room temperature (with the exception of the cinnamoyl complex) and their spectra were very similar to the solution spectra except for the solvent shift. The resolution of the neat spectra was slightly lower due to the higher viscosity of the neat complexes as compared with their SO_2 solutions.

The F^{19} spectra of the complexes shows no C–F resonances, only antimony–fluorine peaks at around ϕ +100 (parts per million from external $CFCl_3$).

Experimental Section

Acryloyl, methacryloyl, and crotonoyl fluorides were prepared from the acids and benzoyl fluoride using the published procedure for cyclopropanecarbonyl fluoride.[4d] Tigloyl, β,β-dimethylacryloyl, and cinnamoyl fluorides were prepared from the corresponding chlorides and anhydrous hydrogen fluoride using the published procedure for preparing cyclobutanecarbonyl fluoride.[4d]

The preparation of the alkenyloxocarbonium ion complexes from alkenoyl fluorides and antimony pentafluoride in 1,1,2-trifluorotrichloroethane solution was carried out according to methods previously described to prepare other types of oxocarbonium complexes.[4a-d]

The techniques of infrared and nmr studies were also analogous to those described previously. Infrared spectra were obtained on a Beckman Model IR 10 spectrophotometer and a PE 337 spectrophotometer. Nmr spectra were obtained on Varian Associates Model HA-60-IL and A56-60A spectrometers equipped with variable-temperature probes. All chemical shifts are relative to external TMS (H^1) or CCl_3F (F^{19}) as references (capillary tubes).

Acknowledgment. Generous support of this work by a grant from the National Institutes of Health is gratefully acknowledged.

Carbodiimide–Sulfoxide Reactions. VI.[1] Syntheses of 2′- and 3′-Ketouridines

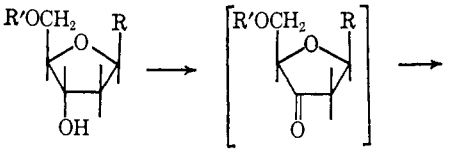
A. F. Cook[2] and J. G. Moffatt

Contribution No. 46 from the Institute of Molecular Biology, Syntex Research, Palo Alto, California. *Received January 23, 1967*

Abstract: Oxidation of 2′,5′-di-O-trityluridine by the dimethyl sulfoxide–dicyclohexylcarbodiimide method gave crystalline 2′,5′-di-O-trityl-3′-ketouridine in good yield. Treatment with hydrogen chloride in chloroform then gave free 3′-ketouridine. In a similar way oxidation of 3′,5′-di-O-trityluridine gave 3′,5′-di-O-trityl-2′-ketouridine which was detritylated to free 2′-ketouridine. These oxidations could also be performed using dimethyl sulfoxide together with acetic anhydride or phosphorus pentoxide. The uridine ketones were very labile toward alkali, being cleaved to uracil. Borohydride reduction of the free or tritylated 2′-ketone led predominantly to the formation of products with the arabinose configuration while similar reduction of the 3′-ketones gave mixures of the corresponding xylosides and ribosides in a ratio of 2:1. Attempts to alkylate the ditrityl 3′-ketone with Grignard reagents, methyllithium, or diazomethane have not as yet been successful. Nuclear magnetic resonance spectra data are presented for the various compounds.

An extremely mild, yet efficient, oxidation of alcohols through their reaction with dimethyl sulfoxide (DMSO) and dicyclohexylcarbodiimide (DCC) has been developed in this laboratory.[3] Since the oxidation proceeds at room temperature, and under essentially neutral conditions (*e.g.*, using pyridinium trifluoroacetate as the proton source), it has found considerable application when dealing with sensitive compounds.[4] A particular merit lies in the oxidation of primary alcohols exclusively to the aldehyde stage,[3] and this property has permitted the oxidation of the 5′-hydroxyl group of protected nucleosides to give the corresponding nucleoside 5′-aldehydes.[3a,5]

