Nucleotides. IV.[1] Conversion of Ribonucleosides to New 2′,3′-Ketal Derivatives[2]

Alexander Hampton,[3] J. C. Fratantoni, P. M. Carroll,[3] and Su-chu Wang

Contribution from the Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, New York, 21, New York, and from the University of Alberta Cancer Research Unit, McEachern Laboratory, Edmonton, Alberta, Canada.
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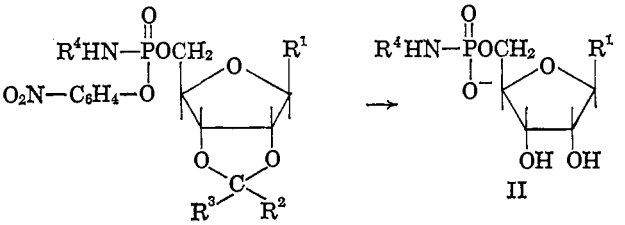
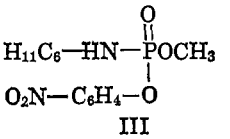
Uridine has been converted to 2′,3′-cyclic ketals derived from benzophenone, diethyl ketone, methyl t-butyl ketone, cyclopentanone, cycloheptanone, cyclooctanone, and crotonaldehyde; the ease with which the blocking groups can be removed with acid increases in the order in which they are listed above and varies over at least a 100-fold range at pH 2 and 25°. The new derivatives are expected to widen the scope of reactions which convert ribonucleosides to 5′-substituted derivatives. In some instances the relative rates of hydrolysis of the cyclic ketals differ from those of corresponding acyclic ketals, and such effects are ascribed to bond-angle strain introduced by the 1,3-dioxolane ring of the cyclic ketals. Synthesis of the cyclic ketals involved treatment of uridine with mixtures of the ketone (as solvent and reactant), its dimethyl ketal (as dehydrating agent), and di-p-nitrophenyl phosphate (as catalyst). Yields were, in general, high. Guanosine also reacted readily with such mixtures. In most cases the dimethyl ketal could be generated in situ with trimethyl orthoformate. The dimethyl ketal of acetone and the dimethyl acetals of benzaldehyde and propionaldehyde converted uridine to products which appear to be 5′-substituted derivatives of the respective 2′,3′-ketals which arise from alcohol exchange reactions; acidic treatment of these derivatives selectively yielded the uridine 2′,3′-ketals. Removal of isopropylidene groups from nucleosides can be markedly promoted by use of ethylene glycol as solvent.

Efficient conversion of ribonucleosides to 5′-monosubstituted derivatives usually requires prior protection of the 2′,3′-*cis*-hydroxyl groups. For this purpose, acid-labile cyclic ketal groups derived from acetone or from benzaldehyde have been employed almost exclusively.[4] The ease with which these two groups can subsequently be removed by acid is of the same order.[5] However, for certain purposes the use of nucleoside 2′,3′-cyclic ketals of higher stability to acid is desirable, *e.g.*, in the preparation of 5′-acetyl nucleosides, during which acetolysis of isopropylidene groups can occur very readily.[6] For other synthetic purposes, the use of 2′,3′-cyclic ketals with relatively high lability to acid is required, *e.g.*, in the preparation from the respective ribonucleosides of the 5′-phosphates of 6-chloropurine nucleoside[7] (the 6 position of which is unstable to acid) and of 3-β-D-ribofuranosyladenine[8] (the glycosidic bond of which is unstable to acid). An acid-labile 2′,3′-nucleoside ketal (anisylideneuridine[6]) has been used in the synthesis of polyribonucleotides. In this laboratory the present studies on new ketal blocking groups arose in the course of investigating a new and potentially shorter route to the ribonucloside 5′-



I

II



III

phosphoramidates (II; R^4 = H, alkyl) which were shown by Chambers and by Khorana to serve as important intermediates for the synthesis of nucleotide coenzymes.[9-11] These phosphoramidates (II) are presently best prepared from nucleoside 5′-phosphates and amines[12]; the alternative route envisaged consists of phosphorylation of 2′,3′-ketal derivatives of nucleosides with *p*-nitrophenyl phosphorodichloridate[13] and treatment of the resulting nucleoside 5′-*p*-nitrophenyl phosphorochloridate with primary or secondary amines.[14] The resulting *p*-nitrophenyl esters of 2′,3′-substituted nucleoside 5′-phosphoramidates (I), upon successive acidic and alkaline treatments, could then

(1) Part III: A. Hampton, E. G. Hampton, and M. E. Eidinoff, *Biochem. Pharmacol.*, **11**, 155 (1962). A preliminary account of part of the present studies has been given: A. Hampton and P. M. Carroll, Abstracts 6th International Congress of Biochemistry, New York, N. Y., 1964, I-71.

(2) This work was supported by funds from the National Cancer Institute, National Institutes of Health, United States Public Health Service (Grant CY-3190), and from the National Cancer Institute of Canada.

(3) University of Alberta Cancer Research Unit.

(4) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press Inc., Ltd., London 1963, pp. 112, 116.

(5) M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 430 (1962).

(6) D. M. Brown, L. J. Haynes, and A. R. Todd, *J. Chem. Soc.*, 3299 (1950).

(7) A. Hampton and H. M. Maguire, *J. Am. Chem. Soc.*, **83**, 150 (1961).

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(9) R. W. Chambers and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 3749 (1958).

(10) R. W. Chambers, P. Shapiro, and V. Kurkov, *ibid.*, **82**, 970 (1960).

(11) S. Roseman, J. J. Distler, J. G. Moffatt, and H. G. Khorana, *ibid.*, **83**, 659 (1961).

(12) J. G. Moffatt and H. G. Khorana, *ibid.*, **83**, 649 (1961).

(13) A. F. Turner and H. G. Khorana, *ibid.*, **81**, 4651 (1959).

