Selective Photoreduction of Nucleotides and Nucleic Acids. II. Mechanism of the Two-Step Reduction of Thymidine

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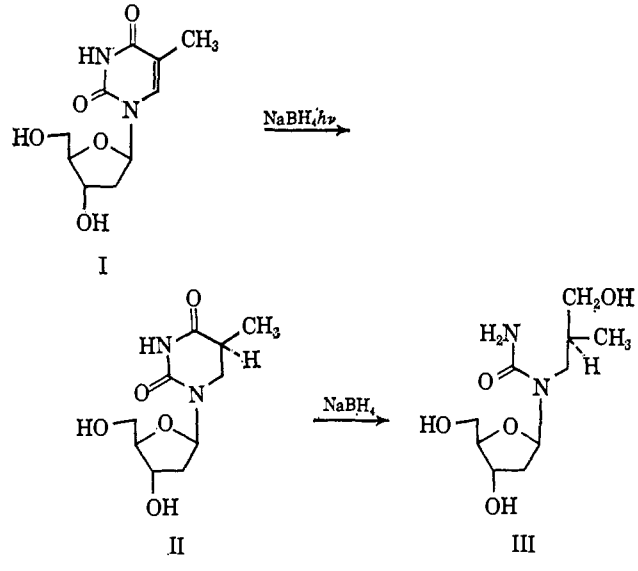
Abstract: Thymidine in its photoexcited state is reduced by sodium borohydride. The two-step reaction involves first photoreduction of the 5,6 double bond and, in a subsequent light-independent step, reductive cleavage between positions 3 and 4 of the dihydrothymine ring. The second reductive step is generally observed with dihydropyrimidine nucleotides. The product of dihydrothymine on reductive ring opening is 3-ureido-2-methylpropanol-1. The structure of this product rests on spectral and chemical evidence and on the synthesis by an independent route. The mechanism of the primary photoreduction was studied by the use of differently labeled reducing systems ($NaBD_4$–H_2O, $NaBH_4$–D_2O, $NaBD_4$–D_2O) combined with nmr spectroscopy. The hydrogen donated by $NaBH_4$ enters the molecule at C-5, whereas the hydrogen added to C-6 originates from the solvent.

Uridine and uridylic acid in their photoexcited state are rapidly reduced by sodium borohydride.[3] In a mixture of the major ribonucleotides uridylic acid was found to be reduced selectively. The reduction is easily followed: (i) by the disappearance of the absorption at 262 mμ, (ii) by the amount of ribose liberated under the conditions of the orcinol assay, and (iii) by the amount of ureido functions generated upon acidic cleavage of the N-glycosidic bond and alkaline opening of the dihydrouracil ring.[4]

This selective photoreduction has permitted a study of the binding characteristics of partially reduced polyuridylic acid[5] and of the template activity of uridylic acid–dihydrouridylic acid copolymers.[6] So far there is no indication that this kind of photoreduction leads to cleavage of polynucleotides as does the selective photooxidative cleavage of pseudouridylic acid residues.[7]

The photoreduction of thymidine (I) with $NaBH_4$ is slower than that of uridine (Figure 1). The expected dihydrothymidine (II)[8,8a] is not found as an end product, but seems to be formed as an intermediate. The dihydrothymidine portion is reductively cleaved by $NaBH_4$ in a second, light-independent step to the two epimers of 3-ureido-2-methylpropanol-1 (III). This secondary reaction was investigated in detail with dihydrothymine as starting material.



I II III

Dihydrothymine (IV) is reduced with sodium borohydride in aqueous solution in the absence of ultraviolet light to a colorless glass (V), which gives a yellow color on chromatograms sprayed with *p*-dimethylaminobenzaldehyde–HCl and a positive Archibald test,[9] indicative of the presence of a ureido group. Spectroscopically this compound is characterized by a carbonyl absorption at 1660 cm^{-1} (acetonitrile) in the infrared, and lack of significant ultraviolet absorption. The nmr spectrum shows signals attributed to a secondary methyl group and two methylene groups attached to nitrogen and oxygen, respectively. The methylene signal at lower field disappears when the reduction is carried out with sodium borodeuteride to give VI. No loss of a C_1 fragment was observed during the reaction, nor was such a fragment formed upon acid or base treatment, which left the compound largely unchanged. Elemental analysis and mass spectrum (Figure 2A) led to the formula $C_5H_{12}N_2O_2$. Unchanged starting material was recovered after treatment with dicyclohexylcarbodiimide in refluxing pyridine as well as on attempted reduction with lithium aluminum hydride in tetrahydrofuran.

(1) Fellow in the visiting program of the U. S. Public Health Service, 1965–1966.

