1 hr. The reaction mixture was poured into crushed ice and an almost colorless substance separated. On crystallization of the substance from benzene, IX, mp 217°, was obtained (20 mg): $\nu_{max}^{KBr}$ 3400, 1750, 1660 cm$^{-1}$.

*Anal.* Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.78; H, 4.82; N, 5.37.

**Decarbonylation of VII.**—The aldehyde VII (40 mg) was mixed with Pd/C (20 mg) and heated in a sealed tube with 1 ml of dry alcohol for 15 min at 270° under vacuum. The residue obtained, after removal of solvent from the alcoholic extract of the reaction product, on crystallization from benzene furnished crystals of X melting at 243°. The compound was soluble in 1% alkali and gave an olive green color with alcoholic ferric chloride solution. This was found identical with 2-hydroxy-3-methylcarbazole (mixture melting point, uv) and different from 2-hydroxy-6-methylcarbazole (mixture melting point 210–220°): $\lambda_{max}^{ethanol}$ 236 mμ (log ε 4.72), 258 (4.31), 302 (4.24).

*Anal.* Calcd for $C_{13}H_{11}NO$: C, 79.17; H, 5.62; N, 7.10. Found: C, 79.03; H, 5.57; N, 7.10.

**Methylation of X.**—The phenol X (40 mg) in methanol (15 ml), on treatment with diazomethane and keeping in a refrigerator for 16 hr, furnished a semisolid mass. This was dissolved in benzene and chromatographed over alumina (3 g). From the fractions collected with benzene as eluent, a colorless solid (Xa) was obtained which on crystallization from a mixture of benzene and petroleum ether melted at 225° (yield, 15 mg).

*Anal.* Calcd for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63. Found: C, 79.54; H, 6.08; N, 6.81.

**Reduction of Dihydromurrayacine to XIII.**—A solution of XII (5 mg) in tetrahydrofuran (10 ml) was slowly added to a suspension of $LiAlH_4$ (1 gm) in tetrahydrofuran (7 ml). The mixture was refluxed for 3 hr. The $LiAlH_4$ was decomposed and the reaction mixture was extracted with ether. The ether layer was washed with water and dried, and solvent was removed from it. A solid, mp 176°, identical with dihydrogirinimbine (mixture melting point, tlc, uv) was obtained. No analysis was possible.

**Zinc Dust Distillation of an α-Hydroxy Acid VIII. Formation of 3-Methylcarbazole.**—The compound VIII (100 mg) was mixed with zinc dust (7.5 g) and distilled by the method and procedure described previously.[4] On working up the reaction product, a compound, mp 207°, was obtained. This was identified as 3-methylcarbazole[5] (mixture melting point, uv).

**Registry No.**—II, 27300-29-4; II 2,4-DNP, 27300-30-7; IVa, 27300-31-8; V, 23095-44-5; VIII, 27300-33-0, IX, 27300-34-1; X, 24224-30-4; Xa, 24224-28-0; XII, 17750-37-7.

**Acknowledgment.**—The authors thank Professor S. M. Sircar, Director, Dr. D. M. Bose, and Dr. A. Sen, Head of Department of Chemistry, for their interest in the work. The financial supports partly by C.S.I.R. and partly by NIS (India) are gratefully acknowledged, The authors acknowledge their indebtedness to Dr. B. C. Das of the Institute-de-Chimie des Substances, Gif-Sur-Yvette, France, for nmr and mass spectra.

## The Preparation and Properties of Some Cytosine Derivatives

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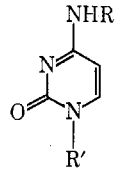
*Received July 6, 1970*

In order to develop a method for the preparation of some O-acylated derivatives of cytosine, in particular, 2′,3′,5′-tri-*O*-benzoylcytidine (IV), a study was made of some 4-N-acylated cytosines in an attempt to obtain a derivative which could be deacylated at 4 N using conditions under which the sugar benzoyl groups would be stable. The only methods available for obtaining partially acylated derivatives of cytidine give the 4-*N*-acyl derivative as the final product[1] or partially O-acylated derivatives.[2] A preliminary communication of part of this work has already appeared,[3] and from this study it was apparent that in aqueous media, of the derivatives investigated (4-*N*-trifluoroacetyl-, trichloroacetyl-, dichloroacetyl-, monochloroacetyl-, and acetylcytosine), only the latter two would be of use as a protecting group for 4 N because of the lability of the other derivatives.