(14) This scheme has analogy in the conversion of 2′,3′-di-O-acetyladenosine to adenosine 5′-phosphoramidate by the successive actions of phenylphosphorodichloridate, ammonia, and alkali (R. W. Chambers and H. G. Khorana, ref. 9).

furnish the required coenzyme intermediates (II). To serve as a model for nucleosides with structure I, methyl *p*-nitrophenyl phosphorocyclohexylamidate (III) was synthesized by reaction of P^1,P^2-methyl-P^1,P^2-*p*-nitrophenyl pyrophosphate with cyclohexylamine; this base was selected because its relatively high basicity indicates[12] that it will give rise to highly reactive amidates of phospho monoesters such as II.[15] Compound III was found to undergo slight decomposition after 18 hr. at pH 1 and 25°. These conditions are comparable to those required to remove an isopropylidene group from a nucleoside,[7] thus indicating that ketal blocking groups of enhanced acid lability would be required for the synthesis of II from I in high yield.

The procedure used for synthesis of most of the new nucleoside ketals was based upon a general method for conversion of nucleosides to isopropylidene derivatives which utilizes the ketone as solvent, di-*p*-nitrophenyl phosphate as catalyst, and the dimethyl ketal of acetone as a dehydrating agent.[16,17] This method requires only mild conditions of temperature (25°) and low concentrations of acid catalyst and gave uniformly high yields with a variety of nucleosides.[16] More recently, it has given good yields of the isopropylidene derivatives of 5-fluorouridine (see Experimental Section), 6-azauridine,[18] pseudouridine,[19] orotidine methyl ester,[20] 3-β-D-ribofuranosyladenine,[21] and 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide.[22] The 2,2-dimethoxypropane used in this procedure can be generated *in situ* by allowing the acetone to react at room temperature for 1 hr. with the stoichiometric amount of trimethyl orthoformate; this reaction is satisfactorily catalyzed by the same amounts of di-*p*-nitrophenyl phosphate as are required for the subsequent formation of isopropylidene nucleosides. By this modified general procedure, isopropylideneuridine, for example, could be isolated in high yield. When the acetone was replaced by cyclopentanone, cycloheptanone, crotonaldehyde, or benzaldehyde, uridine reacted readily with the resulting mixtures of the carbonyl compounds and their dimethyl ketals. Except in the case of benzaldehyde, discussed below, the yields of the uridine 2′,3′-ketals were high. The method was not directly applicable to benzophenone owing to its relative insolubility in trimethyl orthoformate, but when a mixture of benzophenone and preisolated benzophenone dimethyl ketal was employed, uridine was converted quantitatively to 2′,3′-O-diphenylmethylideneuridine. Cyclooctanone did not react to an appreciable extent with trimethyl orthoformate under the above conditions, and it was necessary to heat the ketone with methanol and trimethyl orthoformate in order to form its dimethyl ketal. A mixture of cyclooctanone and its ketal readily converted uridine to its 2′,3′-cyclooctylidene derivative.

Guanosine also reacted readily with the above cyclopentanone and cycloheptanone ketone–ketal mixtures when the amount of di-*p*-nitrophenyl phosphate was increased to that needed in the analogous synthesis of isopropylideneguanosine.[17] Paper chromatography indicated that quantitative conversion to a single product had occurred. The above ketone–ketal procedure hence appears applicable to a variety of nucleosides as well as to a variety of aliphatic and aromatic ketones and aldehydes.

Under conditions similar to those used for the synthesis of uridine cyclic ketals, uridine reacted readily with trimethyl orthoformate to give the recently described 2′,3′-O-methoxymethylideneuridine[23] and a second product or products which appeared to be 5′-orthoester derivatives of 2′,3′-O-methoxymethylideneuridine.[24]

2,2-Dimethoxypropane, like trimethyl orthoformate, gave rise to a 2′,3′,5′-trisubstituted derivative of uridine. Mixtures of acetone, 2,2-dimethoxypropane, di-*p*-nitrophenyl phosphate, and uridine used[16] for the preparation of isopropylideneuridine were analyzed by partition chromatography on an anion-exchange cellulose, and minor amounts of a second, less hydrophilic, product were detected. This material became the major reaction product when the amount of dimethoxypropane in the mixture was increased fivefold. Upon hydrolysis for 30 min. at pH 4, 25°, or at pH 8, 100°, it was completely converted to 2′,3′-O-isopropylideneuridine; its ultraviolet spectrum indicated it to be a ribose-substituted derivative of uridine, and the infrared spectrum showed the absence of sugar hydroxyl groups. This product hence appears to be a mixed ketal of acetone derived from methanol and from the primary hydroxyl of 2′,3′-O-isopropylideneuridine.[25]

The dimethyl ketals of benzaldehyde and of propionaldehyde also gave rise to appreciable amounts of products with the properties of 5′-substituted derivatives of the respective uridine 2′,3′-ketals. The other dimethyl ketals used (except that of acetone) either had little tendency to form 5′-substituted uridines or these derivatives were too labile to permit detection. Under the conditions found suitable for the preparation of isopropylideneguanosine,[17] the dimethoxypropane did not (as with uridine) form detectable amounts of a stable 5′-substituted guanosine derivative.

Other new 2′,3′-O-alkylidene nucleosides have been reported recently. Chladek and Smrt[26] prepared 2′,3′-O-cyclopentylidene, cyclohexylidene, and anisylidene nucleosides by treating nucleosides under anhydrous conditions with 1 to 2 molar equiv. of a diethyl ketal with dimethylformamide as solvent and hydrochloric acid as catalyst. Cramer and co-workers[27] converted ribonucleosides to 2,4-dimethoxy- and 4-dimethylaminobenzylidene derivatives by the action of the substituted benzaldehydes in dioxane solution in the presence of trichloroacetic acid.