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(3) P. Cerutti, K. Ikeda, and B. Witkop, *J. Am. Chem. Soc.*, **87**, 2505 (1965).

(4) R. D. Batt, J. K. Martin, J. M. Ploeser, and J. Murray, *ibid.*, **76**, 3663 (1954).

(5) P. Cerutti, H. T. Miles, and J. Frazier, *Biochem. Biophys. Res. Commun.*, **22**, 466 (1966).

(6) F. Rottman and P. Cerutti, *Proc. Natl. Acad. Sci. U. S.*, **55**, 960 (1966).

(7) M. Tomasz and R. W. Chambers, *Biochemistry*, **5**, 773 (1966).

(8) Dihydrothymidine should exist in two epimeric forms. Two different melting points have been reported: 206–209° [H. T. Miles, *Biochim. Biophys. Acta*, **27**, 46 (1958)] and 152–153° [M. Green and S. S. Cohen, *J. Biol. Chem.*, **225**, 397 (1957)].

(8a) NOTE ADDED IN PROOF. Dr. Y. Kondo in this laboratory has now found that catalytic reduction leads only to one form of dihydrothymidine with mp 155–156° and $[\alpha]^{20}D$ −20.5°, from which only the levorotatory dihydrothymine, mp 263°, $[\alpha]^{20}D$ −7.2°, is obtained. The reported "higher melting form of dihydrothymidine" is a mixture containing free dihydrothymine arising from reductive cleavage of the ribosyl residue.

(9) R. Archibald, *J. Biol. Chem.*, **156**, 121 (1944).

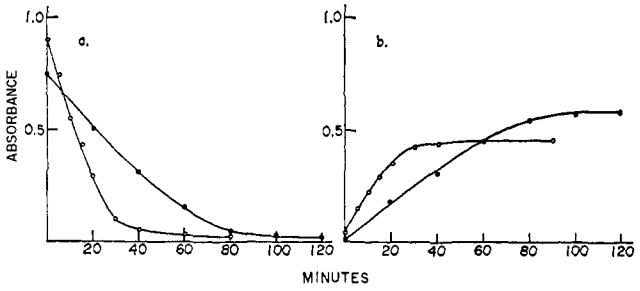
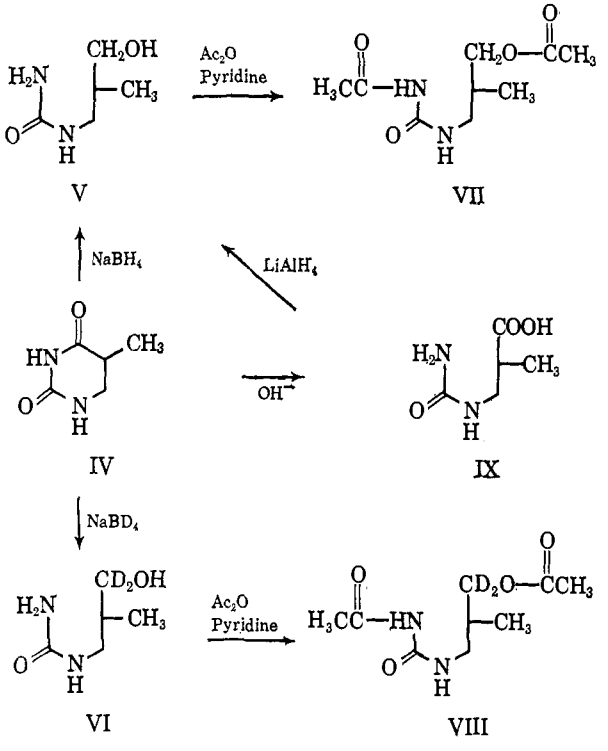


Figure 1. Photoreduction of uridine and thymidine. (a) OD_{260}: O–O–O, uridine, ●–●–●, thymidine. (b) Pentose assay: O–O–O, ribose (orcinol method); ●–●–●, 2-deoxyribose (diphenylamine method).

On treatment with acetic anhydride in pyridine a crystalline diacetate, $C_9H_{16}N_2O_4$ (VII), was obtained. The molecular weight (216) was determined osmometrically and from the mass spectrum (Figure 2B). The diacetate VIII, obtained from the $NaBD_4$ reduction



product VI, had a molecular weight of 218. In the mass spectrum (Figure 2C) several peaks are shifted upwards by 2 mass units. This indicates that two deuterium atoms have entered the molecule.