Our earlier observations[3b] showed that the reaction of deoxynucleosides, such as thymidine, substituted at the 5′ position by phosphate, acetyl, or *p*-nitrobenzoyl groups (**1**, R = thymine, R′ = PO_3H_2, Ac, *p*-nitrobenzoyl) with DMSO and DCC in the presence of anhydrous orthophosphoric acid led to the rapid and complete cleavage of the N-glycosidic bond with release of thymine. Such a degradation undoubtedly proceeds *via* oxidation to the 5′-substituted 3′-ketonucleoside **2** which then undergoes β elimination of the heterocyclic base. The elimination step was apparently extremely rapid and no sign of the intermediate ketones **2** could be detected chromatographically. Similar results have been encountered by others during attempted oxidation of the 3′-hydroxyl group of protected deoxynucleosides with manganese dioxide,[6] chromium trioxide in pyridine,[7] or platinum and oxygen under forcing conditions.[8]



1 **2**

R + sugar fragments

(1) For part V see M. G. Burdon and J. G. Moffatt, submitted for publication.

(2) Syntex Postdoctoral Fellow, 1964–1966.

(3) (a) K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, **85**, 3027 (1963); (b) K. E. Pfitzner and J. G. Moffatt, *ibid.*, **87**, 5661 (1965); (c) K. E. Pfitzner and J. G. Moffatt, *ibid.*, **87**, 3670 (1965).

(4) See, *e.g.*, (a) J. D. Albright and L. Goldman, *J. Org. Chem.*, **30**, 1107 (1965); (b) B. R. Baker and D. H. Buss, *ibid.*, **30**, 2304 (1965); (c) A. G. Brook and J. B. Pierce, *ibid.*, **30**, 2566 (1965).

(5) A detailed account of these studies is in preparation by G. H. Jones and J. G. Moffatt.

(6) A. S. Jones, R. T. Walker, and A. R. Williamson, *J. Chem. Soc.*, 6033 (1963).

(7) A. S. Jones, A. R. Williamson, and M. Winkley, *Carbohydrate Res.*, **1**, 187 (1965).

(8) Personal communication from Dr. G. M. Tener of the University of British Columbia.

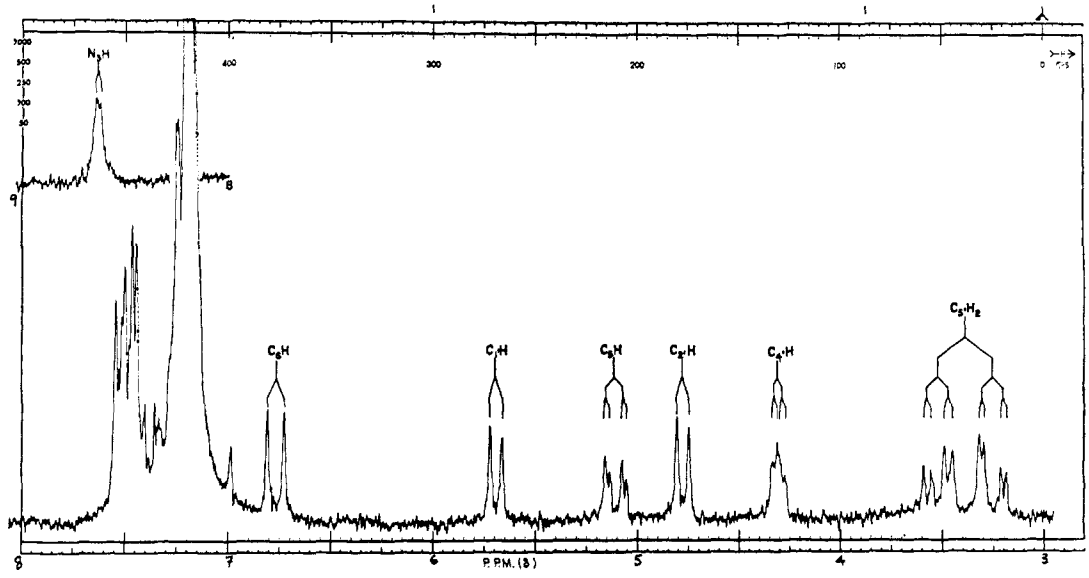
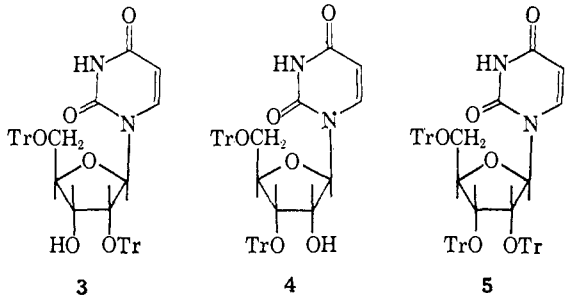