(15) GMP-cyclohexylamidate, prepared from GMP, has been used to prepare GDP-glucose by J. Baddiley, N. A. Hughes, and A. L. James, *J. Chem. Soc.*, 2574 (1961).
(16) A. Hampton, *J. Am. Chem. Soc.*, **83**, 3640 (1961).
(17) A. Hampton, *Biochem. Prepn.*, **10**, 91 (1963).
(18) R. H. Hall and R. Thedford, *J. Org. Chem.*, **28**, 1056 (1963).
(19) R. W. Chambers, V. Kurkov, and R. Shapiro, *Biochemistry*, **2**, 1192 (1963).
(20) J. G. Moffatt, *J. Am. Chem. Soc.*, **85**, 1118 (1963).
(21) N. J. Leonard and R. A. Laursen, *ibid.*, **85**, 2026 (1963).
(22) G. Shaw, D. V. Wilson, and C. P. Green, *J. Chem. Soc.*, 2650 (1964).
(23) M. Jarman and C. B. Reese, *Chem. Ind.* (London), 1493 (1964). 2′,3′-O-Ethoxymethylidene nucleosides have been synthesized by J. Zemlicka, *ibid.*, 581 (1964).
(24) C. B. Reese and J. E. Sulston, *Proc. Chem. Soc.*, 214 (1964).
(25) The reaction of sorbose with 2,2-diethoxypropane has, similarly, given a mixed ketal of acetone derived from ethanol and from the primary hydroxyl of the sugar (K. Tokuyama and E. Honda, *Bull. Chem. Soc. Japan*, **37**, 591 (1964)).
(26) S. Chladek and J. Smrt, *Collection Czech. Chem. Commun.*, **28**, 1301 (1963).
(27) F. Cramer, W. Saenger, K. Scheit, and J. Tennigkeit, *Ann.*, **679**, 156 (1964).

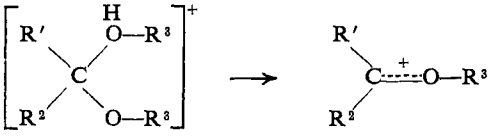
Rates of hydrolysis of the uridine ketals are given in Table I. Considerable evidence indicates that the rate-determining step in the acid hydrolysis of ketals (eq. 1)

Table I. Rates of Hydrolysis of Uridine 2′,3′-Ketals at pH 2, 26°

2′,3′ Substituent	Half-life of ketal,[a] hr.
Methoxymethylidene[23]	0.08[b]
Crotonylidene	0.5
Cyclooctylidene	2.5
Cycloheptylidene	3
Cyclopentylidene	4
Methyl-*t*-butylmethylidene	20
Isopropylidene	20
Diethylmethylidene	40
Diphenylmethylidene	>40[c]

[a] The hydrolysis mixtures initially consisted of 0.01 *M* solutions of the nucleoside ketals in 0.01 *M* HCl. [b] The product is a mixture of 2′- and 3′-formyluridine.[23] [c] Not sufficiently soluble in the aqueous hydrolysis medium. A 0.01 *M* solution in methanol–concentrated HCl (2:1) underwent 80% hydrolysis in 25 hr. at 26°.

involves an enlargement of the angle R^1CR^2 owing to formation of a transition state with much carbonium ion character.[28] Thus, the diethyl ketal of methyl *t*-



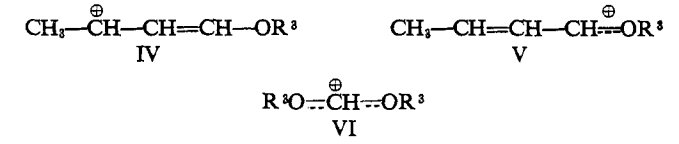
(1)

butyl ketone is hydrolyzed much more rapidly than the diethyl ketal of acetone, since the strain of steric repulsion between R^1 and R^2 in the starting state is released upon attainment of the transition state. The uridine ketal of methyl *t*-butyl ketone, however, is not hydrolyzed faster than isopropylideneuridine (Table I); this implies that the strain due to repulsion between the methyl and *t*-butyl groups could be largely nullified in the starting state itself by an increase in the R^1CR^2 bond angle mediated by the ribofuranose and 1,3-dioxolane rings. Such an effect of the two rings can help to explain also the acid lability of cyclopentylideneuridine relative to isopropylideneuridine. The analogous diethyl ketals of acetone and cyclopentanone, on the other hand, are hydrolyzed at about the same rates, and evidence indicates that conversion of the cyclopentanone ketal to the transition state entails a decrease in ring torsional strain which is offset by an increase in bond-angle strain.[28] With cyclopentylideneuridine, activation for hydrolysis could well be facilitated if bond-angle strain (a tendency for R^1CR^2 to increase) is imposed on the starting state by the 1,3-dioxolane ring as postulated above. Cycloheptylideneuridine hydrolyzes more rapidly than cyclopentylideneuridine, and the same order of reactivity holds true for the diethyl ketals of the two ketones.[28] No report seems to be available on the ease of hydrolysis of an acyclic ketal of cyclooctanone, but much data indicate that among carbocyclic compounds the maximum reactivity (or inertness) toward a variety of reagents is shown by the eight- to ten-membered rings.[29]

(28) M. M. Kreevoy, C. R. Morgan, and R. W. Taft, Jr., *J. Am. Chem. Soc.*, 82, 3064 (1960).
(29) H. C. Brown, *J. Chem. Soc.*, 1248 (1956).

That cyclooctylideneuridine is more labile to acid than cycloheptylideneuridine agrees with this general trend.