These observations suggest structure V for the reduction product of dihydrothymine. The final structural proof was provided by the synthesis of V following an independent route. Dihydrothymine was converted into β-ureidoisobutyric acid IX by treatment with base.[4] Upon refluxing with lithium aluminum hydride in tetrahydrofuran, IX yielded 3-ureido-2-methylpropanol-1, the diacetate of which was identical with the diacetate VII.

The $NaBH_4$ reduction of dihydrothymine may well start with the addition of hydride ion to the carbonyl group at C-4. The intermediate X takes up a proton from the reaction medium and opens to the aldehyde XI, which is then reduced to the primary alcohol V.

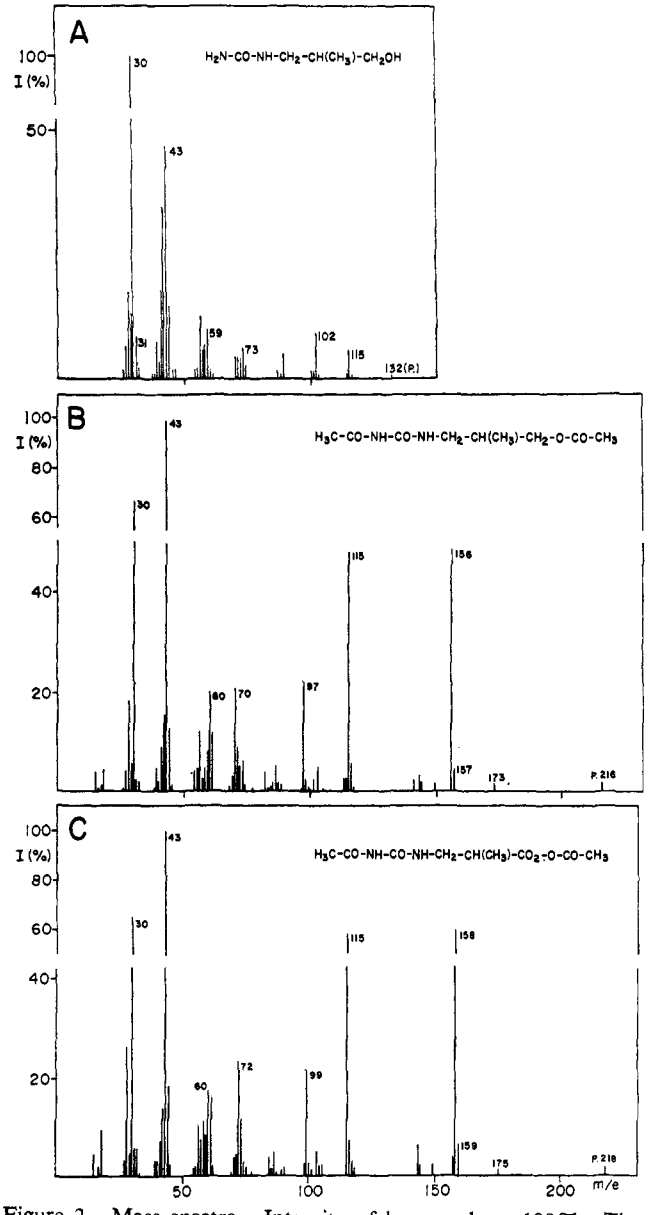
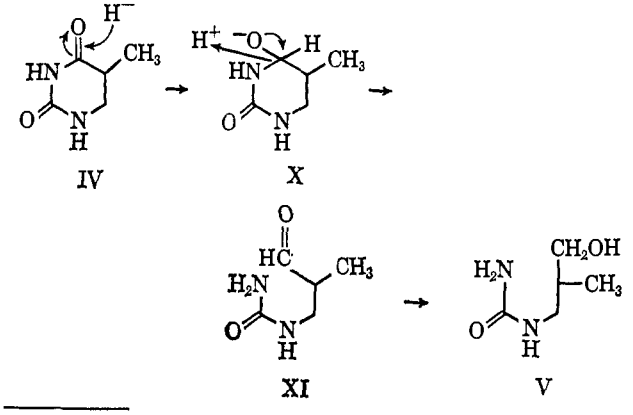


Figure 2. Mass spectra. Intensity of base peak = 100%. The peaks at *m/e* 31, 59, and 73 (A) are assigned to fragments $[CH_2OH]^+$, $[CH(CH_3)CH_2OH]^+$, and $[CH_2CH(CH_3)CH_2OH]^+$, respectively.

This reaction, which proceeds with unexpected ease in the case of dihydrothymine, is reminiscent of the cleavage and reduction of 1-*p*-toluenesulfonyl-L-pyrrolid-5-one-2-carboxamide,[10] of N-acylindoles and -car-



(10) Z. Pravda and J. Rudinger, *Collection Czech. Chem. Commun.*, 20, 1 (1955).