4-*N*-Chloroacetylcytosine (I) was prepared as previously described.[3] This was easily converted into cytosine under mild acidic conditions, the time for 50% hydrolysis in 0.1 *N* HCl at 20° being 13.5 min, compared with 130 min for the hydrolysis of 4-*N*-acetylcytosine (I). The compound was indefinitely stable in dry ethanol and dry pyridine. It has previously been reported[4] that the acidic deacylation of 4-*N*-acetylcytosine (80% acetic acid) gave a mixture of cytosine and uracil in equal amounts. In the present study, no chromatographic evidence was found for the presence of any uracil (<5%) in the acidic hydrolysate of either 4-*N*-chloroacetyl- or 4-*N*-acetylcytosine.

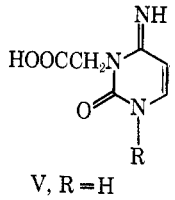


I, $R^1$ = H; R = $CH_2ClC(=O)-$

II, $R^1$ = 2′,3′,5′-tri-*o*-chloroacetylribose; R = $CH_2ClC(=O)-$

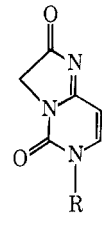
III, $R^1$ = 2′,3′,5′,-tri-*o*-benzoylribose; R = $CH_3C(=O)-$

IV, $R^1$ = 2′,3′,5′,-tri-*o*-benzoylribose; R = H



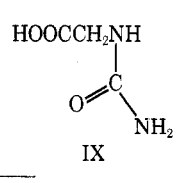
V, R = H

VI, R = ribose

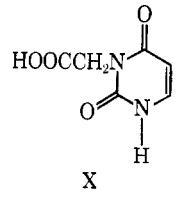


VII, R = H

VIII, R = 2′,3′,5′-tri-*o*-chloroacetylribose



IX



X

(1) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *J. Amer. Chem. Soc.*, **85**, 3821 (1963); K. A. Watanabe and J. J. Fox, *Angew. Chem.*, **78**, 579 (1966); G. Keith and J. P. Ebel, *Biochim. Biophys. Acta*, **166**, 16 (1968).

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The normal method for the removal of the 4-*N*-acetyl group from cytosine is to use alkaline conditions, the time for 50% hydrolysis in 0.01 *N* KOH at 20° being 340 min; and in 15% $NH_3$ in methanol, 57 min.[3] It was hoped that the 4-*N*-chloroacetyl group would be removed even faster under these conditions, particularly as it had been shown that this was sufficient to instantaneously deacylate the trifluoroacetyl-, trichloroacetyl-, and dichloroacetylcytosines. However, a product was isolated in fair yield which was shown by its uv and nmr spectra and elemental analysis to be the imidazopyrimidine (VII), 1*H*-2,3-dihydro-2,5-dioxo[1,2-*c*]pyrimidine, formed by a cyclization reaction between $N^3$ of the pyrimidine ring and the chloroacetyl group with the elimination of hydrogen chloride. Confirmation of the identity of this compound was obtained from its acidic hydrolysis to 3-carboxymethylcytosine (V) and its alkaline hydrolysis to 3-carboxymethyluracil (X). Ueda and Fox[5] have observed a similar cyclization with 4-*N*-chloropropionylcytosine. The bicyclic product gave only cytosine on acidic or alkaline hydrolysis, which is surprising in view of the results obtained here. The same paper[5] described the synthesis of several pyrimido[1,2-*c*]pyrimidines and those which contained the 4-*N*-acyl-$N^3$-alkylcytosine structure were easily hydrolyzed to 3-(2-carboxyethyl)-cytosine and the corresponding uracil derivative. Similar derivatives of some *C*-alkylcytosines[6] and some purines[7] have been reported.

Thus the chloroacetyl group might be of use as an amino blocking group for cytosine, where a group which is more readily removed under acidic conditions than acetyl is required; however, the cyclization of 4-*N*-chloroacetylcytosine under basic conditions is a serious disadvantage to its use because it is impossible to regenerate cytosine from the cyclized product.