The relative ease of hydrolysis of 2′,3′-O-crotonylideneuridine and 2′,3′-O-methoxymethylideneuridine (Table I) is ascribable to resonance stabilization of the transition state by structures such as IV, V, and VI (R^3 = uridine 2′- or 3′-). Synthesis of crotonylidene-



glucitols have recently been reported and their acid lability noted.[30] 2′,3′-O-Diphenylmethylideneuridine is appreciably more stable to hydrolysis than isopropylideneuridine; similarly, the ethylene glycol ketal of benzophenone is more stable than the ethylene glycol ketal of acetone.[31] A product with the chromatographic and spectral properties of propionylideneuridine was obtained from the reaction of uridine with propionaldehyde and trimethyl orthoformate, and was found to resemble the diphenylmethylideneuridine in its resistance to hydrolysis. This is in accord with the stability of the ethylene glycol acetal of acetaldehyde relative to the corresponding ketal of acetone.[31]

Removal of isopropylidene groups (and, presumably, ketal blocking groups in general) can be promoted by alcohol exchange reactions. Thus, for a given concentration of acid (0.01 *N*), conversion of isopropylideneuridine to uridine at 25° proceeded ten times more rapidly in ethylene glycol solution than in water. The more volatile ethylene dithioglycol was about equally effective (but was a poor solvent for isopropylidene nucleosides), while methanol and ethanol were significantly less effective. The usefulness of ethylene glycol was further tested with 2′,3′-O-isopropylideneuridine 5′-di-*p*-nitrophenyl phosphate[32] since removal of the isopropylidene group of this compound required relatively drastic conditions and the usual methods led to the concomitant liberation of *p*-nitrophenol. When ethylene glycol was substituted for aqueous methanol or dioxane, paper chromatographic analysis demonstrated that removal of the isopropylidene group could be accomplished under milder conditions and without formation of *p*-nitrophenol. This transformation is closely analogous to those required for the proposed syntheses of the nucleotide coenzyme intermediates II.

Experimental Section

Solvent systems for paper chromatography were (A) 2-propanol–NH_4OH–water (7:1:2), (B) 2-propanol–NH_4OH–0.1 *M* aqueous boric acid (7:1:2), and (C) 2-propanol–water (7:3). *cis*-Glycol systems were located on papers by the Schiff–periodate[33] and benzidine–periodate[34] spray procedures.

Evaporations were carried out under reduced pressure in a rotating evaporator. Unless otherwise specified,

(30) T. G. Bonner, E. J. Bourne, and D. Lewis, *ibid.*, 3375 (1963).
(31) O. Cedar, *Arkiv Kemi*, 6, 523 (1954).
(32) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, 79, 3741 (1957).
(33) J. G. Buchanan, C. A. Dekker, and A. G. Long, *J. Chem. Soc.*, 3162 (1950).
(34) J. A. Cifonelli and F. Smith, *Anal. Chem.*, 26, 1132 (1954).

substances were dried *in vacuo* at room temperature over sodium hydroxide. Melting points (capillary method) are uncorrected. Analyses were by the Schwarzkopf Laboratory, Woodside, N. Y., and by A. Bernhardt, Mulheim, Germany.

2',3'-O-Isopropylidene-5-fluorouridine. A mixture of 5-fluorouridine (105 mg., 0.4 mmole), acetone (4 ml.), 2,2-dimethoxypropane (0.4 ml.), and di-*p*-nitrophenyl phosphate (13.6 mg., 0.04 mmole) was stirred for 1 hr. The solution was added to an excess of dilute ammonia and the mixture was evaporated to dryness. To a solution of the residue in water (20 ml.) was added 0.15 ml. of Dowex-1 (Cl^-) ion-exchange resin. After 0.5 hr. (stirring) the resin was filtered off and washed with water. The combined filtrate and washings were concentrated to *ca.* 1 ml., yielding long white needles, 95 mg., m.p. 195–195.5°. The product had the same R_f as isopropylideneuridine in system A.

Anal. Calcd. for $C_{12}H_{15}N_2O_6F$: C, 47.68; H, 5.00; N, 9.27; F, 6.29. Found: C, 48.08; H, 5.10; N, 9.35; F, 6.24.

2',3'-O-Isopropylideneuridine from Acetone and Trimethyl Orthoformate. A solution of di-*p*-nitrophenyl phosphate (7 mg.) in a mixture of reagent grade acetone (2 ml.) and trimethyl orthoformate (0.3 ml.) was stored for 1 hr. at 25° in a stoppered flask. Uridine (49 mg.) was added and the mixture was stirred magnetically until solution was complete (30 min.). After a further 1.5 hr. the solution was concentrated under vacuum to about one-third its volume and added to 3 ml. of 1 *N* NH_4HCO_3. Volatiles were removed *in vacuo* and the residue was extracted with warm chloroform. Removal of solvent and addition of two drops of acetone caused the residue to form a crystalline mass, 49 mg. (86% yield), m.p. 160–161° (undepressed in admixture with authentic isopropylideneuridine). The product showed the expected R_f value (0.32) upon chromatography in system A.

2',3'-O-Diethylmethylideneuridine. Dry HCl was bubbled for 0.5 hr. into a suspension of uridine (200 mg.) in anhydrous *p*-dioxane (7 ml.) and diethyl ketone (10 ml.; dried with Drierite and distilled; b.p. 100–101.5°). During the first 10 min. the mixture became warm and the solid dissolved. The mixture was kept at 25° for 1 hr., during which solid separated and was evaporated to near dryness. The residual oil was dissolved in 10 ml. of ethanolic 2 *N* NH_4OH; solvent was removed, the residue was dissolved in chloroform, and the cloudy solution was clarified. Chromatography in system A showed one ultraviolet-absorbing component and a trace of uridine. After removal of solvent the product was warmed and triturated with water (2 ml.), then chilled, giving 170 mg. of white solid, m.p. 144–146°. Crystallization from water gave 105 mg. of small white needles, m.p. 149–150°.