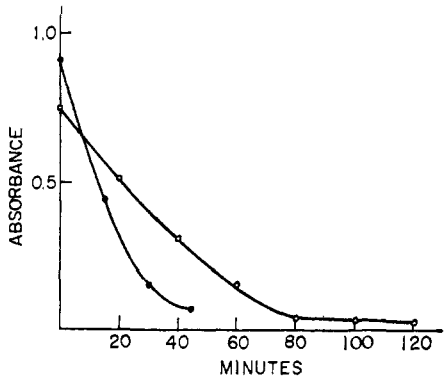


Figure 3. Photoreduction of thymidine (OD_{260}), O–O–O. Dark reduction of dihydrothymidine (Archibald assay), ●–●–●.

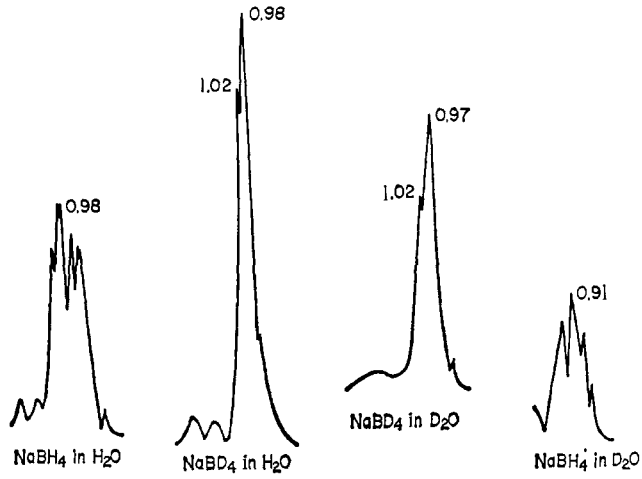


Figure 4. Nmr signals of the secondary methyl group of the photoreduction product.

bazoles,[11] and of certain imides[12] with complex metal hydrides. The scope and limitations of this reaction are under study.

When dihydrothymidine (II) was reduced under analogous conditions, a product was obtained that showed a carbonyl absorption at 1650 cm^{-1} and nmr signals for the secondary methyl group and the two methylene groups at the expected positions. One of the latter again disappeared when the reduction was carried out with $NaBD_4$. The Archibald test was practically negative. Chromatography in several systems revealed that the product was not uniform. Acid hydrolysis yielded 2-deoxyribose and 3-ureido-2-methylpropanol-1 (V), chromatographically identical with authentic samples. We therefore assign structure III (one of two epimers) to the main reduction product of dihydrothymidine.

The photoreduction of thymidine proceeds considerably slower than the light-independent $NaBH_4$ reduction of dihydrothymidine. This is shown in Figure 3, where the rates of the two reactions are compared at equal substrate and $NaBH_4$ concentrations. If dihydrothymidine is formed as the primary product of the photoreduction, it is immediately consumed by the faster secondary reaction. The final product yields upon acid hydrolysis 2-deoxyribose, 3-ureido-2-methylpropanol-1, and small amounts of unidentified products. The main component of the photoreduction product is III. We have not yet been able to clarify why this compound gives a negative Archibald test, which is positive in the case of V.

Relevant information about the sequential steps of the photoreduction was expected from the nmr spectra of the products obtained by using differently labeled reducing systems. Especially the presence of a methyl group at C-5 in thymidine, which becomes secondary upon saturation of the double bond, lends itself to an investigation of the mechanism of the photoreduction.

When, accordingly, the photoreduction of thymidine was carried out with $NaBH_4$ and $NaBD_4$ in H_2O and D_2O as solvents, the nmr spectra of the isolated products showed methyl and methylene signals which vary significantly with the nature of the reducing species and the reaction medium. The spectra show conclusively that the hydrogen donated by $NaBH_4$ (as hydride ion or hydrogen atom[13]) enters the molecule at the 5 position during photoreduction, whereas the hydrogen added to C-6 originates from the solvent. The splitting pattern of the methyl peaks (Figure 4) is not surprising, since the attack of hydrogen can occur from both sides of the pyrimidine ring, leading to two diastereomers. Thus, two superimposed nmr signals for the two unequivalent methyl groups are actually observed.

Our data and information on the physical and chemical properties of excited thymine and its derivatives permit no final formulation of the mechanism of the photoreduction. There is, however, considerable support for a mechanism including the following steps: (i) excitation of the thymine moiety (thymine anion) leading to a metastable triplet state, (ii) formation of a partially reduced thymidine radical (unpaired electron at C-5) upon addition of hydrogen from the solvent, (iii) reaction of this radical with $NaBH_4$ to dihydrothymidine. In the subsequent step the dihydrothymine ring is then opened and further reduced by $NaBH_4$.

