Photolysis of 16. An aqueous solution (500 ml) of the ylide **16** (1.0 g) was irradiated with a high-pressure mercury lamp for 3 hr. After removal of the aqueous solvent, the products were purified by chromatography (silica gel, MeOH–$CHCl_3$, 3:5). The major product was 2-methyl-4-hydroxypyrimidine (100 mg, 22%) which was converted to the hydrochloride and identified by direct comparison with an authentic sample. A small amount of 1-arabinosyl-2-methyl-4(1*H*)-pyrimidinone (**19**) was also obtained.

Dimethyloxosulfonium 1-(5-*O*-Trityl-2-deoxy-β-D-xylofuranosyl)-5-methyl-1,4-dihydro-4-oxo-2-pyrimidinemethylide (22). To the ylide prepared from NaH (0.85 g) and trimethyloxosulfonium chloride (5.5 g) in THF (150 ml) was added 2,3′-anhydro-1-(5-*O*-trityl-2-deoxyxylofuranosyl)thymine[26] (4.5 g) and the mixture was gently refluxed overnight. The precipitate was collected by filtration and washed several times with cold water to leave a colorless solid (2.0 g, 37%), which was recrystallized from MeOH–ether or aqueous EtOH to give colorless crystals: mp 203–205°; $[\alpha]^{25}D$ −9.8° (*c* 20.5, MeOH); ir 3250 (OH), 1640 (C_2), 1550, 1170 cm^{-1}; uv λ_{max}^{MeOH} 280, 233 nm (log ε 4.33, 4.31); λ_{min}^{MeOH} 256 nm (log ε 4.06).

Anal. Calcd for $C_{32}H_{34}N_2O_5S \cdot 0.5H_2O$: C, 67.72; H, 6.17; N, 4.93; S, 5.64. Found: C, 67.54; H, 6.34; N, 4.97; S, 5.21.

The filtered THF solution was evaporated *in vacuo* and the residue was chromatographed on silica gel ($CHCl_3$–MeOH, 5:1) to give another product (1.4 g; λ_{max}^{MeOH} 266 nm; λ_{min}^{MeOH} 246 nm) as a colorless amorphous solid, which was hydrolyzed with HCl to give 3-methylthymine: mp 208–210° (lit.[27] 209–210°); λ_{max}^{MeOH} 264 nm; λ_{min}^{MeOH} 234 nm.

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2,5-Dimethyl-1-(5-*O*-trityl-2-deoxy-β-D-xylofuranosyl)-4-(1*H*)-pyrimidinone (24). Desulfurization of the above ylide **22** (0.2 g) with Raney nickel (2 g) in aqueous EtOH (20 ml) at room temperature for 30 min left a colorless solid (mp 208–213°) which was recrystallized from aqueous methanol to give an analytical sample as colorless prisms: mp 223–225°; uv λ_{max}^{MeOH} 238 nm (log ε 4.20); ir 3200, 1645, 1620 cm^{-1}.

Anal. Calcd for $C_{30}H_{30}N_2O_4$: N, 5.81. Found: N, 5.74.

2,3′-Anhydro-1-(5-*O*-trityl-2-deoxy-β-D-xylofuranosyl)uracil. This compound was obtained from 5′-*O*-trityl-2′-deoxyuridine (5 g) in 65% yield, analogously to the corresponding thymidine.[26] Recrystallization from aqueous EtOH gave colorless needles, mp 138–140°; the uv spectrum showed only end absorption.

Anal. Calcd for $C_{28}H_{24}N_2O_4$: C, 74.32; H, 5.35; N, 6.19. Found: C, 74.46; H, 5.12; N, 6.23.

Dimethyloxosulfonium 1-(5-*O*-Trityl-2-deoxy-β-D-xylofuranosyl)-1,4-dihydro-4-oxopyrimidinemethylide (23). The above anhydrouracil (2.3 g) was treated with the ylide **1** prepared from NaH (0.5 g) and trimethyloxosulfonium chloride (3.3 g) in THF. The product was purified by chromatography on silica gel (MeOH–$CHCl_3$, 5:3) and recrystallized from aqueous EtOH to give the pyrimidinemethylide **23** as colorless crystals (0.84 g, 30% yield): mp 170–175°; $[\alpha]^{25}D$ −7.5° (*c* 0.3, MeOH); uv λ_{max}^{MeOH} 279, 230 nm (log ε 4.37, 4.38); λ_{min}^{MeOH} 255 nm (log ε 4.00); nmr (DMSO-d_6) 6.42 (6 H, s, $S(CH_3)_2$).

Anal. Calcd for $C_{31}H_{32}N_2O_5S \cdot 0.5H_2O$: C, 67.27; H, 5.97; N, 5.05. Found: C, 66.87; H, 5.90; N, 4.98.

Acid treatment in EtOH gave dimethyloxosulfonium 4-hydroxypyrimidinemethylide hydrochloride (**5**), mp 155–157°, identical with an authentic sample.

Hydrogenolysis and Stereochemistry of Photodimers of Thymine and Thymidine

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Abstract: In analogy to dihydrouracil, -thymine, or 5,6-cyclopropyluracils the cis-syn photodimer of thymine with excess aqueous sodium borohydride at room temperature is easily hydrogenolyzed to the dialcohol 3,4-(α)-*cis*-bishydroxymethyl-3,4(β)-*cis*-dimethyl-1,2(α)-*cis*-dicarbamidocyclobutane (mp 207–208°, 61%), the dicarbinol 4,5-*trans*-dihydroxy-4a,4b-dimethyltetrahydrocyclobuta[1,2-*d*:4,3-*d*′]dipyrimidine-2,7(1*H*,6*H*)-dione (mp 260°, 18%), the monoalcohol 6,7-dimethyl-7-hydroxymethyl-8-ureido-2,4-diazabicyclo[4.2.0]octane-3,5-dione (mp 207°, 5%), and the monocarbinol 4a,4b-dimethyl-5-hydroxyoctahydrocyclobuta[1,2-*d*:4,3-*d*′]dipyrimidine-2,4,7(3*H*)-trione (mp 285–288°, trace). The trans-anti photodimer of thymine gave only one product by complete hydrogenolysis, *viz.*, 2,4-*trans*-bishydroxymethyl-2,4-*trans*-dimethyl-1,3-*trans*-dicarbamidocyclobutane, in analogy to facile ring opening in 0.1 *N* NaOH to *trans*-1,3-dimethyl-2,4-*trans*-dicarbamidocyclobutane-1,3-*trans*-dicarboxylic acid (mp 244°, 80%). The isomeric cis-syn acid was obtained on oxidation of the dialcohol with $KMnO_4$. Catalytic oxygenation (Pt, O_2) of the dicarbinol gave back cis-syn dimer and the monocarbinol (mp 285–288°). The monoalcohol (mp 207°), stable on irradiation, gave on permanganate oxidation a monoacid, *viz.*, 6,7-dimethyl-3,5-dioxo-8-ureido-2,4-diazabicyclo[4.2.0]octane-7-carboxylic acid (mp 238–240°) which on reductive photolysis gave 5,6-dihydrothymine and, presumably, β-ureidoisobutyric acid. The optically active trans-syn photodimers of thymine **3a**, $[\alpha]^{25}D$ +94.1° (H_2O), and **3b**, $[\alpha]^{25}D$ −92° (H_2O), obtained from thymidine dimer precursors, on hydrogenolysis with $NaBH_4$ gave the optically inactive 2,4(α)-*cis*-bishydroxymethyl-2,4(β)-*cis*-dimethyl-1,3(α)-*cis*-dicarbamidocyclobutane, as required by theory.