Anal. Calcd. for $C_{14}H_{20}N_2O_6$: C, 54.01; H, 6.45; N, 9.00. Found (for material dried at 100°): C, 54.39; H, 6.50; N, 9.38.

2',3'-O-(Methyl-t-butyl)methylideneuridine. To a suspension of uridine (122 mg.) in chloroform (10 ml.) was added methyl *t*-butyl ketone (3 ml.), ethyl orthoformate (1.65 ml.), and one drop of concentrated H_2SO_4. After 3 days at 25° the brown mixture was shaken with 10 ml. of 5% aqueous $NaHCO_3$. The chloroform was washed twice with small portions of the $NaHCO_3$ solution, then dried and evaporated. To a solution of the residual brown oil in chloroform (2 ml.) was added light petroleum, yielding white needles, 58 mg., m.p. 170–175°. Recrystallization raised the melting point to 176°.

Anal. Calcd. for $C_{15}H_{22}N_2O_6$: C, 55.20; H, 6.80. Found: C, 55.41; H, 7.09.

2',3'-O-Cyclopentylideneuridine. Method A. Cyclopentanone (10 ml.) was added to a suspension of uridine (0.5 g.) in anhydrous dioxane (25 ml.). Dry HCl was passed in at a brisk rate for 20 min., at which time the solid dissolved. After a further hour volatiles were removed *in vacuo* and 2 *N* ethanolic ammonia (40 ml.) was evaporated from the residue. To a solution of this residue in chloroform (30 ml.) was added charcoal (25 mg.); after 1 hr. the solution was filtered and evaporated (finally at 0.1 mm. and 50°). Trituration of the gum with benzene (20 ml.) induced its crystallization. A solution of this product in aqueous ethanol was clarified with Celite and concentrated *in vacuo* to *ca.* 15 ml. and chilled, giving white needles (430 mg.), m.p. 169–169.5°.

Anal. Calcd. for $C_{14}H_{18}N_2O_6$: C, 54.18; H, 5.85; N, 9.03. Found: C, 54.00; H, 6.04; N, 8.80.

Method B. When cyclopentanone was substituted for cycloheptanone in the procedure described below for the preparation of 2',3'-O-cycloheptylideneuridine, the uridine reacted readily (conversion time, 2 hr.). The product was isolated as described for the heptylidene analog and obtained in 80% yield as white needles, m.p. 169° (undepressed in admixture with material from preparation A).

2',3'-O-Cycloheptylideneuridine. A solution of di-*p*-nitrophenyl phosphate (14 mg.) in a mixture of cycloheptanone (4 ml.) and trimethyl orthoformate (0.6 ml.) was stored for 2 hr. at 25° in a stoppered vessel. Uridine (98 mg.) was added and the suspension was stirred magnetically; the solid dissolved after 2 hr. After an additional 3 hr. the solution was added to an excess of 2 *N* ethanolic ammonia and the mixture was evaporated to dryness (finally at 1 mm., 40°). Chloroform (10 ml.) was added and the solution was concentrated to *ca.* 5 ml. by boiling, cooled, and filtered (after addition of Celite filter-aid) to remove ammonium di-*p*-nitrophenyl phosphate. The chloroform was removed under reduced pressure and the oily residue was extracted rapidly three times with preheated water (5, 2, and 2 ml.). The combined aqueous solutions were clarified with Celite filter-aid and concentrated at 10 mm. to small volume, yielding 119 mg. (88% yield) of white needles, m.p. 163.5–165°. In water, the product showed an ultraviolet absorption maximum at 261 mμ.

Anal. Calcd. for $C_{16}H_{22}N_2O_6$: C, 56.80; H, 6.56; N, 8.28. Found: C, 57.14; H, 6.77; N, 8.41.

In initial experiments the product separated from water in the form of white needles, m.p. 111–113°. Aqueous solutions of this material, when seeded with the crystals of m.p. 165°, yielded only the higher melting form.

2',3'-O-Cyclooctylideneuridine. Cyclooctanone (3.15 g., 25 mmoles), trimethyl orthoformate (redistilled, 3.3 ml., 30 mmoles), methanol (5 ml., 150 mmoles), and di-*p*-nitrophenyl phosphate (30 mg.) were refluxed for 1.5 hr. protected against moisture. Methyl formate was removed by fractional distillation and the

residual solution was concentrated (finally at 1 mm. and 25°) to a yellow oil. This material showed almost no absorption at 1700 cm.$^{-1}$ (carbonyl band of cyclooctanone) and possessed a strong doublet at 1100 cm.$^{-1}$ (C–O–C–O–C group) and a strong band at 1050 cm.$^{-1}$ (aliphatic ether), both of which were absent from cyclooctanone. The dimethyl ketal of cyclooctanone is not described, but the diethyl ketal has been made[35] by heating the ketone with ethanol and triethyl orthoformate in the presence of acid.

Cyclooctanone (8 g.) was dissolved at room temperature in 0.8 ml. of the above oil. Uridine (200 mg.) and di-*p*-nitrophenyl phosphate (56 mg.) were added and the mixture was stirred magnetically; solution was almost complete after 3.5 hr. A sample was taken after 18 hr., added to an equal volume of pyridine, evaporated under reduced pressure, taken up in methanol, re-evaporated, and then chromatographed on DEAE-cellulose paper in 2-propanol–1% aqueous NH_4HCO_3 (7:3 v./v.). No uridine was seen, and only one product spot. After 20 hr. a twofold excess of 2 *N* methanolic NH_4OH was added to the reaction mixture and the solution was evaporated to dryness under reduced pressure (finally at 0.1 mm., 50°). The residue was dissolved in the minimum volume of 2-propanol–H_2O (7:3) and applied to a column of Whatman DEAE-cellulose, 5 cm. high × 2.5 cm. diam., prepared in the same solvent. The column was eluted with this solvent, under a pressure of 1–2 p.s.i., and 10-ml. fractions were collected. Fractions 3, 4, 5, and 6 were ultraviolet absorbing. Fractions 3 and 4 were evaporated under reduced pressure. Residue 3 was extracted with 1–2 ml. of heptane, then twice with 1–2-ml. portions of light petroleum (b.p. 40–50°) and twice with 1–2-ml. portions of diethyl ether. Residue 4 was extracted once with 1–2 ml. of heptane. The two residues were crystallized from ethanol by adding a few drops of cyclohexane and then gradually adding light petroleum during several hours. After several days at 4° fraction 3 yielded 97 mg., m.p. 161.5–163°, and fraction 4 yielded 45 mg., m.p. 150–151°. When cooled to 130° and reheated, product 4 gradually formed a white solid which melted at 160–163°.