The following data are in favor of this mechanism. It has been shown by phosphorescence and esr studies (at alkaline pH, at 77 °K, in an ethylene glycol–water glass)[14,15] that thymine and some of its derivatives are excited by ultraviolet light to a metastable triplet state, which seems to be the result of $\pi \rightarrow \pi^*$ rather than an $n \rightarrow \pi^*$ transition. The activated centers are preferentially located at the 5,6 double bond of thymine.[16] An important reaction of the excited thymine is presumably the addition of hydrogen from the solvent to C-6, leading to a partially reduced thymine radical with the unpaired electron at C-5. The predominant formation of this radical has been demonstrated by esr spectroscopy in ultraviolet-irradiated thymine, thymidine, poly-dAT, and DNA. No stable products from this radical precursor have so far been obtained.[17] The isotope distribution in the products obtained from

(11) K. Bannholzer, T. W. Campbell, and H. Schmid, *Helv. Chim. Acta*, **35**, 1577 (1952).

(12) B. Witkop and J. B. Patrick, *J. Am. Chem. Soc.*, 74, 3861 (1952).

(13) Recent experimental observations as well as quantum-mechanical considerations suggest that in some cases borohydride in water may be a donor of hydrogen atoms rather than of hydride ions.

(14) T. W. Longworth, R. O. Rahn, and R. G. Shulman, personal communication.

(15) O. Rahn, R. G. Shulman, and T. W. Longworth, *Proc. Natl. Acad. Sci. U. S.*, **53**, 893 (1965).

(16) This result is in agreement with molecular orbital calculations of the spin-density distribution for the first excited state; *cf.* M. T. Mantione and B. Pullman, *Biochim. Biophys. Acta*, **91**, 387 (1964).

(17) P. S. Pershan, R. G. Shulman, B. T. Wyluda, and T. Eisinger, *Science*, **148**, 378 (1965).

the photoreduction of thymidine with differently labeled reducing systems ($NaBD_4$–H_2O, $NaBD_4$–D_2O, $NaBH_4$–D_2O) suggests that the partially reduced C-5-thymidine radical might well be an important intermediate in the photoreduction of thymidine.[18]

The addition of other groups to the 5 or 6 position of photoexcited uracil is of relevance for the formulation of a unifying mechanism. While the mercapto group of cysteine attacks the 5 position of photoexcited uracil,[19] hydroxyl[20] and possibly also cyanide groups[21] add in the 6 position. Under the more severe conditions of γ irradiation, ethanol adds in the 6 position to give a secondary alcohol derived from 6-substituted uracil.[22] The interaction of DNA with photoactivated aromatic carcinogens is of special interest in this connection.[23]

Ionizing radiation damage, light-induced mutations, as well as other effects that are of the highest genetic import, are concentrated on thymine groups.[24] The results presented here on the reductive photochemistry of monomeric derivatives of thymine are suggestive of numerous applications to thymidine-containing polynucleotides.

Experimental Section

Apparatus and Methods. Infrared spectra were recorded on Perkin-Elmer spectrophotometers Model 21 and Model 237B; nmr spectra on a Varian A-60 spectrometer with sodium 3-(trimethylsilyl)-1-propanesulfonate in D_2O as external standard, unless otherwise stated. Chemical shifts are given in δ units. Ultraviolet spectra and optical densities were measured with a Cary Model 14 recording spectrophotometer. Melting points, determined with the Büchi apparatus, are uncorrected. Chromatography was carried out in the following systems: (A) methanol–chloroform (1:4 and 3:7) on silica gel G (Merck), buffered with 0.1 *N* sodium acetate, (B) 1-propanol–concentrated NH_4OH (8:1) on silica gel G, (C) 1-butanol–acetic acid–water (4:1:1) on Schleicher & Schuell Paper No. 2041, ascending technique, (D) ethyl acetate–pyridine–water (2:1:2) on Paper No. 2041, ascending technique.