Part of the radiation damage done to nucleic acids by the action of ultraviolet light is caused by the formation of cyclobutane-type dimers[2,3] and of pyrimidine bases, which play an important role in photoreactivation,[4] photoreversal,[5] and repair processes[6,7] in biological systems. Photodimerizations of pyrimidines have

(1) Fellow in the Visiting Program, U. S. Public Health Service, 1967–1970.
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(3) Recent review: E. Fahr, *Angew. Chem., Int. Ed. Engl.*, **8**, 578 1969).
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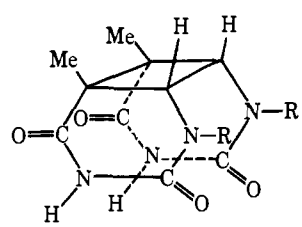
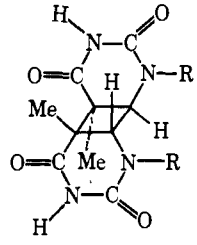
(7) R. P. Boyce and P. Howard-Flanders, *Proc. Nat. Acad. Sci. U. S.*, **51**, 293 (1964).

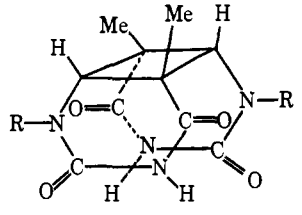
been extensively studied under a wide variety of conditions at the level of mono-,[6-8] di-,[9] and polynucleotides including DNA.[10,11] After the first demonstration of stable thymine dimer **1** in 1960 as a major product from the uv irradiation of a frozen solution of thymine,[8] the four possible thymine dimers **1–4** have been isolated,[12]



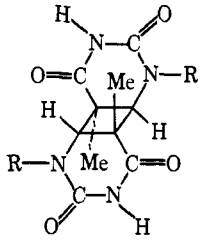
1, cis-syn



2, cis-anti



3a, b, trans-syn



4, trans-anti

of which some dimers have been structurally confirmed by chemical transformations[13] and X-ray analysis.[14] Recently a novel photoadduct of thymine was isolated[15] and characterized by X-ray analysis.[16] So far, information on the chemical degradation of these dimers has been scarce.[3] In this paper, we describe a smooth reductive cleavage of thymine dimers to novel cyclobutane derivatives by sodium borohydride. This method is mild and selective enough to permit localization and quantitation of photolesions in irradiated polynucleotides and nucleic acids.[17-19]

Hydrogenolysis of Photodimers of Thymine. When the *cis-syn*-thymine dimer **1** (R = H) obtained from uv irradiation of frozen aqueous solutions[8] was reduced with excess sodium borohydride in aqueous solution at room temperature, three products, **5**, **6**, and **7** (see Scheme I), were isolated by careful chromatography on silica gel ($CHCl_3$–MeOH, 1:1). All products gave a pink coloration with *p*-dimethylaminocinnamaldehyde, the modified Ehrlich reagent, indicative of the presence of a ureido group or its equivalent. The major product (61% yield) was identified as a novel cyclobutane derivative **7**, *viz.*, 3,4(α)-*cis*-bishydroxymethyl-3,4(β)-*cis*-dimethyl-1,2(α)-*cis*-dicarbamidocyclobutane, mp 207–208°, on the basis of the nmr spectrum (Table I). The two other products, **5** and **6**, were intermediates on the way to the final product **7**; on independent hydrogenolysis with sodium borohydride both of them gave **7** as the sole product. The reduction product **6** (18% yield) is represented as a dimer of 4-hydroxy-2-pyrimidinone, mp 258–260°, on the basis of the nmr data (Table I). Singlet peaks at τ 4.97 and 4.87 (in D_2O) are consistent with 4.90, the value reported for the hydroxymethine proton in 4-hydroxytetrahydro-2-(1*H*)-pyrimidinone nucleoside.[20] The uv spectrum showed only end absorption both in neutral and basic media. In the preliminary communication[17] structure **6**, *viz.*, 4,5-*trans*-dihydroxy-4a,4b-dimethyltetrahydrocyclobuta[1,2-*d*:4,3-*d'*]dipyrimidine-2,7(1*H*,6*H*)-dione, which has no plane of symmetry, was erroneously formulated as the half-alcohol **5**. The real half-alcohol **5**, *viz.*, 6,7-dimethyl-7-hydroxymethyl-8-ureido-2,4-diazabicyclo[4.2.0]octane-3,5-dione, mp 207°, was originally misinterpreted as a lactone.[17] Its nmr spectrum (D_2O) showed two singlet peaks at τ 8.68 and 8.71 due to methyl protons, and two pairs of doublet peaks at 5.93 and 5.52 (J = 10.0 Hz) and 5.78 and 5.50 (J = 8.5 Hz) as AB patterns, each assignable to hydroxymethylene and cyclobutane ring protons, respectively. The uv spectrum, end absorption in neutral media and λ_{max} 236 nm in 0.1 *N* NaOH, supports the dihydro-2,4(1*H*,3*H*)-pyrimidinedione structure.[21,22] The use of less sodium borohydride, 2–4 instead of 10 mol, gave **6** and **7** in ratios of 7:1 and 16:1, respectively. However, dimer **1** (R = H) was unreactive to the sodium borohydride–pyridine reagent, which is known to reduce certain amides effectively.[23]

On the other hand, the *trans-anti*-thymine dimer **4** (R = H) which is easily isolated from thymine solutions irradiated in the presence of sensitizers, such as acetone or benzophenone,[24] was easily hydrogenolyzed to the cyclobutane derivative **13**, mp >300°, as the sole isolable product. The same conditions convert dimer **1** (R = H) to a mixture of **6** and **7** in a ratio of 7:1. The nmr spectrum (D_2O–trifluoroacetic acid) of **13** showed singlet peaks at τ 9.11, 6.53, and 6.11 with intensity ratios of 3:2:1.