Anal. Calcd. for $C_{17}H_{24}N_2O_6$: C, 58.00; H, 6.86; N, 7.96. Found (product 3): C, 58.09; H, 6.83; N, 8.12. Found (product 4): C, 58.13; H, 6.85; N, 8.14.

The two products had the same R_f values on chromatograms on DEAE-cellulose paper in 2-propanol–1% aqueous NH_4HCO_3 (7:3) and on Whatman No. 1 paper in 2-propanol–NH_4OH–0.1 *M* H_3BO_3 (7:1:2). The periodate–benzidine spray reactions for *cis*-glycols were negative. Both compounds showed infrared hydroxyl absorption at *ca.* 3500 cm.$^{-1}$.

2',3'-O-Diphenylmethylideneuridine. Benzophenone (8.16 g.), benzophenone dimethyl ketal (1.44 g.), and uridine (200 mg.) were ground together and stirred magnetically in a stoppered flask in a bath at 47–48°. When the mixture liquefied, di-*p*-nitrophenyl phosphate (112 mg.) was added; the uridine dissolved after 3 hr. After 48 hr. the solution started to become turbid. After 72 hr. a sample (0.2 ml.) was added to 2 *N* ethanolic NH_4OH (0.3 ml.) and the solution was evaporated. A solution of the residue in 2 *N* aqueous NH_4OH (2 ml.) was extracted with light petroleum (three 2-ml. portions). Chromatography of the aqueous solution showed that no uridine was present. Light petroleum (90 ml.) was added to the reaction flask and the mixture was poured into aqueous NH_4HCO_3 (120 mg. in 4 ml.). A white solid was filtered off and washed successively with water, light petroleum, and water; yield 298 mg. (89%), m.p. 207–208°. The product (43 mg.) was dissolved in 2 *N* ethanolic NH_4OH (2 ml.) with warming and filtered from insolubles, and the solution was allowed to evaporate. When crystals began to form, a few drops of water were added and the solution was refrigerated overnight. The crystals were washed with 50% aqueous ethanol; yield 27 mg., m.p. 211–213°. The R_f in solvent A was 0.85.

Anal. Calcd. for $C_{22}H_{20}N_2O_6$: C, 64.70; H, 4.94; N, 6.86. Found: C, 65.73; H, 5.26; N, 7.04.

2',3'-O-Crotonylideneuridine. Di-*p*-nitrophenyl phosphate (28 mg.), crotonaldehyde (8 ml., dried over Na_2SO_4 and distilled; b.p. 100–104°), and trimethyl orthoformate (1.2 ml., redistilled) were stirred at room temperature for 0.5 hr. in a stoppered flask. Uridine (196 mg.) was added to the yellow solution; it dissolved after 10 min. A sample was withdrawn after 0.75 hr., neutralized with aqueous alcoholic NH_4OH, and chromatographed in system A. No uridine was present and a single ultraviolet-absorbing product, R_f 0.60, had formed. One hour after the addition of uridine a white precipitate began to form. The reaction mixture was refrigerated overnight. The solid was collected by filtration and washed with crotonaldehyde and with small portions of diethyl ether; yield 150 mg., m.p. 199–201° dec. In ethanol the nucleoside showed absorption maxima at 262 and 208 mμ (ϵ 10.3 × 10^3 and 9.3 × 10^3, respectively) and a minimum at 233 mμ. The product (140 mg.) was dissolved in warm 2 *N* aqueous NH_4OH (5 ml.), filtered from insolubles, concentrated under reduced pressure to small volume, and stored overnight at 2°; white needles (106 mg.), m.p. 197–198°, were obtained.

Anal. Calcd. for $C_{13}H_{16}N_2O_6$: C, 52.7; H, 5.45; N, 9.46. Found: C, 52.6; H, 5.39; N, 9.55.

2',3'-O-Benzylideneuridine. Di-*p*-nitrophenyl phosphate (50 mg.) was dissolved in benzaldehyde (4 ml.) containing trimethyl orthoformate (1.2 ml.). The mixture became warm. After 45 min. uridine (200 mg.) was added. The uridine dissolved in the stirred mixture after 3 hr., whereupon aqueous 2 *N* NH_4OH (2 ml.) and diethyl ether (20 ml.) were added. The white crystalline precipitate of benzylideneuridine which formed in the stirred mixture was collected and washed with ether and with water; yield 85 mg. (30%), m.p. 190–193° (reported[36] m.p. 189–190°). The product showed only one component upon paper chromatography in solvent A and on DEAE paper in 2-propanol–1% aqueous NH_4HCO_3 (7:3). A portion of the above ethereal solution was freed of volatiles at 0.1 mm.; chromatography of the gummy residue in the DEAE paper system showed that the major ultraviolet light absorbing component had a higher R_f than benzylideneuridine; the only other component was benzylideneuridine.

(35) U. Schmidt and P. Grafen, *Ann.*, **656**, 97 (1962).

(36) M. Smith *Biochem. Prepn.*, **8**, 130 (1961).