For irradiation a U-shaped Hanovia low-pressure mercury lamp, No. 87A-45, with an intensity of 4.3 w at 253.7 mμ was employed. The D_2O (Volk) used for photoreduction experiments and nmr spectra was 99.8% pure. The orcinol and diphenylamine assays for ribose and 2-deoxyribose, respectively, were performed according to established methods.[25]

Reduction of Dihydrothymine with Sodium Borohydride. 3-Ureido-2-methylpropanol-1 (V). To a solution of 4.86 g of dihydrothymine (38 mmoles) in 800 ml of water was added 7.22 g of sodium borohydride (190 mmoles). The solution was stirred until the absorption at 234 mμ[4] of a sample in 0.1 *N* alkali had disappeared (2 hr). The reaction mixture was then adjusted to pH 3 by addition of Dowex 50 W-H^+, stirred for 10 min, filtered, and lyophilized. The residue was taken up in methanol and the solution was taken to dryness. This procedure was repeated until all the boric acid had been removed. Finally, the residue was again taken up in methanol and insoluble matter was removed by centrifugation. The insoluble fraction was twice washed with methanol and the combined extracts were evaporated to leave a viscous residue which was dissolved in water. A white turbidity was removed by filtration through a layer of Celite and the solution was lyophilized to give 4.55 g of a pale yellow oil, which solidified upon cooling in a Dry Ice bath but melted again at room temperature.

In order to ascertain the absence of volatile reduction products, nitrogen was passed through the reduction solution of a second run, in which 1.28 g of dihydrothymine in 250 ml of water was reduced with 1.90 g of $NaBH_4$. The gas was led into a trap, cooled with Dry Ice. After the reaction was complete, Dowex 50 W-H^+ was added to the reaction mixture and nitrogen was used to drive the evolved hydrogen gas through a second cooled trap. The yield of nonvolatile reduction product was 1.18 g. No volatile products, such as methanol, formaldehyde, or formic acid, could be detected in the two traps. The reduction product was further purified by trituration with tetrahydrofuran, which left about 5% of solid inorganic material undissolved. On evaporation, the solution yielded a colorless glass, which gave one major spot on chromatography in systems A, B, and C, and a trace of less polar material. Spraying with a 1% solution of *p*-dimethylaminobenzaldehyde in 1.0 *N* HCl (Ehrlich's reagent) produced an immediate yellow color. The compound did not react with ninhydrin.

Anal. Calcd for $C_5H_{12}N_2O_2$: C, 45.45; H, 9.09; N, 21.21. Found: C, 45.35; H, 9.10; N, 20.44; mol wt, 132 (mass spectrum).

The infrared spectrum showed 3330 cm^{-1} (film); 1660, 1612, 1542 cm^{-1} (acetonitrile). The nmr spectrum (D_2O) showed a doublet centered at 0.89 ppm, J = 7 cps (2-methyl); sextet at 1.80 ppm (2-H); doublet at 3.06 ppm, J = 7 cps (–CH_2N<); doublet at 3.49 ppm, J = 6 cps (CH_2O–).

Reduction with Sodium Borodeuteride. 3-Ureido-2-methyl-1,1-dideuteriopropanol-1 (VI). To a solution of 900 mg of dihydrothymine in 300 ml of water 1.4 g of $NaBD_4$ was added. The product, a colorless glass (VI), was isolated in the same fashion as V. Its nmr spectrum showed the same signals as V except for the doublet at 3.49 ppm, which had disappeared.

N,O-Diacetyl-3-ureido-2-methylpropanol-1 (VII). To a solution of 1.07 g of the ureido alcohol (V) in 10 ml of pyridine was added 10 ml of acetic anhydride. After 40 hr at room temperature the solution was diluted with cold water and extracted with chloroform. The chloroform extract was washed with water, dried with sodium sulfate, and evaporated to yield a yellow solid, soluble in hot cyclohexane except for a minor impurity. After three crystallizations 901 mg of colorless platelets, mp 85°, was obtained. The platelets changed into prisms on prolonged standing under cyclohexane.

Anal. Calcd for $C_9H_{16}N_2O_4$: C, 50.0; H, 7.40; N, 12.96; acetyl, 39.8; mol wt, 216. Found: C, 50.03; H, 7.33; N, 12.89; acetyl, 39.58; mol wt, 216 (osmometer, mass spectrum).

The infrared spectrum showed: 3440, 3330, 1720, 1560 cm^{-1} (chloroform). The nmr spectrum ($CDCl_3$ with TMS as internal standard) showed a doublet at 0.99 ppm, J = 7 cps (2-methyl); singlets at 2.10 and 2.16 ppm (acetyl); triplet at 3.33 ppm (–CH_2-NHAc); doublet at 4.05 ppm, J = 6 cps (–CH_2OAc); and NH signals at 8.68 and 10.37 ppm. The signal for the 2 proton, a sextet centered about 2.10 ppm, is partially buried under the acetyl peaks.

N,O-Diacetyl-3-ureido-2-methyl-1,1-dideuteriopropanol-1 (VIII). When 150 mg of the $NaBD_4$ reduction product VII was treated with 2 ml of acetic anhydride in 2 ml of pyridine at room temperature, 107 mg of crystalline diacetate IX, mp 83°, was obtained. A mass spectrum showed the molecular weight to be 218.

