**,[18] the stereochemistry assigned to **3** is 5(*R*)-iodo-O^6,5′-cyclo-5,6(*S*)-dihydrothymidine. Furthermore, making the reasonable assumption that the epimerization of **2b** takes place at C-5, it then follows that the stereochemical representations of **2a** and **2b** are those shown above.

Treatment of **3** with base or silver nitrate produced **4**. Recrystallization from water afforded a good yield (>90%) of **4**: mp 219–220°; uv max 0.1 *N* HCl 269.5 nm (ε 12,550), H_2O 269.5 nm (ε 12,500), 0.1 *N* NaOH 268.5 nm (ε 9600); uv min 0.1 *N* HCl 236 nm (ε 2880), H_2O 236 nm (ε 2880), 0.1 *N* NaOH 244 nm (ε 4000); pK_a = 9.68 ± 0.05; pmr (DMSO-d_6) δ 1.72 (s, 3, 5-methyl), 3.88 and 4.63 (pair d, 2, $J_{5'a,5'b}$ = 12.5 Hz, H-5′), 6.74 (m, 1, H-1′), 11.17 (broad s, 1, N*H*); mass spectrum (70 eV) *m/e* (rel intensity) 240 (47), 222 (2), 212 (3), 194 (6), 179 (3), 168 (46), 142 (8), 140 (17), 124 (75), 98 (7), 97 (19), 96 (10), 83 (56), 81 (100), 69 (30). *Anal.* Calcd for $C_{10}H_{12}N_2O_5$: C, 50.00; H, 5.00; N, 11.62. Found: C, 50.20; H, 5.04; N, 11.55. Mild acid hydrolysis of **4** gave 6-hydroxythymidine and 5-methylbarbituric acid, its mass spectrum exhibits a fragmentation pattern characteristic of O^6,5′-cyclonucleosides,[19] and the pmr spectrum is in agreement with the assigned structure, including the fact that the H-5′ pattern is characteristic of cyclonucleosides which have an oxygen cyclonucleoside bond to C-5′,[3,20]

The conversion of **2b** through **3** to **4** is consistent with the premise that the base-catalyzed conversion of 5-halogenonucleosides to O^6,5′-cyclonucleosides involves an *addition–elimination* mechanism. It should be emphasized, however, that the 5-methyl group in **2b** and **3** precludes the possibility of a keto–enol equilibrium, in which C-5 participates, intervening in their transformation to **4**. This is in direct contrast to a conversion such as 5-iodouridine to O^6,5′-cyclouridine by means of base. In this latter case, such a keto–enol equilibrium involving a proton on C-5, rather than a methyl group, is possible in the corresponding saturated 5,6 adduct. The stereochemically most stable intermediate will be formed, therefore, regardless of the initial mode of addition in the formation of the 5,6 adduct.

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to thymidine by catalytic hydrogenation [R. Duschinsky, *et al.*, *J. Med. Chem.*, **10**, 47 (1967)].

(18) X-Ray examination of the crystals showed that they were orthorhombic, belonging to the unique space group $P2_12_12_1$. Their density (2.026 g/ml at 25°), as determined by flotation, and their unit cell dimensions corresponded to four molecules per unit cell, with cell parameters a = 14.28, b = 12.14, and c = 6.87 Å.

The intensities of 1600 unique reflections (corresponding to the copper sphere) were measured on a Picker diffractometer with a molybdenum target and a graphite monochromator. A Patterson synthesis showed the position of the iodine and a Fourier synthesis based on the iodine indicated 18 peaks, of which the 17 highest were shown by least-squares refinement to be nonhydrogen atoms. The structure refined to an *R* factor of 0.11 when isotropic temperature factors were used and it refined to 0.075 anisotropically. A complete description of the X-ray determination of the structure of **3** will be presented in a forthcoming publication by Demetrius Tsernoglou and Jaime A. Rabi.

(19) The mass spectra of three cyclonucleosides (O^6,5′-cyclouridine, O^6,5′-cyclodeoxyuridine, and O^6,5′-cyclothymidine) show a number of characteristic features when compared with the corresponding parent nucleosides. Some of these characteristics are: (1) they have large parent peaks; (2) sugar residue (S) peaks are absent; (3) B + 2H peaks are very small or absent; and (4) an important peak, at *m/e* 154 for the two uracil derivatives and at *m/e* 168 for the thymine derivative, which is due to a fragment containing portions of both the base and sugar. The mass spectroscopy of nucleosides, cyclonucleosides, and derivatives of these will be treated in detail in a forthcoming publication (E. G. Lovett and D. Lipkin).

(20) I. Doerr and J. J. Fox, *J. Amer. Chem. Soc.*, **89**, 1760 (1967).

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Additions and Corrections

Photochromism of [2.2]Metacyclophan-1-enes and the Thermal Isomerization of 4,5,15,16-Tetrahydropyrenes [*J. Amer. Chem. Soc.*, **92**, 3681 (1970)]. By CHESTER E. RAMEY and V. BOEKELHEIDE, Department of Chemistry, University of Oregon, Eugene, Oregon.

At the beginning of the paragraph at the top of the second column on page 3682, the line should read: Because of the ease with which the photoisomers (**6**, **7**, and **8**) revert back....

Additionally, in the Experimental Section, the opening sentence should read: Two solutions each were prepared by dissolving **3** (5.8 and 5.9 mg, respectively), **4** (5.8 and 4.6 mg, respectively), and **5** (5.0 and 5.4 mg, respectively) in 5.5 ml of cyclohexane in each case.

Nuclear Magnetic Resonance Evidence for *cis*-Peptide Bonds in Proline Oligomers [*J. Amer. Chem. Soc.*, **92**, 6191 (1970)]. By C. M. DEBER, F. A. BOVEY, J. P. CARVER, and E. R. BLOUT, Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts 02115, and Bell Telephone Laboratories, Inc., Murray Hill, New Jersey 07974.

On page 6192, column two, the fourth sentence of the first paragraph should read: By correlating the nmr spectra with optical rotation changes, it is apparent that the lower field peak (in $CDCl_3$) at τ 5.25 corresponds to the *trans* form.

Application of Solvent Effects to the Study of Diamagnetic and Paramagnetic Ring Currents [*J. Amer. Chem. Soc.*, **93**, 556 (1971)]. By F. A. L. ANET and G. E. SCHENCK, Department of Chemistry, University of California, Los Angeles, California 90024.