The corresponding acylated nucleoside was investigated in order to see whether the same properties were present. 4-*N*-chloroacetyl-2′,3′,5′-tri-*O*-chloroacetylcytidine (II) was prepared by reacting cytidine with chloroacetic anhydride in DMF in the presence of potassium carbonate. The product, which was isolated in 80% yield, could not be obtained in a crystalline form but was chromatographically pure and had the elemental analysis and uv spectrum required for the tetrachloroacetylcytidine. As the compound was insoluble in water, the cyclization had to be done in DMF, but the cyclized product was less stable than the cytosine derivative. Very dilute alkali (0.001 *N* KOH) or acid (0.01 *N* HCl) gave 3-carboxymethylcytidine (VI), and the presence of the cyclized compound (VIII) could only be demonstrated by the uv spectrum of the solution. Acidic hydrolysis of the cyclized compound, followed by a mild alkaline hydrolysis to remove any *O*-acyl groups remaining, resulted in the isolation of 3-carboxymethylcytidine (VI) in fair yield (27%). No evidence for the production of any cytosine, uracil, or their 3-carboxymethyl derivatives was obtained. Thus no deamination or glycosyl bond cleavage had occurred during the acidic hydrolysis. Alkaline hydrolysis (1 *N* KOH at 100°) of the cyclized product gave 3-carboxymethylcytidine which, in turn, rapidly decomposed. The only product which could be detected in the hydrolysate apart from a small amount of 3-carboxymethycytidine was a compound which gave a positive reaction with Ehrlich's reagent when subjected to tlc and was therefore considered to be a urea derivative. This compound was also an acid and was thought likely to be *N*-carboxymethylurea (IX), formed from the typical alkaline degradation of 1,3-disubstituted pyrimidines.[8,9]

All attempts to selectively 4-N-acylate cytidine with chloroacetic anhydride failed, under conditions which have been used to selectively acetylate or benzoylate cytidine[2] or deoxycytidine.[10]

It appeared from the properties of the derivatives so far described that the best route to a 2′,3′,5′-tri-*O*-acylcytidine with the amino group unprotected would be the selective acid 4-N-deacylation of a 4-*N*-acyl-2′,3′,5′-tri-*O*-acylcytidine. It was found that the *O*-benzoyl group of 2′,3′,5′-tri-*O*-benzoyluridine[11] was stable to 0.1 *N* HCl at room temperature for up to 3 weeks, and thus the 4-N-debenzoylation of tetrabenzoylcytidine was attempted using 0.1 *N* HCl. The deacylation was followed by the changing uv spectrum of the solution and was seen to be almost complete in 12 days at room temperature. 2′,3′,5′-Tri-*O*-benzoylcytidine (IV) was isolated in 40% yield (32% overall yield from cytidine), and, in contrast to results obtained with the deoxycytidine derivative,[12] only 10% deamination to 2′,3′,5′-tri-*O*-benzoyluridine was detected in the reaction solution. No glycosyl bond cleavage was found.

2′,3′,5′-Tri-*O*-benzylcytidine was more readily prepared by the selective 4-N-deacylation of 4-*N*-acetyl-2′,3′,5′-tri-*O*-benzoylcytidine (III) which was made by the benzoylation of cytidine which had been selectively 4-N-acetylated by the method of Watanabe and Fox.[2] Only traces of 4-*N*-benzoyl-2′,3′,5′-tri-*O*-benzoylcytidine were produced, and the pure product (III) (53% overall yield from cytidine) was obtained after recrystallization from ethanol. This method is quicker and gives a better yield than the method of Fox, *et al.*,[13] which involves the condensation of 1-chloro-2,3,5-tri-*O*-benzoyl-D-ribofuranose with the mercury derivative of 4-*N*-acetylcytosine.

The acidic hydrolysis of 4-*N*-acetyl-2′,3′,5′-tri-*O*-benzoylcytidine was achieved in 0.1 *N* HCl at room temperature for 3 days. At the end of this time, the uv spectrum of the solution showed that no 4-N-acylated product was left. 2′,3′,5′-Tri-*O*-benzoylcytidine (IV) was isolated in almost quantitative yield (40% overall yield from cytidine), and no deaminated product or products formed by glycosyl bond cleavage were detected.