This facile hydrogenolysis is paralleled by the ease of alkaline hydrolysis of dimer **4**. In contrast to dimer **1** (R = H) which—unlike dihydrothymine, dihydrouracil,[25] and cyclothymine[26]—is stable to alkali, at room temperature, dimer **4** (R = H) easily opens up in 1.0 *N* NaOH at room temperature to yield the cyclobutanedicarboxylic acid (**14**), mp 244°, in >80% yield.

(8) R. Beukers and W. Berends, *Biochim. Biophys. Acta*, **41**, 550 (1960); S. Y. Wang, *Nature (London)*, **190**, 690 (1961); R. F. Stewart, *Biochim. Biophys. Acta*, **75**, 129 (1963).

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(11) D. Weinblum, *Biochem. Biophys. Res. Commun.*, 27, 384 (1967).

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(16) I. L. Karle, S. Y. Wang, and A. J. Varghese, *ibid.*, **164**, 183 (1969); *cf.* J. L. Flippen and I. L. Karle, *J. Amer. Chem. Soc.*, **93**, 2762 (1971); S. Y. Wang, *ibid.*, **93**, 2768 (1971).

(17) T. Kunieda and B. Witkop, *J. Amer. Chem. Soc.*, **89**, 4232 (1967).

(18) B. Witkop, *Photochem. Photobiol.*, **7**, 813 (1968).

(19) T. Kunieda, L. Grossman, and B. Witkop, *Biochem. Biophys. Res. Commun.*, **33**, 453 (1968).

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(25) E. G. Sander, *J. Amer. Chem. Soc.*, **91**, 3629 (1969).

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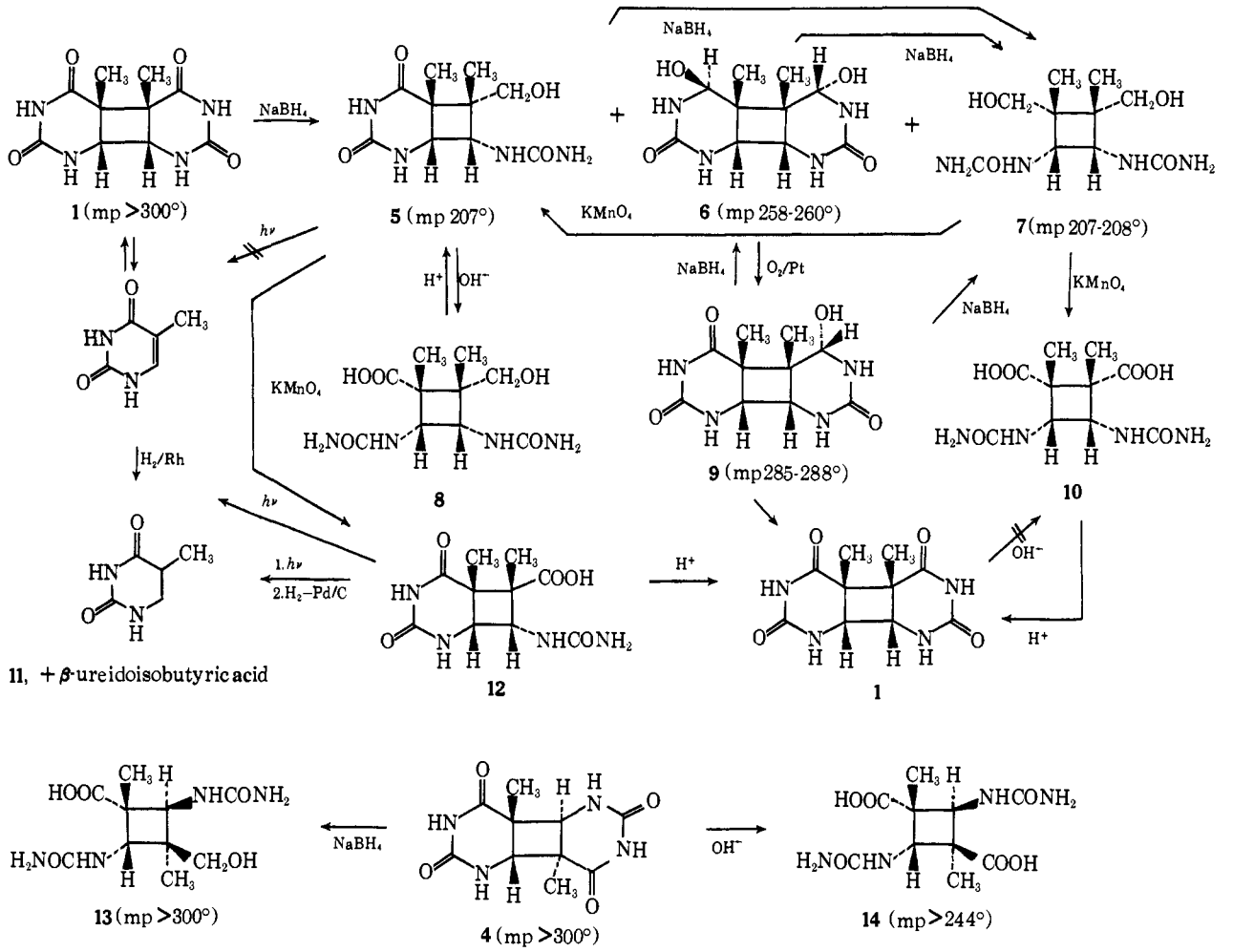
Scheme I



Opening occurs even with 0.01 *N* NaOH solution, and in 0.1 *N* NaOH is almost complete within 1 hr. Dimer **3** (R = H) is stable to dilute alkaki at room temperature (Figure 1).

On the other hand, monoalcohol **5**, in which only one pyrimidine ring is hydrogenolyzed, underwent smooth ring opening in 1.0 *N* NaOH at room temperature to afford **8** which easily reverted to **5** on treatment with dilute hydrochloric acid. Ring opening of **5**, which was followed by the decrease of λ_{max} 236 nm in dilute alkali, paralleled that of dihydrothymine,[27] which is known to change from a second- to a zero-order dependence on HO^-, as the concentration of alkali increases.[25]

The monoalcohol **5** is oxidized by potassium permanganate in neutral solutions to give the monoacid **12**. This compound gave back dimer **1** (R = H) on treatment with dilute hydrochloric acid. Photolysis of **12**, in contrast to the photostable monoalcohol **5**, gave thymine. When the irradiated mixture was catalytically hydrogenated (Pd/C), dihydrothymine, mp 261°,[27] was isolated in addition to a compound with a strongly positive reaction to the modified Ehrlich reagent, with the same R_f as 3-ureidoisobutyric acid,[28] which, however, was not obtained in a pure form.