Synthesis of 3-Ureido-2-methylpropanol-1 *via* β-Ureidoisobutyric Acid (IX). A solution of 2 g of dihydrothymine in 25 ml of 0.5 *N* aqueous baryta was left at room temperature until the optical density at 234 mμ in 0.1 *N* alkali was practically zero (2 hr). The solution was then adjusted to pH 3.5 by the addition of 1.0 *N* sulfuric acid. The precipitated barium sulfate was removed by centrifugation. The supernatant was lyophilized and the residue was recrystallized from a mixture of ethanol and ethyl acetate to give 1.75 g of β-ureidoisobutyric acid as colorless crystals, mp 120–121° (lit.[5] 98–100°). The acid IX gives a yellow color upon spraying with Ehrlich's reagent: infrared, 3440, 3350, 1695, 1665, 1630, 1595, 1558 cm^{-1} (KBr); nmr (D_2O), doublet at 1.12 (J = 7.5 cps, α-methyl), sextet at 2.65 (α-H), doublet at 3.23 ppm (J = 6 cps β-methylene).

Anal. Calcd for $C_5H_{10}N_2O_3$: C, 41.1; H, 6.85; N, 19.18. Found: C, 41.29; H, 6.75; N, 18.91.

A suspension of 500 mg of β-ureidoisobutyric acid in 60 ml of tetrahydrofuran was refluxed for 24 hr in the presence of excess

(18) A concerted mechanism, involving complex formation of the borohydride with the 4-carbonyl function and addition of a hydride ion to C-6, analogous to the mechanism suggested for the unusual ground-state reduction of iresin with $NaBH_4$ [*cf.* C. Djerassi and W. Rittel, *J. Am. Chem. Soc.*, 79, 3528 (1957)], can clearly be excluded on the basis of our experiments.

(19) K. C. Smith and R. T. Aplin, *Biochemistry*, **5**, 2125 (1966). We are indebted to Dr. K. C. Smith for a copy of the manuscript prior to publication.

(20) S. Y. Wang, *J. Am. Chem. Soc.*, **80**, 6196 (1958); *Photochem. Photobiol.*, **1**, 37 (1962).

(21) A. M. Moore, *Can. J. Chem.*, **37**, 1287 (1959).

(22) P. E. Brown, M. Calvin, and J. F. Newmark, *Science*, **151**, 68 (1966).

(23) *Cf.* S. A. Rapaport and P. O. P. Ts'o, *Proc. Natl. Acad. Sci. U. S.*, **55**, 381 (1966).

(24) D. Shugar, *Israel J. Med. Sci.*, **1**, 1347 (1965).

(25) E. Volkin and W. Cohn, *Methods Biochem. Analy.*, **1**, 287 (diphenylamine assay), 298 (orcinol assay) (1954).

lithium aluminum hydride. Excess reagent was destroyed by careful addition of aqueous tetrahydrofuran; the precipitate was removed by centrifugation and washed with tetrahydrofuran and subsequently with hot methanol. The methanol extract was evaporated and the residue was triturated with tetrahydrofuran.

The combined THF solutions yielded on evaporation 321 mg of a faintly pink oil. The oil was dissolved in propanol-ammonia (8:1), poured on a column of 50 g of silica gel, and eluted with the same solvent mixture to give 308 mg of colored material, which was rechromatographed on 10 g of alumina. Chloroform–methanol (4:1) eluted 88 mg of a pale yellow oil, identical with regard to infrared and chromatography (systems A, B, C) with V. Acetylation in pyridine–acetic anhydride gave a product which was identical in every respect with the diacetate VII. After three crystallizations from cyclohexane, the mp was 85–86°, undepressed on admixture with VII, 85°.

Dark Reduction of Dihydrothymidine. A solution of 500 mg of dihydrothymidine in 150 ml of water was stirred with 450 mg of $NaBH_4$ for 1 hr. After this period, the optical density (234 mμ) in 0.1 *N* alkali had fallen to zero, and the Archibald test[7] was negative. The reaction solution was neutralized by stirring with Dowex 50 W-H^+, filtered, and lyophilized. Boric acid was removed by repeated evaporation with methanol and the residue was taken up in water. The solution was cleared by centrifugation and again lyophilized to yield a colorless glassy material which was not uniform and could not be crystallized (III): infrared, 3400, 1650, 1510 cm^{-1} (Nujol); nmr (D_2O), poorly resolved multiplet at 0.95 (*sec*-methyl group), doublets at 3.02 (J = 6.5 cps, $-CH_2N<$), and 3.54 ppm (J = 6 cps, $-CH_2O-$).