### Experimental Section

**4-*N*-Acetylcytosine** was prepared by the method of Wheeler and Johnson.[14] 2′,3′,5′-Tri-*O*-benzoyluridine was prepared by

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(10) B. A. Otter and J. J. Fox, "Synthetic Procedures in Nucleic Acid Chemistry," W. W. Zorbach and R. S. Tipson, Ed., Interscience, New York, N. Y., 1968, p 285.
(11) J. J. Fox, D. V. Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Amer. Chem. Soc.*, **81**, 178 (1959).
(12) H. G. Khorana, A. F. Turner, and J. P. Vizsolyi, *ibid.*, **83**, 686 (1961).
(13) J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *ibid.*, **79**, 5060 (1957).
(14) W. L. Wheeler, and T. B. Johnson, *Amer. Chem. J.*, **29**, 492 (1903).

the method of Fox, *et al.*[11] 4-*N*-Benzoyl-2′,3′,5′-tri-*O*-benzoylcytidine was prepared by the method of Brown, *et al.*[4] 4-*N*-Chloroacetylcytosine was prepared as previously described.[3]

**Acidic Hydrolysis of 4-*N*-Chloroacetylcytosine (I).**—I was dissolved in 0.1 *N* HCl to give a solution with an optical density at 244 nm of about 1.0. The uv spectrum of the solution at 20° was followed by observing the appearance of a new peak at 275 nm and the disappearance of the maximum at 298 nm. In this way the time taken for 50% hydrolysis was determined. The value was 13.5 min compared with 130 min for 4-*N*-acetylcytosine, as determined under the same conditions.

**Mild Alkaline Hydrolysis of 4-*N*-Chloroacetylcytosine (I).**— I (1.6 g) was heated in distilled water (200 ml) on a boiling water bath. The mixture was stirred vigorously and the pH of the solution was kept at 8 by the addition of 1 *N* KOH. When complete solution had been achieved and the pH was constant, the solution was concentrated to 80 ml and allowed to stand at room temperature. The crystals which formed were removed by filtration, washed with a little cold water, and dried to give a slightly brown crystalline product (0.7 g). The product was further decolorized and recrystallized from hot water using decolorizing charcoal to give a white crystalline product which was 1*H*-2,3-dihydro-2,5-dioxoimidazo[1,2-*c*]pyrimidine (VII): darkens rapidly above 80°, does not melt below 300°; λmax (at pH 1) 300 nm (ε 20,800); λmin (at pH 1) 260 nm (ε 3600); λmax (at pH 5) 302 nm (ε 21,000); λmin (at pH 5) 242 nm (ε 2600); λmax (at pH 14) 318 nm (ε 27,000); λmin (at pH 14) 270 nm (ε 1600); nmr spectrum (in trifluoroacetic acid) τ 5.31 (singlet, two protons), 3.4 (doublet, 5 H of pyrimidine ring), 1.8 (doublet, 4 H of pyrimidine ring) ($J_{4H,5H}$ = 7 cps). The compound did not move on electrophoresis at pH 3.5. *Anal.* Calcd for $C_6H_5N_3O_2$: C, 47.67; H, 3.34; N, 27.81. Found: C, 47.56; H, 3.10; N, 27.40.

**Hydrolysis of 1-*H*-2,3-Dihydro-2,5-dioxoimidazo[1,2-*c*]pyrimidine (VII). A. Acidic.**—VII (0.4 g) was dissolved in 1 *N* HCl (20 ml) and the solution allowed to stand at 37° for 14 hr. The solvent was removed by evaporation and the remaining crystalline solid redissolved in water and evaporated to dryness several times to remove most of the hydrochloric acid. The residue was dissolved in a small volume of water and acetone was added to give a slightly turbid solution. The solution, on standing at room temperature, deposited fine needles of 3-carboxymethylcytosine (V, 0.21 g) (as the hydrochloride): mp >300°; λmax (at pH 1) 277 nm (ε 7960); λmin (at pH 1) 242 nm (ε 2030); λmax (at pH 10.5) 297 nm (ε 9640); λmin (at pH 10.5) 250 nm (ε 1590);[5] nmr spectrum (in [$^2H_6$] dimethyl sulfoxide) τ 5.32 (singlet, two protons), 3.8 (doublet, 5 H), 2.3 (doublet, 4 H) ($J_{4H,5H}$ = 7 cps). The compound did not move on electrophoresis at pH 3.5. *Anal.* Calcd for $C_6H_7N_3O_3 \cdot HCl$: C, 35.04; H, 3.93; N, 20.44. Found: C, 35.20; H, 4.11; N, 20.82.