The analogous oxidation of dialcohol **7** with potassium permanganate gave the monoalcohol **5**, mp 207°, and the diacid **10** in a ratio of 3:1, in addition to dimer **1**

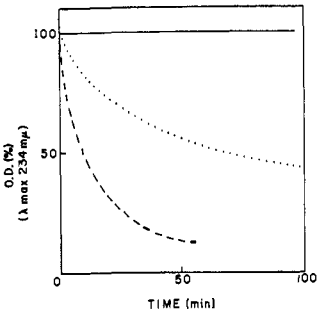


Figure 1. Stability of thymine dimers **1** and **3** to base (—) and easy alkaline ring opening of dimer **4** by 0.01 *N* NaOH (···) and 0.1 *N* NaOH (- - -) at room temperature (1.6×10^{-4} mol/l.).

(R = H). Dicarboxylic acid **10** quantitatively recyclized to dimer **1** on acid treatment.

Catalytic oxygenation ($Pt-O_2$) of **6** gave back thymine dimer **1** and another product, mp 285–288° dec, formulated as the monocarbinol, 4a,4b-dimethyl-5-hydroxyoctahydrocyclobuta[1,2-*d*;4,3-*d*']dipyrimidine-2,4,7-(3*H*)-trione (**9**). This product, which is easily isolated because of its low solubility in water, was also observed among the reaction products of **1** (R = H) with sodium borohydride and was converted to a mixture of **6** and **7** with sodium borohydride.

Photodimers of Thymidine. Three thymidine dimers, **1** (mp 184–188°, $[\alpha]_D$ +25° (MeOH)), **3a** (impure), and **3b**, $[\alpha]_D$ +45° (MeOH) (R = deoxyribo-

(27) Y. Kondo and B. Witkop, *J. Amer. Chem. Soc.*, 90, 764 (1968).
(28) W. E. Cohn and D. G. Doherty, *ibid.*, 78, 2863 (1956).

Table I. Nmr Spectra of Thymine Dimers and Their Products of Hydrolysis, Hydrogenolysis, and Oxidation[a]

	⟩CCH₃		⟩C—CH₂		—C(H)—C(H)—		
Compd	DMSO-d_6	D_2O	DMSO-d_6	D_2O	DMSO-d_6	D_2O	Remarks
1	8.67 (6 H, s)				6.24 (2 H, s)		
5		8.71 (3 H, s) 8.68 (3 H, s)		5.93 (1 H, s) J = 10.0 Hz 5.52 (1 H, s) J = 10.0 Hz		5.50 (1 H, d) J = 8.5 Hz 5.78 (1 H, d) J = 8.5 Hz	AB pattern for ⟩CCH_2– and cyclobutane protons
6	8.97 (3 H, s) 8.93 (3 H, s)	8.91 (3 H, s) 8.79 (3 H, s)			6.23 (2 H, broad multiplet)	5.98 (2 H, s)	Carbinol protons, 4.97 and 4.87 (1 H, s, D_2O) 5.30 and 5.15 (1 H, d, J = 5.0 Hz, DMSO-d_6)
7	8.97 (6 H, s)	8.84 (6 H, s)	6.57 (4 H, broad singlet)	6.25 (4 H, s)	6.17 (2 H, d, J = 4.5 Hz)	5.85 (2 H, s)	Addition of D_2O changes cyclobutane doublet to singlet at 6.11
10		8.71 (6 H, s)				5.90 (2 H, s)	
12		8.66 (3 H, s) 8.60 (3 H, s)				6.16 (1 H, d) 5.90 (1 H, d) J = 6.0 Hz	AB pattern for cyclobutane protons
13		9.11 (6 H, s)		6.53 (4 H, s)		6.13 (2 H, s)	Solvent, D_2O and trifluoroacetic acid (1:1)
14	8.77 (6 H, s)				5.55 (2 H, d) J = 10 Hz		$-NH_2$, 4.20 (4 H, s) >NH, 3.15 (2 H, d) J = 10.0 Hz
15		8.74 (6 H, s)		6.23 (4 H, s)		6.33 (2 H, s)	

[a] The spectra were measured on a Varian A-60 spectrometer. Chemical shifts are given in τ values (tetramethylsilane as internal or external (D_2O) standard).

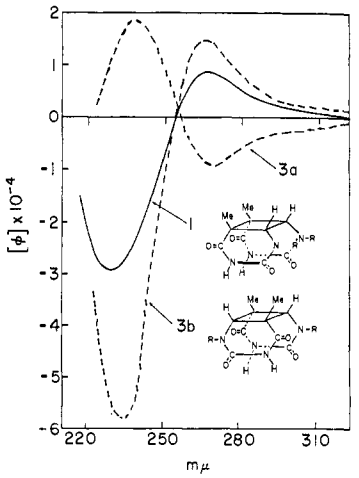


Figure 2. ORD curves of thymidine dimers **1** (—) and **3a** and **3b** (- - -) (R = 2-deoxyribofuranosyl) in MeOH.

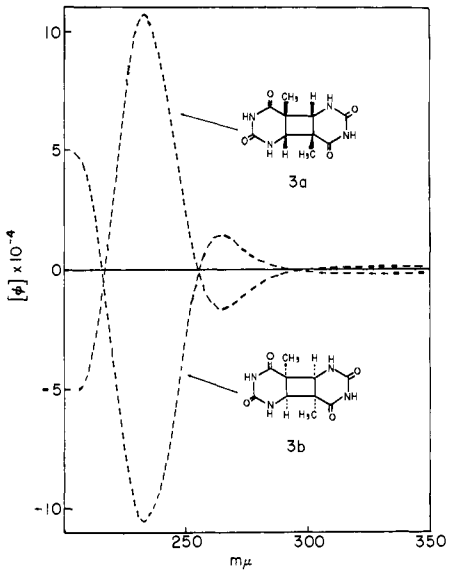


Figure 3. ORD curves of the optically active photodimers **3a** and **3b**. The assignments are tentative.