Hydrolysis was effected by heating the photoreduction product in 0.3 *N* sulfuric acid for 1 hr in a boiling-water bath. After neutralization with baryta the solution was cleared by centrifugation and the supernatant evaporated. 3-Ureido-2-methylpropanol-1 and 2-deoxyribose could be identified by chromatography in systems A, B, C, and D. Similarly 200 mg of dihydrothymidine was treated with 200 mg of $NaBD_4$ in 50 ml of water. In the nmr spectrum (D_2O) of the isolated product the doublet at 3.54 ppm had disappeared.

Photoreduction of Thymidine. The reaction was carried out in semicircular quartz cells of approximately 1-cm thickness, each with a capacity of 100 ml. Two cells were fitted around a cylindrical quartz cooler, in the center of which the ultraviolet lamp was suspended. This apparatus was enclosed in a polished stainless steel cylinder. A solution with starting concentrations of 2 μmoles/ml of thymidine and 10 μmoles/ml of $NaBH_4$ showed less than 1% of the initial optical density at 260 mμ after irradiation for 2 hr at room temperature. In the control experiment, the optical density (260 mμ) of a thymidine solution irradiated in the absence of $NaBH_4$ remained unchanged over this period. The photoreduction of thymidine was carried out in the same manner with $NaBH_4$ in D_2O, $NaBD_4$ in water, and $NaBD_4$ in D_2O. The reaction was worked up in the same fashion as the reduction of dihydrothymine. The photoreduction product had the following properties: infrared absorption at 1650 cm^{-1} (Nujol); nmr (D_2O), two doublets centered at 0.97 and 1.03 ppm (J = 6.5 cps, secondary methyl group). The product was not uniform on chromatography in systems A and C. Hydrolysis yielded 3-ureido-2-methylpropanol-1 (V), 2-deoxyribose, and urea, identified by chromatography in systems A, B, C, and D.

Acknowledgment. We are greatly indebted to Dr. Udo Axen, Massachusetts Institute of Technology, for measuring the mass spectra and to Dr. R. G. Shulman for stimulating discussions.

A Study of the Synthesis and Properties of 2H-Benz[*cd*]azulene and Related Compounds[1,2]

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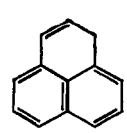
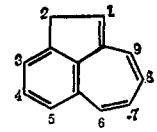
Abstract: A synthesis of 2H-benz[*cd*]azulene (II) is described. As predicted from molecular orbital calculations, the behavior of 2H-benz[*cd*]azulene on treatment with the appropiate reagents indicates easy formation of the corresponding carbonium ion, carbanion, and free-radical species. Various derivatives of 2H-benz[*cd*]azulene are described. The carbonyl derivatives have properties analogous to those of perinaphthenone and are relatively strong bases.

The synthesis[3] and studies[3-5] of the properties of perinaphthene (I) have demonstrated that the anion, carbonium ion, and radical derived from perinaphthene are all relatively stable. The usual explanation for this behavior is that perinaphthene, being an odd-alternant hydrocarbon, has a nonbonding orbital so that whether zero, one, or two electrons, corresponding to carbonium ion, radical, and anion, are placed in the nonbonding orbital there is little change in the delocalization energy.[6]



I



II

The ions or radical derived from perinaphthene, however, have a high degree of symmetry and the question of the degree to which symmetry plays a role in stabilizing these species is a pertinent one. For this reason we became interested in 2H-benz[*cd*]azulene (II) since it is isomeric with perinaphthene and is an odd-nonalternant hydrocarbon with a lower degree of symmetry.

Molecular orbital calculations for 2H-benz[*cd*]-azulene have been made both by the simple Hückel

(1) A preliminary announcement of this work was made at the 146th National Meeting of the American Chemical Society, Denver, Colo., Jan 20–22, 1964; *cf.* Abstracts, p 34C.

(2) We thank the Petroleum Research Fund of the American Chemical Society (Grant No. 914) for support of this research.

(3) V. Boekelheide and C. E. Larrabee, *J. Am. Chem. Soc.*, 72, 1245 (1950).

(4) R. Pettit, *ibid.*, **82**, 1972 (1960).

(5) (a) D. H. Reid, *Tetrahedron*, **3**, 339 (1958); (b) *Tetrahedron Letters*, **No. 15**, 530 (1961).

(6) A. Streitwieser, Jr., "Molecular Orbital Theory for Organic Chemists," John Wiley and Sons, Inc., New York, N. Y., 1961, p 46.