**B. Alkaline.**—VII (0.6 g) was dissolved in 1 *N* NaOH (20 ml), and the solution was heated at 100° for 1 hr and was passed down a column of Dowex 50 ($H^+$ form) which was washed with water until no more uv-absorbing material was eluted. The combined washings were evaporated to dryness, the residue was dried and dissolved in dry ethanol, and *n*-hexane added slowly to the solution. Crystals of 3-carboxymethyluracil (X, 0.3 g) were obtained: mp 222–224°; λmax (at pH 5.0) 260 nm (ε 7120); λmin (at pH 5.0) 229 nm (ε 2280); λmax (at pH 14) 285 nm (ε 10,730); λmin (at pH 14) 245 nm (ε 2280 );[6,9] nmr spectrum (in [$^2H_6$] dimethyl sulfoxide) τ 5.68 (singlet, two protons), 4.45 (doublet, 5 H), 2.64 (triplet, 4 H), 1.08 (doublet, 3 H). The compound had a mobility of 4.2 cm/hr on electrophoresis at pH 3.5. *Anal.* Calcd for $C_6H_6N_2O_4$: C, 42.36; H, 3.56; N, 16.47. Found: C, 42.20; H, 3.50; N, 16.80.

**4-*N*-Chloroacetyl-2′,3′,5′-tri-*O*-chloroacetylcytidine (II).**—Anhydrous cytidine (1 g) and chloroacetic anhydride (10 g) were dissolved in dry DMF (30 ml). Anhydrous potassium carbonate (10 g of a finely divided powder) was added to the stirred solution which was cooled in an ice bath. After the initial reaction had subsided, the mixture was allowed to stand at room temperature for 15 min. The potassium salts were removed by filtration, and the filtrate was poured into water (300 ml). After stirring for 1 hr, the mixture was extracted with chloroform, the extract dried with anhydrous magnesium sulfate, and the chloroform removed by evaporation to yield an oil which on repeated evaporation with dry methanol gave a stable dry foam. The foam was washed with ether and dried to give II (1.77 g) as a pale yellow powder. The product could not be obtained crystalline: λmax (in ethanol) 248 nm (ε 14,500) and 299 (5850); λmin (in ethanol) 227 nm (ε 2150) and 274 (1560). *Anal.* Calcd for $C_{17}H_{17}N_3O_9Cl_4$: C, 37.18; H, 3.13; N, 7.65; Cl, 25.81. Found: C, 37.76; H, 3.45; N, 8.00; Cl, 25.86.

An attempt was made to prepare 4-*N*-chloroacetylcytidine by the selective 4-N-acylation of cytidine. Cytidine (0.1 g) was dissolved in hot methanol (10 ml) and chloroacetic anhydride (0.1 g) was added. The solution was heated under reflux and examined at intervals by tlc and uv spectroscopy. No evidence for the presence of any uv-absorbing material other than cytidine was obtained at any time. More anhydride (0.3 g) was added and the experiment was repeated at room temperature, but no reaction was obtained.

**Preparation of the Imidazopyrimidine VIII.**—II (0.25 g) was dissolved in dry DMF (10 ml) and the solution heated at 90°. After 5 hr tlc on silica (2% ethanol in chloroform) showed the presence of only two uv-absorbing components. The slower components (70% of the total product) had the uv spectrum expected for 4-*N*-acyl-$N^3$-alkylcytidine with a λmin 258 nm and λmax 305 nm and was presumably the imidazopyrimidine VIII. The other faster component (30% of the total product) was unchanged starting material. All attempts to isolate VIII failed, as it partially decomposed on taking the solution to dryness and also decomposed on a silica plate to a compound which had the properties of 3-carboxymethyl-2′,3′,5′-tri-*O*-chloroacetylcytidine, which on treatment with ammonia gave 3-carboxymethylcytidine (VI) (see below).