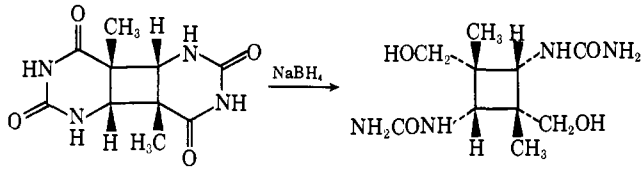
furanosyl), were isolated from the irradiated frozen solution of thymidine[12] and purified by chromatography on silica gel. Their ORD spectra are shown in Figure 2. Mild acid hydrolysis of these dimers gave optically inactive dimer **1** (R = H), (+)-dimer **3a**, $[\alpha]^{25}D$ +94° (H_2O), and (−)-dimer **3b**, $[\alpha]^{25}D$ −92° (H_2O). In addition we observed the formation of a new fluorescent product, λ_{max}^{MeOH} 318 nm, which presumably corresponds to Wang's adduct ($\lambda_{max}^{H_2O}$ 316 nm).[15,16]

The ORD spectra of (+)-**3a** (R = H) and (−)-**3b** (R = H) exhibited Cotton effects with a strong intensity, *i.e.*, $|[\phi]_P - [\phi]_T|/100$ ($a = 1.2 \times 10^5$), around 250 nm (Figure 3), probably due to the intensive n–π^* transitions of the ureidocarbonyl group in analogy to dihydrothymine[27] and cyclothymine nucleosides.[26] Al-

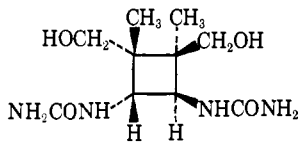
though ORD spectra of nucleosides and bound nucleotides have been recorded and correlated with conformational structures in connection with biological problems,[29] no such data have been available on ORD spectra of thymine dimers and their derivatives. Absolute configurations of these dimers are tentative at present and are assigned as shown on the basis of the ORD curve of (*S*)-(−)-dihydrothymine whose configuration has been established recently.[27]

The ORD curves of thymidine dimers **1**, **3a**, and **3b** (R = 2-deoxyribofuranosyl) also showed Cotton effects in the same region (Figure 2). The sign of the Cotton effect depends only on the configuration of the dimeric pyrimidine part.

In the same way as dimer **4**, optically active thymine dimer **3b** (R = H) smoothly and quantitatively underwent reductive ring opening with excess sodium borohydride to give a cyclobutane derivative, mp 211°. The nmr spectrum (D_2O) showed three sharp singlet peaks at τ 8.70, 6.31, and 6.20 with an intensity ratio of 3:1:2, assignable to methyl, cyclobutane ring methine, and methylene protons, respectively.



(−)-**3b** **15**



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The optical inactivity, shown by the ORD technique, of **15** provides conclusive evidence of the trans-syn structure for both **15** and dimer **3**. The cis-anti dimer **2** (R = H) should be optically active and would have retained optical activity on ring opening to **16**. Another unequivocal proof for the racemic trans-syn dimer has been given recently by X-ray analysis on the fully methylated dimer.[14]

On hydrogenolysis with sodium borohydride, thymidine dimers **1** and **3** (R = 2-deoxyribofuranosyl) gave several spots, which on spraying with the modified Ehrlich reagent gave pink spots on tlc. This suggests hydrogenolysis of the pyrimidine rings, but the paucity of the products did not permit isolation and further characterization. These hydrogenolytic reactions with irradiated nucleotides and sodium borotritide have shown a linear relationship between irradiation and incorporation of tritium.[19]

Experimental Section

Melting points are uncorrected. Infrared spectra were measured in Nujol mulls. Nmr spectra were determined on a Varian A-60 spectrometer and chemical shifts and coupling constants (J) are given in τ values and hertz, respectively. Ultraviolet spectra and ORD curves were taken on a Cary 15 and a Cary 60 spectrophotometer, respectively. The modified Ehrlich reagent was prepared by dissolving *p*-dimethylaminocinnamaldehyde (1%) in 0.5 *N* hydrochloric acid.

Thymine Dimer (1, R = H). This dimer was obtained by irradiation of a frozen solution of thymine. Recrystallization from water gave colorless needles, mp >300°. The spectrum showed end absorption only. The ir spectrum was identical with that of Weinblum's dimer A:[12] nmr (DMSO-d_6) 8.67 (6 H, s, CH_3), 6.24 (2 H, s, cyclobutane ring protons). This dimer was stable to acid and base at room temperature.

Thymine Dimer (4, R = H). A solution of thymine (2 g) in 80% acetone (500 ml) was irradiated internally with a water-cooled Hanovia high-pressure mercury lamp for 20 hr.[24] The precipitate (400 mg) was collected by filtration. Recrystallization from water gave dimer **4** as colorless prisms, mp >300°. The ir spectrum was identical with that of Weinblum's dimer D.[12]

4,5-*trans*-Dihydroxy-4a,4b-*cis*-dimethyldecahydrocyclobuta[1,2-*d*:4,3-*d'*]dipyrimidine-2,7-dione (6). A suspension of thymine dimer **1** (R = H, 2.8 g) and of excess sodium borohydride (1.8 g) in water (250 ml) was stirred at room temperature overnight. The reaction mixture became a clear solution after several hours. Excess hydride was decomposed with acetone and the mixture was filtered through a column of Amberlite, IRC-50 (H^+) resin. The filtrate was evaporated to dryness *in vacuo* and boric acid was removed by repeated flash evaporation with methanol. The residue was washed with hot methanol and the insoluble dicarbinol **6** (2.4 g) was collected. The methanol-soluble products were chromatographed on silica gel and eluted with methanol–chloroform (1:1) to give more **6** (120 mg) in addition to the monoalcohol **5** (150 mg) and the dialcohol **7** (300 mg), to be described below.

The combined fractions containing **6** were recrystallized from water or aqueous ethanol to give colorless prisms: mp 258–260° dec; yield 2.1 g (74%); ir 3400 (s), 3200 (m), 1695 (m), 1670 (s), 1645 (m), 1620 (s), 1530 (s), 1020 (s) cm^{-1}. The mass spectrum showed no parent ion peak.

Anal. Calcd for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29; N, 21.87. Found: C, 46.66; H, 6.16; N, 21.56.

6,7-Dimethyl-7-hydroxymethyl-8-carbamido-2,4-diazabicyclo-[4.2.0]octane-3,5-dione (5). This was obtained as a minor product from the reaction of dimer **1** (R = H) with sodium borohydride as described above. Recrystallization from ethanol gave **5** as colorless crystals, mp 207–208°. The uv spectrum showed λ_{max} 238 nm in 1.0 *N* NaOH, which disappeared in a few minutes; ir 3400, 1760, 1680, 1630, 1560 cm^{-1}.

Anal. Calcd for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29; N, 21.87. Found: C, 46.84; H, 6.22; N, 21.69.