Reaction of Uridine with Propionaldehyde. Di-*p*-nitrophenyl phosphate (14 mg.) was dissolved in propionaldehyde (4 ml.) and trimethyl orthoformate (0.6 ml.). After 0.5 hr., uridine (100 mg.) was added; it did not dissolve after 20 hr. of stirring. Extra catalyst (126 mg.) and anhydrous dioxane (0.8 ml.) were added; solution of the uridine was complete by 42 hr. Paper chromatography on DEAE-cellulose showed that after 44 hr. almost all the uridine had reacted. Two products were present with R_f 0.64 and 0.84 in 2-propanol–1% aqueous NH_4HCO_3 (7:3) and R_f 0.35 and 0.52 in system C. The ratio of the products (fast:slow) was 2:3. Prolonging the reaction for 96 hr. did not affect this ratio. The products were eluted from a chromatogram into water; both showed absorption maxima at 212 and 261 mμ, A_{212}/A_{261} 0.56.

The propionylideneuridines were freed of acid catalyst with DEAE-cellulose (as described for the preparation of cyclooctylideneuridine) and obtained as a colorless gum. The chromatographically faster-running ketal was converted to the slower-running ketal within 10 min. by 47% ethanolic 5 *N* HCl. Under these conditions, the latter ketal was converted to material with the R_f of uridine within 17 hr.

Reaction of Uridine with 2,2-Dimethoxypropane. Uridine, anhydrous acetone, 2,2-dimethoxypropane, and di-*p*-nitrophenyl phosphate were mixed in the proportions described[16] for the preparation of isopropylideneuridine. After 1 hr. the solution was neutralized with NH_4OH and chromatographed on DEAE-cellulose in 2-propanol–1% NH_4HCO_3 (7:3). In addition to di-*p*-nitrophenyl phosphate (R_f 0.26) and isopropylideneuridine (R_f 0.70), an ultraviolet light absorbing component of R_f 0.84 was visible, the amount being approximately one-half that of the isopropylideneuridine. An ethanol eluate of the component of R_f 0.84 had spectroscopic properties (maxima at 259 and 204 mμ of *ca.* equal intensity, minimum at 233 mμ) similar to those of uridine.[37] The infrared spectrum (KCl disk) of the solid obtained from removal of the ethanol was compared with that of uridine and isopropylideneuridine. It showed no primary hydroxyl absorption at 3300 or 1050 $cm.^{-1}$ and enhanced absorption at 1390 $cm.^{-1}$ (ascribable to extra *gem*-dimethyl groups).

An aqueous 2.5% solution of the above reaction product was heated at 100° and analyzed by chromatography in systems A and B. A single ultraviolet-absorbing product corresponding in R_f to isopropylideneuridine was formed. Conversion was 50% after 5 min. and essentially complete after 30 min.

When the amount of 2,2-dimethoxypropane in the reaction mixture was increased 20-fold, paper chromatography showed that the product consisted almost exclusively of the above 5′-substituted isopropylideneuridine. When the acetone in the reaction mixture was replaced by 2,2-dimethoxypropane, the time required to dissolve the uridine increased from 20 min. to more than 3 days; after 3 days the mixture contained uridine, isopropylideneuridine, and 5′-substituted isopropylideneuridine in the ratio 1:1:10. Inclusion in the reaction mixture of 30% methanol likewise slowed the reaction, and after 20 hr. the proportions of the foregoing three components were 1:10:1.

(37) D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, *Biopolymers*, 1, 193 (1963).

Methyl p-Nitrophenyl N-Cyclohexylphosphoramidate. To a solution of 50 mg. of P^1,P^2-dimethyl-P^1,P^2-di-*p*-nitrophenyl pyrophosphate[32] in 20 ml. of anhydrous benzene was added 40 mg. (3 molar equiv.) of freshly distilled cyclohexylamine with exclusion of moisture. A fine precipitate formed immediately. The mixture was kept at room temperature overnight and filtered from cyclohexylammonium methyl *p*-nitrophenyl phosphate (m.p. 145–146°; reported[32] m.p. 150–151°) and the filtrate was washed in succession with 20-ml. portions of 0.1 *N* HCl, 1% $NaHCO_3$, and water. Evaporation of the solvent gave a gummy residue solution of which in a minimum of chloroform (two drops) and addition of an equal volume of cyclohexane rapidly produced colorless crystals (18.5 mg., 53%), m.p. 112.5–114°; R_f in solvent C, 0.90.

Anal. Calcd. for $C_{13}H_{19}N_2O_5P$: C, 52.34; H, 6.42; N, 9.39. Found: C, 52.11; H, 6.62; N, 9.33.

Use of Ethylene Glycol for Removal of Isopropylidene Groups. A. From Isopropylideneuridine. Isopropylideneuridine (5 mg.) was dissolved in ethylene glycol (1 ml.) which had been made 0.01 *N* with respect to HCl by addition of concentrated aqueous HCl. Aliquots were neutralized with ethanolic NH_4OH and chromatogrammed in solvent A; 50% conversion of the isopropylidene compound to uridine occurred after 2 hr. at 25°. When the hydrolysis mixture contained 10% of water and was 0.1 *N* with respect to HCl, 60% hydrolysis occurred after 2 hr.

B. From 2′,3′-O-Isopropylideneuridine 5′-Di-p-nitrophenyl Phosphate. The above uridine derivative[32] (8 mg.) was warmed in ethylene glycol (1 ml.) to 100°, at which point it dissolved. To the cooled (25°) solution was added 0.04 ml. of concentrated HCl. At intervals aliquots were evaporated from a bath at 50° and 0.2 mm. to remove ethylene glycol, and the residues were dissolved in dioxane and subjected to paper chromatography in 1-butanol–4% boric acid (86:14). Conversion of the isopropylidene derivative (R_f 0.88) to uridine 5′-di-*p*-nitrophenyl phosphate (R_f 0.70) was 40% complete within 20 min. When the chromatograms were placed in a mixture of ammonia, water, and dioxane vapors, the spots corresponding to these two nucleoside 5′-di-*p*-nitrophenyl phosphates became yellow during 10 min. due to the formation of *p*-nitrophenol (as expected under these conditions[7]). The use of paper which had been soaked in 4% boric acid, blotted, and dried gave R_f 0.57 for uridine 5′-di-*p*-nitrophenyl phosphate and R_f 0.89 for its isopropylidene derivative.