**Hydrolysis of the Imidazopyrimidine VIII. A. Acidic.**—4-*N*-Chloroacetyl-2′,3′,5′-tri-*O*-chloroacetylcytidine (II, 1 g) was dissolved in DMF (20 ml) and heated for 5 hr at 90°. The solution was taken to dryness, the residue dissolved in acetone (40 ml), and 4 *N* HCl added to make the solution 1 *N* with respect to acid, and the solution was left at 37° for 24 hr. The solvent was removed by evaporation under reduced pressure and the HCl was removed by repeated evaporation to dryness with acetone. The residue was dissolved in water, ammonia (sp gr 0.880, 1 ml) was added, and the solution was allowed to stand for 1 hr, after which time the uv spectrum of the solution showed a maximum (pH 1) at 280 nm only. The solution was evaporated to dryness, redissolved in water (50 ml) which was adjusted to pH 9 with ammonia and applied to a column of Deacidite FF ($Cl^-$ form), and eluted with water (pH 5) until no more cytidine was obtained. The column was then eluted with 0.01 *N* acetic acid. One major uv-absorbing component was present in the eluate and this was further purified on a column (22 × 3 cm) of microcrystalline cellulose eluted with propan-2-ol–water (70:30). The fractions containing the major component were combined and evaporated to dryness, and the residue recrystallized from aqueous acetone to give 3-carboxymethylcytidine (VI) as colorless platelets (0.2 g, 27%): mp 225–227° dec; λmax (at pH 1) 279 nm (ε 8040); λmin (at pH 1) 240 nm (ε 2420); λmax (at pH 14) 266 nm (ε 7020); λmin (at pH 14) 245 nm (ε 2470).[15] *Anal.* Calcd for $C_{11}H_{15}N_3O_7$: C, 43.84; H, 5.03; N, 13.95. Found: C, 43.76; H, 5.09; N, 14.27. No evidence for the presence of cytosine, uracil, uridine, or 3-carboxymethyluridine was obtained.

**B. Alkaline.**—II (50 mg) was dissolved in DMF, heated at 90° for 5 hr, and then made 0.01 *N* with respect to NaOH. The compound (VIII) with a uv absorption spectrum characteristic of a 4-*N*-acyl-$N^3$-alkylcytidine was immediately replaced by a compound identified as 3-carboxymethylcytidine (V). In 1 *N* NaOH at 100° for 30 min, this compound rapidly decomposed in a manner typical of an $N^1$,$N^3$-disubstituted pyrimidine,[8] and the only compound which would be detected in the hydrolysate apart from a trace of 3-carboxymethylcytidine was a compound which gave a positive reaction with Ehrlich's reagent and which on electrophoresis in formate buffer (pH 4) exhibited the mobility of a monocarboxylic acid (5.5 cm/hr). The compound was likely to be *N*-carboxymethylurea (IX).

**Acidic Hydrolysis of 2′,3′,5′-Tri-*O*-benzoyluridine and 4-*N*-Benzoyl-2′,3′,5′-tri-*O*-benzoylcytidine.**—2′,3′,5′-Tri-*O*-benzoyluridine[14] (10 mg) was dissolved in ethanol (10 ml), 1 *N* HCl (1.1 ml) was added, and the solution was allowed to stand at room temperature. The material could be recovered unchanged after 3 weeks.