3,4(α)-*cis*-Hydroxymethyl-3,4(β)-*cis*-dimethyl-1,2(α)-*cis*-dicarbamidocyclobutane (7). A. From Dimer 1. Dimer **1** (R = H, 0.8 g) was treated with sodium borohydride (1.3 g) in water at room temperature for 20 hr. After the work-up described for **6**, the products were separated by chromatography on silica gel with $CHCl_3$–MeOH (4:6) to give **5** and **6** (140 mg), and **7** (510 mg). Recrystallization of the main product from methanol gave the cyclobutane **7** as colorless prisms: mp 207–208°; ir 3400 (NH, OH), 1665 and 1645 (ureido), 1560 (amide II), and 1040 (C–O) cm^{-1}.

Anal. Calcd for $C_{10}H_{20}N_4O_4$: C, 46.14; H, 7.75; N, 21.53. Found: C, 45.91; H, 7.45; N, 21.54.

B. By Hydrogenolysis of Monoalcohol 5. Monoalcohol **5** (50 mg) was stirred with sodium borohydride (100 mg) in water (10 ml) at room temperature overnight. The crude product was obtained as a semisolid free of starting material and showed the properties of dialcohol **7** on tlc (silica gel). Recrystallization from ethanol gave colorless prisms, mp 205–210°, identical with **7** obtained from dimer **1** (ir spectrum).

C. By Hydrogenolysis of the Dicarbinol 6. Dicarbinol **6** (0.9 g) was treated with sodium borohydride (3.0 g) as described above. After purification by chromatography on silica gel (MeOH–$CHCl_3$, 1:1), the cyclobutane **7** was obtained in 71% yield (ir spectrum).

Treatment of **7** with trifluoroacetic acid followed by evaporation gave a colorless solid, mp 165°, presumably the bistrifluoroacetate of **7**, whose ir spectrum showed peaks at 1780 and 1650 cm^{-1} and no band at 1040 cm^{-1}.

Attempted Hydrogenolysis with Sodium Borohydride in Pyridine.[23] A mixture of dimer **1** (R = H, 80 mg) and sodium borohydride (100 mg) was gently refluxed in pyridine (8 ml) for 20 hr. Pyridine was removed *in vacuo*. The residue was dissolved in water and passed through a Bio-Rex 70 column (H^+). The filtrate was evaporated *in vacuo* to leave a colorless solid (60 mg) identical with

(29) T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *Biochemistry*, 6, 843 (1967), and references cited therein.

the starting material **1** (ir spectrum). Tlc showed only traces of the reduction products.

2,4-*trans*-Dihydroxymethyl-2,4-*trans*-dimethyl-1,3-dicarbamidocyclobutane (13). A mixture of thymine dimer **4** (R = H, 100 mg) and sodium borohydride (65 mg) in water (50 ml) was stirred at room temperature for 5 hr. The precipitate (60 mg) was collected by filtration and the filtrate was passed through an IRC-50 column (H^+). The eluent was evaporated to dryness *in vacuo* and the residue repeatedly flash evaporated with methanol. Extraction with methanol gave more **13**, in all 90 mg (90%). Recrystallization from aqueous methanol gave dialcohol **13** as colorless small prisms, mp >300°. The ir spectrum showed strong bands at 3400, 3250, 1655 (ureido carbonyl), 1530 (amide II), and 1050 (C–O) cm^{-1}.

Anal. Calcd for $C_{10}H_{20}N_4O_4$: C, 46.14; H, 7.75; N, 21.53. Found: C, 46.22; H, 8.06; N, 21.31.

In analogy to **7**, treatment of **13** with trifluoroacetic acid gave a bistrifluoroacetate as a colorless solid, mp 185°, which showed bands at 1790 and 1650 cm^{-1} and no band at 1050 cm^{-1} in the ir spectrum.

1,3-Dimethyl-2,4-diureido-1,3-cyclobutanedicarboxylic Acid (14). Thymine dimer **4** (R = H, 100 mg) was dissolved in 1.0 *N* NaOH (5 ml) and kept at room temperature for 2 hr. The solution was passed through a Dowex-50 column (H^+) and the eluent evaporated to dryness *in vacuo*. The residue was crystallized from water to give cyclobutanedicarboxylic acid **14** as colorless prisms (80% yield), mp 243–244° dec (lit.[24] mp *ca.* 270°). The ir spectrum showed bands at 3450, 3320 (NH), 1720 (COOH), 1640 (ureido carbonyl), and 1530 cm^{-1} (amide II).

Anal. Calcd for $C_{10}H_{16}N_4O_6$: C, 41.66; H, 5.59; N, 19.44. Found: C, 41.76; H, 5.31; N, 19.28.

2-Hydroxymethyl-1,2-*cis*-dimethyl-3,4-*cis*-diureidocyclobutanecarboxylic Acid (8). Monoalcohol **5** (100 mg) was dissolved in 1.0 *N* NaOH (10 ml) and kept at room temperature overnight. The uv spectrum indicated the rapid disappearance of absorption at 238 nm, suggestive of the opening of the pyrimidine ring. The solution was passed through an IRC-50 (H^+) column and the eluent lyophilized to leave a colorless solid, which was contaminated with a small amount of the starting material **5**.

Attempts to obtain compound **8** crystalline were unsuccessful, but acid treatment resulted in quantitative reclosure to **5**.

6,7-Dimethyl-3,5-dioxo-8-ureido-2,4-diazabicyclo[4.2.0]octane-7-carboxylic Acid (12). To a suspension of **5** (280 mg) in water (50 ml) was added an aqueous solution of $KMnO_4$ (500 mg). The mixture was stirred at room temperature for 22 hr. After decomposition of excess permanganate with methanol, the precipitated manganese dioxide was filtered off and washed with hot water. The combined filtrate and washing were passed through an IRC-50 column (H^+). The eluent was evaporated *in vacuo* to give a colorless solid, which was washed with methanol to give dimer **1** (R = H, 20 mg). The methanol-soluble products were chromatographed on silica gel with MeOH–$CHCl_3$ (1:1) to give, in addition to starting material **5**, the monoacid **12** (70 mg, mp 238–240°), which gave a pink color with the modified Ehrlich reagent: uv $\lambda_{max}^{1.0\ N\ NaOH}$ 236 nm; ir 3350, 1710, 1670, 1580, and 1520 cm^{-1}.

When compound **12** was heated in 10% HCl for 2 hr and evaporated and the residue crystallized, dimer **1** (R = H) was quantitatively recovered (R_f = 0.26; BuOH–AcOH–H_2O, 80:12:30).