The reaction was analyzed also by chromatography in solvent A in which the phospho triesters were rapidly and completely hydrolyzed, giving rise to *p*-nitrophenol (R_f 0.77), uridine 5′-*p*-nitrophenyl phosphate (R_f 0.52), and its isopropylidene derivative (R_f 0.71). Of the two uridine derivatives, only the one of R_f 0.52 reacted positively toward the spray test[31] for *cis*-glycol systems. With the above-described boric acid impregnated paper, uridine 5′-*p*-nitrophenyl phosphate had R_f 0.41 and its isopropylidene derivative had R_f 0.72 in solvent A.

Hydrolysis of Uridine 2′,3′-Ketals at pH 2. The results given in Table I were obtained by adding portions of the nucleoside ketal solution at intervals to an excess of dilute ammonia, evaporating the mixture

to dryness, dissolving the residue in *ca.* one-half its original volume of aqueous 50% methanol, and chromatographing the solution in system A. Spots were eluted into aqueous 50% methanol and assayed spectrophotometrically at 260 mμ. Rate constants were calculated for hydrolysis of isopropylideneuridine and cyclopentylideneuridine, and both reactions were found to follow pseudo-first-order kinetics.

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Luminescence of Purines[1]

Beverly J. Cohen and Lionel Goodman

Contribution from Whitmore Chemical Laboratory, Pennsylvania State University, University Park, Pennsylvania. *Received June 17, 1965*

The phosphorescence and polarized phosphorescence excitation spectra have been studied for purine, the purine anion, adenine, and some alkyl-substituted adenines. The results indicate that, in all cases, the triplet → singlet transition moment is perpendicular to the molecular plane, and that the lowest singlet excited state in purine is (n, π), and in the purine anion and adenines, (π, π*). The emission properties of 9-n-butyladenine in nonpolar media are reported and briefly discussed in terms of an energy-transfer process.*

There is considerable interest in the location of the n → π* absorption bands in isolated nucleic acid bases, as such knowledge is important in understanding both energy transfer and hypochromism in polynucleotides. Clark and Tinoco[2] found a weak absorption between 290 and 320 mμ in purine in hydrocarbon solvent at 353°K., which they assume to be an n → π* transition from its disappearance in polar solvent. A similar phenomenon is observed in 9-methylpurine.[3] Stewart and Davidson[4] have definitely identified an n → π* band in 9-methyladenine, by studying the polarized absorption spectra in the crystalline adenine–thymine dimer. Borresen[5] concluded that the lowest singlet excited state in both adenine and purine should be (n, π*), from their nonfluorescence in water at room temperature. His reasoning involved the well-known rule that (π, π*) states fluoresce, whereas (n, π*) states do not.[6] However, the number of established exceptions are sufficiently numerous to indicate that no general statement can be made regarding nonfluorescence of (n, π*) states. N-Heterocyclics which have fluorescent (n, π*) states are 3,4-benzocinnolin[7a] pyridazine and its derivatives,[7b] *s*-tetrazine,[8] and pyrimidine.[5] Thus the character of the lowest excited state cannot be conclusively identified by the presence or lack of fluorescence. In the case of adenine, Callis, Rosa, and Simpson[9] have quite rigorously ascertained, by a polarized fluorescence excitation experiment, that the lowest singlet excited state in ethylene glycol–water solvent at 196°K. is (π, π*). However, this experiment cannot distinguish between a higher energy n → π* absorption and higher π → π* absorptions.

As the first step in a program of the characterization of excited states in polynucleotides, it is necessary to have an unequivocal assignment of the lowest energy bands in the simple bases. The present work is an attempt to conclusively identify the n → π* bands in the bases, purine, adenine, 9-*n*-butyladenine, and 9-methyladenine through a study of polarized phosphorescence excitation spectra.

Experimental Section

Materials. Purine and adenine, A grade, were obtained from the California Corp. for Biochemical Research, and used without further purification. Adenine, obtained from the Nutritional Biochemicals Corp., was recrystallized twice from water. These two samples of adenine gave identical results. 9-Methyladenine, grade I, was purchased from the Cyclo Chemical Corp., and the sample of 9-*n*-butyladenine was generously donated to us by Dr. John A. Montgomery of the Southern Research Institute. These were used without further purification.

EPA (5:5:2 parts by volume of ether, isopentane, and ethanol), methylcyclohexane–isopentane (5:1), ethanol–NaOH (20:1),[10] butanol–isopentane (3:7), and mixtures of hydrocarbon and triethylamine were used as solvents for emission studies. All concentrations were between 10^{-3} and 10^{-5} *M*.

Procedure. The emission and polarization spectra were obtained with a Baird-Atomic fluorescence spectrophotometer, Model SF1. Radiation from a 150-w. xenon source was dispersed by a double-grating monochromator and irradiated the sample which was at 77°K. The emission, observed perpendicularly to the excitation beam, was focused through a second double-grating monochromator and was detected with an RCA Type 1P28 phototube. The signal was recorded on an X–Y recorder. Polarization measure-

(1) Supported by a grant from the National Science Foundation.
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(9) P. R. Callis, E. J. Rosa, and W. T. Simpson, *J. Am. Chem. Soc.*, **86**, 2292 (1964).
(10) The NaOH solution is 0.5% by weight of NaOH.