4-*N*-Benzoyl-2′,3′,5′-tri-*O*-benzoylcytidine[4] (0.5 g) was dissolved in a solvent containing chloroform (20 ml), ethanol (150 ml), and 1 *N* HCl (19 ml). The solution was allowed to stand at

(15) G. W. Kenner, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 855 (1955); T. Ueda and J. J. Fox, *J. Amer. Chem. Soc.*, **85**, 4024 (1963).

room temperature for 12 days and then evaporated to dryness under reduced pressure, and the ethyl benzoate present was removed by repeated evaporation with ethanol. Tlc on silica (10% ethanol in benzene) showed the presence of three uv-absorbing components in the mixture. Two minor compounds accounting for about 10% of the total product were unchanged starting material and 2′,3′,5′-tri-*O*-benzoyluridine. The third compound was isolated in a pure state by recrystallization of the hydrolysis products from boiling chloroform–ethanol (1:4) to which petroleum ether (60–80° fraction) had been added to incipient cloudiness, and the solution was allowed to cool. A flucculent white precipitate of 2′,3′,5′-tri-*O*-benzoylcytidine (IV) (as the hydrochloride) was collected and dried (0.22 g, 32% overall yield from cytidine): mp 226–227° dec; λmax (in ethanol) 230 nm (ε 29,500) and 280 (9500); λmin (in ethanol) 253 nm. *Anal.* Calcd for $C_{30}H_{25}N_3O_8 \cdot HCl$: C, 60.87; H, 4.44; N, 7.10. Found: C, 60.80; H, 4.43; N, 7.11. No evidence for any glycosyl bond cleavage was obtained.

**4-*N*-Acetyl-2′,3′,5′-tri-*O*-benzoylcytidine (III).**—Cytidine was selectively 4-N-acylated using acetic anhydride in methanol as described by Watanabe and Fox,[2] and the product was benzoylated as follows. 4-*N*-Acetylcytidine (0.9 g) was suspended in dry pyridine (25 ml) and benzoyl chloride (2 ml) was added. The mixture was stirred at room temperature for 2 hr and then poured into 200 ml of 0.01 *N* HCl. After stirring for 1 hr, the sticky precipitate was extracted with chloroform, washed with sodium bicarbonate solution, dried over anhydrous magnesium sulfate, and evaporated to dryness. The residue was dissolved in hot chloroform and *n*-hexane was added to give a slightly turbid solution. On standing, the product crystallized to give a mixture which was mainly the desired compound III but which contained traces of 4-*N*-benzoyl-2′,3′,5′-tri-*O*-benzoylcytidine. Recrystallization from ethanol gave the chromatographically pure product III (1.3 g, 45% overall yield from cytidine): mp 191–192°; λmax (in ethanol) 231 nm (ε 40,400) and 284 (7500), shoulder at 295 nm. *Anal.* Calcd for $C_{32}H_{27}N_3O_9$: C, 64.30; H, 4.56; N, 7.03. Found: C, 64.64; H, 4.42; N, 6.50.

**Acid Hydrolysis of 4-*N*-Acetyl-2′,3′,5′-tri-*O*-benzoylcytidine (III).**—III (1.3 g) was dissolved in chloroform–ethanol (3:1, 100 ml) and 1 *N* HCl (11 ml) was added. The solution was allowed to stand at room temperature for 3 days, at the end of which time the uv absorption of the solution at 300 nm had dropped to zero. The solvent was removed under reduced pressure and ethanol was added to the residue which was evaporated to dryness several times to remove the HCl. Tlc on silica (10% ethanol in benzene) showed the presence of a single uv-absorbing compound which had the same $R_f$ as a marker of IV, prepared as described above. The product was recrystallized as described before to give 2′,3′,5′-tri-*O*-benzoylcytidine (1.1 g, 40% overall yield from cytidine) (as the hydrochloride): mp 226–227° dec; λmax (in ethanol) 230 nm (ε 29,700) and 280 (9650); λmin (in ethanol) 253 nm. *Anal.* Calcd for $C_{30}H_{25}N_3O_8 \cdot HCl$: C, 60.87; H, 4.44; N, 7.10. Found: C, 60.54; H, 4.41; N, 7.10.

**Registry No.**—II, 27391-02-2; III, 27391-03-3; IV HCl, 20649-51-8; V HCl, 27415-59-4; VI, 27391-05-5; VII, 14630-99-0; X, 14383-43-8.

**Acknowledgment.**—The authors thank Professor A. S. Jones for his interest and encouragement and the Science Research Council for a research studentship (to R. S. G.).