Photolysis of 12. A solution of **12** (20 mg) in water (20 ml) was irradiated for 30 min. The uv spectrum of the mixture showed the appearance of strong absorption at 265 nm, due to the formation of thymine. The irradiated mixture was hydrogenated on 10% Pd/C (50 mg) at 25 lb of hydrogen pressure for 20 hr at room temperature. Dihydrothymine, mp 261°,[27] was isolated by chromatography on silica gel (MeOH–$CHCl_3$). The second product corresponded to β-ureidoisobutyric acid on tlc (silica gel) in two different solvent systems (MeOH–$CHCl_3$–AcOH, 10:10:1, and *n*-ProOH–H_2O, 7:3).

1,2-*cis*-Dimethyl-3,4-*cis*-diureido-1,2-cyclobutanedicarboxylic Acid (10). To a solution of diol **7** (300 mg) was added permanganate (450 mg) in water (30 ml) and the mixture was stirred at room temperature for 17 hr. After the decomposition of excess permanganate with ethanol, the precipitate was filtered off. The combined filtrate and washings (pH 8–9) were passed through an IRC-50 (H^+) column. The eluent was evaporated *in vacuo* to give a colorless glass which showed two spots on silica gel tlc ($CHCl_3$–MeOH, 1:3). This solid was chromatographed on silica gel. Compound **5** was eluted with a mixture of $CHCl_3$ and MeOH (1:4) as the major product (220 mg), mp 207–208°. The diacid **10** (85 mg) was eluted with a mixture of *n*-propyl alcohol and water (10:1) in impure form. The ir spectrum showed bands at 3300, 1650, and 1530 cm^{-1}.

When **10** was dissolved in 10% HCl (10 ml) and heated at 100° for 2 hr, removal of the solvent and recrystallization from water gave thymine dimer **1** (R = H) as colorless needles, whose ir spectrum was identical with that of an authentic sample.

4a,4b-*cis*-Dimethyl-5-hydroxyoctahydrocyclobuta[1,2-*d*:4,3-*d'*]dipyrimidine-2,4,7(3*H*)-trione (9). Oxygen gas was bubbled into an aqueous solution of compound **6** (100 mg) in the presence of Pt black (200 mg) under vigorous stirring overnight. The catalyst was filtered off and washed with hot water. The combined filtrate and washings were evaporated to dryness *in vacuo*. The resulting solid was washed with a mixture of methanol and water to leave a colorless solid, mp 280–285° (*ca.* 60 mg). Recrystallization from water gave colorless prisms, mp 285–288° dec. The ir spectrum showed bands at 3500, 3350 (OH, NH), 1730, 1670 (ureido carbonyl), and characteristic bands at 970, 960, 930, and 910 cm^{-1}.

Aminocarbinol **9** was also formed as a minor product during the hydrogenolysis of dimer **1** (R = H) with sodium borohydride and as a main product in the oxidation of **6** with permanganate in 1.0 *N* potassium hydroxide.

Thymidine Dimers. These dimers were prepared by irradiation of frozen solutions of thymidine and separated into three fractions (A, B, and C[12]) by chromatography on Dowex 1-X8 (formate) resin with gradient formate as an eluent. Each fraction was neutralized by passing through an IR-120 (H^+) column and subsequently a CG-45 column. Evaporation of the solvent under 40° *in vacuo* gave a pale yellow oil.

Thymidine Dimer 1 (R = 2-Deoxyribofuranosyl). The combined residues from the first and second fractions (A and B) were dissolved in methanol, chromatographed on silica gel, and eluted with *n*-propyl alcohol–ammonia (10:1). The product eluted first was rechromatographed on silica gel in methanol to afford a colorless hygroscopic solid. Recrystallization from ethanol gave thymidine dimer **1** as colorless crystals, mp 184–188°, $[\alpha]^{25}D$ +25° (*c* 0.4, methanol). The uv spectrum showed end absorption only. After uv irradiation for 10 min, absorption at λ_{max}^{MeOH} 267 nm (ε 6200) returned, indicative of regeneration of thymidine monomer: ORD (*c* 0.4, MeOH) $[\alpha]_{267}$ +1800 (p), $[\alpha]_{254}$ 0, $[\alpha]_{228}$ −6000 (t), $[\alpha]_{220}$ −4400.

Anal. Calcd for $C_{20}H_{28}N_4O_{10}\cdot H_2O$: C, 47.81; H, 5.98; N, 11.16. Found: C, 48.03; H, 6.02; N, 11.64.

Hydrolysis with 10% HCl gave pure optically inactive thymine dimer **1** (R = H) whose identity was confirmed by comparison of ir spectra and R_f values (R_f = 0.45, isopropyl alcohol–H_2O–ammonia, 7:2:1).

Thymidine Dimer 3a (R = 2-Deoxyribofuranosyl). Dimer **3a**, obtained from the same column by further elution with methanol, was rechromatographed on silica gel in ethanol to afford a colorless hygroscopic solid which still showed two spots on paper chromatography (R_f = 0.54 and 0.61, isopropyl alcohol–H_2O–ammonia, 7:2:1). The compound with R_f = 0.61 showed strong fluorescence under uv light. Dimer **3a** was further purified by chromatography (silica gel–methanol) to give a colorless hygroscopic solid, which was still contaminated with a small amount of the fluorescent compound: ORD (*c* 0.7, MeOH) $[\alpha]_{268}$ −1900 (t), $[\alpha]_{256}$ 0, $[\alpha]_{238}$ +4000 (p), $[\alpha]_{225}$ +800.

Anal. Calcd for $C_{20}H_{28}N_4O_{10}$: C, 49.58; H, 5.83; N, 11.57. Found: C, 49.90; H, 6.13; N, 11.45.

The strongly fluorescent compound (R_f = 0.61) was extracted with methanol from paper chromatograms and showed λ_{max}^{MeOH} 318 nm.

Thymidine Dimer 3b (R = 2-Deoxyribofuranosyl). Dimer **3b** was obtained from fraction C and purified by chromatography on silica gel in analogy to dimer **3a**. Dimer **3b** had mp 150° and was hygroscopic: $[\alpha]^{25}D$ +45° (*c* 0.8, MeOH); ORD (*c* 0.6, MeOH) $[\alpha]_{266}$ +3300 (p), $[\alpha]_{254}$ 0, [a]$_{234}$ −12,000 (t), $[\alpha]_{225}$ −8200. Dimer **3b** showed only end absorption which on irradiation became a peak at 265 nm.

Anal. Calcd for $C_{20}H_{28}N_4O_{10}$: N, 11.57. Found: N, 11.15.