## Nucleophilicities toward *n*-Propyl Tosylate in Dimethyl Sulfoxide

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*Received August 27, 1970*

An important contribution to the understanding of solvent effects on reaction rates has been the recent calculation of solvent activity coefficients[1,2] ($^{O}\gamma^{S}_{\pm}$) and enthalpies of transfer[3] ($\Delta\Delta H_{ts}$) of transition states. $^{O}\gamma^{S}_{\pm}$ for SN2 reactions can be calculated[1] from the rates in the two solvents of interest (O and S)

$$\log k^S/k^O = \log {}^{O}\gamma^{S}_{Y^-} + \log {}^{O}\gamma^{S}_{RX} - \log {}^{O}\gamma^{S}_{\pm}$$

provided that the solvent activity coefficients for the transfer of each reactant from solvent S to the reference solvent O have been measured. Parker[1] has found that, in the transfer from methanol to dipolar aprotic solvents, the rate increase may be assisted or resisted by the change in transition state solvation.

The purposes of the present study are (1) to establish the relative reactivities of a series of nucleophiles toward *n*-propyl tosylate under sufficiently similar experimental conditions to permit comparisons, and (2) to estimate $^{O}\gamma^{S}_{YRX\pm}$ values for the reactions where possible. Attention has been given to the small anions hydroxide, methoxide, and fluoride, for which few rate data are available under comparable conditions.

### Experimental Section

The nucleophile sources were tetrabutylammonium thiosulfate, hydroxide, fluoride, chloride, bromide, and iodide, sodium methoxide, phenoxide, and thiocyanate, and *N*-methylaniline. The substrate was *n*-propyl tosylate in all reactions except that with fluoride, where *n*-hexyl tosylate was used. Nucleophile concentrations were about 0.03 *M*, and the tosylate was about 0.015 *M*, except in the case of fluoride, and the chloride rate at −4.5°, in which concentrations were 0.45 *M* in the nucleophile and 0.25 *M* in the substrate (to facilitate gas chromatographic determination of hexyl fluoride). Rate measurements were made at −4.5, 20, 30, or 40°. The solvent in each case was dimethyl sulfoxide containing no more than 0.05% water, except that the rates at −4.5° were measured in 45% DMSO–55% tetramethylene sulfoxide (v/v).

The reaction rates were followed by potentiometric titration of aliquots (*vs.* standard iodine, sulfuric acid, or silver nitrate solutions). In the fluoride reaction the appearance of hexyl fluoride was followed by gas chromatography (DC 710 on Chromosorb W).

Tetrabutylammonium thiosulfate (mp 60.1°) and tetrabutylammonium fluoride (mp 58°) were prepared from the bromide by anion exchange. The other nucleophile sources are commercially available.

The rates of reaction of the chloride ion were measured under each condition of temperature, substrate, concentration, and solvent. The slightly inexact assumption has been made that other nucleophiles are affected by these experimental differences to the same extent as is the chloride reaction, and corrections have been made on this basis. The rates of the bromide runs with $Bu_4NBr$ and KBr are, within experimental error, the same. The effect of cation variation is insignificant with this anion, and this is probably true also for the majority of the other nucleophiles. With the small anions, $OH^-$, $F^-$, and $CH_3O^-$, appreciable differences in rate would be anticipated as a function of the cation, due to ion-pair association of the salts in nonaqueous media. It is extremely difficult to obtain $Bu_4NF$ free from water and $NaOCH_3$ free from methanol. The low melting point of $Bu_4NF$ (58°) suggests that the sample is a hydrate, probably with 3–5 molecules of water. Solvation of the anions by hydroxylic solvents should decrease nucleophilic reactivity. Our rates should be considered only as establishing for $OH^-$, $F^-$, and $CH_3O^-$ the *lower limit* of the reactivity which would be observed at infinite dilution in the absence of water or methanol. The rates of reaction with chloride in DMSO and in 45% DMSO–55% tetramethylene sulfoxide are substantially the same. In the hydroxide reaction with *n*-propyl tosylate, 48% of the product (by gc) is 1-propanol, and the remainder is the E2 product propylene. The rate of substitution by hydroxide was taken as 0.48 × the overall rate of disappearance of hydroxide as de-

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