Dextro- and Levorotatory Thymine Dimers (3a and 3b, R = H). Thymidine dimers **3a** and **3b** (R = 2-deoxyribosyl) were hydrolyzed with 10% HCl at 90° for 20 min and, after removal of the solvent, the residue was crystallized from water to give thymine dimers (+)-**3a** and (−)-**3b** as colorless prisms. The ir spectra were identical with those of Weinblum's dimer C:[12] (+)-**3a**, $[\alpha]^{25}D$ +94.1° (*c* 0.55, H_2O); ORD (*c* 0.55, H_2O) $[\alpha]_{298}$ 0, $[\alpha]_{265}$ −6400 (t), $[\alpha]_{256}$ 0, $[\alpha]_{233}$ +42,000 (p), $[\alpha]_{217}$ 0, $[\alpha]_{210}$ −17,000; (−)-**3b**; $[\alpha]^{25}D$ −92° (*c* 0.64, H_2O); ORD (*c* 0.47, H_2O) $[\alpha]_{307}$ 0, $[\alpha]_{265}$ +5900 (p), $[\alpha]_{256}$ 0, $[\alpha]_{233}$ −41,200 (t), $[\alpha]_{217}$ 0, $[\alpha]_{210}$ +16,000.

2,4(α)-*cis*-Dihydroxymethyl-2,4(β)-*cis*-dimethyl-1,3(α)-*cis*-dicarbamidocyclobutane (15). A mixture of (−)-thymine dimer **3b** (R = H, 20 mg, $[\alpha]_D$ −92° (H_2O)) and sodium borohydride (64 mg) in water (3 ml) was stirred at room temperature for 22 hr. After decomposition of excess hydride with acetone, the solution was adjusted to pH ~5 by the addition of IRC-50 (H^+) resin. Boric acid was removed as methyl ester and the product was purified by chromatography on silica gel and eluted with MeOH–$CHCl_3$ (1:1). The product, a colorless solid (12 mg), was shown to be completely optically inactive in the region 210–700 nm. Recrystallization from methanol gave colorless prisms, mp 210–211°. The ir spectrum had absorption peaks at 3350 (NH, OH), 1650 (ureido carbonyl), 1580, 1530, and 1030 cm^{-1}, which were similar to those of **13**.

Anal. Calcd for $C_{10}H_{20}N_4O_4 \cdot 0.5H_2O$: C, 44.61; H, 7.81; N, 20.82. Found: C, 44.96; H, 7.64; N, 20.74.

Hydrogenolysis of Thymidine Dimers 1 (R = 2-Deoxyribofuranosyl). A mixture of dimer **1** (100 mg) and sodium borohydride (150 mg) in water (15 ml) was stirred at room temperature for 40 hr. After the usual work-up, several products which gave a pink color with the modified Ehrlich reagent were detected on tlc (silica gel; MeOH–$CHCl_3$, 1:1). Three products (mp 149–151, ~150, and 149–154°) were obtained in addition to 2-deoxyribose (chromatography on silica gel, $CHCl_3$–MeOH, 3:2). Acid hydrolysis of the products yielded the monoalcohol **5** and the dicarbinol **6** (tlc on silica gel), spraying with modified Ehrlich reagent. Hydrogenolysis of thymidine dimer **3** (R = 2-deoxyribofuranosyl) gave several products which were positive to the modified Ehrlich reagent.

Free Energies of Hydrolysis of Amides and Peptides in Aqueous Solution at 25°

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Abstract: The free energies of hydrolysis of a series of formamides have been determined at 25°. The equilibrium constants (based on the concentration of un-ionized reagents and on an activity of 1.0 for water) for the formation of formamides of hydrazines and primary and secondary alkylamines follow the equation $\log K_{eq} = 0.5 + 0.51 \cdot pK_{a(amine)}$, while the amides of anilines and hydroxylamines are 1.4 kcal, and of ammonia 3 kcal, less stable than predicted. The results are extended to the general case of peptide formation. The "α" effect is discussed and shown to be independent of the stability of the ground-state reagents in the case of hydrazinolysis.

A knowledge of the free energies of hydrolysis of amides and peptides is fundamental to the understanding of biochemical equilibria. However, due to the experimental difficulty of very slow uncatalyzed rates of reaction, a few random equilibrium constants only have been accurately determined. Apart from one study at elevated temperatures,[1] the effect of the structure of the amine moiety on the hydrolysis equilibria of amides has not been systematically investigated.

The few equilibrium constants known do not fall into a simple pattern, except for the generalization that they are virtually independent of the structure of the acyl portion of the amide.[1,2]

Much attention has been paid to the enzymatic catalysis of amide and peptide hydrolysis through the studies on the mechanism of action of the proteolytic enzymes, notably chymotrypsin. However, due to the experimental difficulties of very slow rates and the masking of the water reaction by the acid- and base-catalyzed reactions, there is little known about the solvolysis of amides, apart from some interesting intramolecular acyl transfers.[3]

In a recent paper[4] we have emphasized the use of equilibrium constants for acyl-transfer reactions, derived from free energy of hydrolysis data, to calculate rate constants for otherwise inaccessible reactions. The determination of the amide equilibria also gives the solvolysis rates, provided the ester aminolysis rates are known.

Experimental Section

Materials. Organic reagents were distilled or recrystallized prior to use. Deionized water was used throughout. *N*-Formylmorpholine (bp 92.5° (3.5 mm) (lit.[5] 103–104° (13 mm)); ir (film) ν_{max} 1675 cm^{-1} (lit.[5] 1675 cm^{-1})) and formylhydrazine (mp 57–59° (lit.[5] 57–59°)) were synthesized by the method of Blackburn and Jencks.[5] Formhydroxamic acid (mp 75–77° (lit.[6] 77.5°)) was synthesized by the method of Bernhard, *et al.*[6]

Equilibrium Constants. Solutions were thermostated at 25.0 ± 0.01° either in stoppered test tubes in a constant-temperature bath or in the cuvettes in the Gilford 2400 spectrophotometer.

The equilibria between semicarbazide or thiosemicarbazide and the formyl derivatives were measured by assaying directly for the semicarbazide or thiosemicarbazide, the experimental concentrations being given in Table I.

The following assay was developed. Semicarbazide and thiosemicarbazide were found to give adducts with 1:2 napthaquinone-4-sulfonic acid (NSA) giving visible maxima at 460 nm. (This reagent has been used as a spot test.)

To 5.0 ml of 10^{-3} *M* NSA were added an aliquot of sample and sufficient sodium hydroxide to give a final concentration of 10^{-2} *M* hydroxide ion on addition of water to give a final volume of 10 ml. (Both the formation and decomposition of the adduct are base catalyzed and the concentration of 10^{-2} *M* NaOH was found to be most satisfactory.)

The absorbance at 460 nm was measured after 2.0 min. Under these conditions the semicarbazide adduct has an extinction coefficient of 8.2×10^3 and the thiosemicarbazide 13×10^3. Beer's

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