Figure 1. Nuclear magnetic resonance spectrum of 2′,5′-di-O-trityl-3′-ketouridine in deuteriochloroform at 100 Mc.

In this paper we describe the successful oxidation of some doubly blocked uridine derivatives and the isolation of free 2′- and 3′-ketouridines.

The starting materials in this work were the well-known 2′,5′-di-O-tritylуridine (**3**) and 3′,5′-di-O-tritylуridine (**4**) which were obtained in yields of 39 and 19% by reaction of uridine with excess triphenylmethyl chloride in pyridine at 100° essentially according to Yung and Fox[9a] and Žemlička.[9b] Much of the 2′,5′-dityrityl compound could be directly isolated by crystallization and the 3′,5′-dityrityl isomer was obtained by chromatography of the mother liquors on a silicic acid column. In addition to these two products a small amount (2%) of the previously undescribed 2′,3′,5′-tri-O-tritylуridine (**5**) was obtained in crystalline form and its structure was confirmed by elemental analysis and nuclear magnetic resonance spectroscopy. The presence of the third trityl group on a sugar hydroxyl rather than on N_3 of the uracil ring was shown by the ultraviolet spectrum which was typically that of a uridine derivative [λ_{max} 261 mμ (ε 9990)]. In addition, the nmr spectrum of **5** showed an interesting, and previously undescribed, coupling (J = 2 cps) of the N_3 and C_5 protons of the uracil ring. This effect, which was readily confirmed by spin-decoupling studies, was also noted with **3** and **4** as well as with the tritylated ketonucleosides to be described below.



3 **4** **5**

Treatment of **3** with DMSO and DCC in the presence of pyridinium trifluoroacetate at room temperature led to the smooth formation of a new, less polar product together with a little unreacted **3**, as demonstrated by thin layer chromatography. Direct crystallization from methanol gave a 46% yield of pure 2′,5′-di-O-trityl-3′-ketouridine (**6**) and preparative thin layer chromatography of the mother liquors on 1-m-long glass plates coated with a 1.3-mm layer of silicic acid[10] gave a further 20% yield of the pure product. This compound had an untraviolet spectrum typical of uridine derivatives [λ_{max} 261 mμ (ε 9600)] and had an infrared spectrum similar to that of **3** except for the presence of an additional carbonyl band at 1775 cm^{-1}.[11] Under nonaqueous conditions the product exists in the keto form as shown by its elemental analysis and its nmr spectrum. The latter is particularly revealing since the keto group effectively insulates the protons at $C_{2'}$ and $C_{4'}$ and thus leads to a spectrum in which all the protons are clearly resolved and readily analyzed. The 100-Mc spectrum of **6** is shown as Figure 1 and all assignments have been confirmed by spin-decoupling studies. Figure 1 clearly shows the previously mentioned coupling of C_5H and N_3H of the uracil ring, these protons occurring as a doublet of doublets ($J_{5,6}$ = 8 cps; $J_{5,3}$ = 2 cps) at δ 5.11 and a doublet (J = 2 cps) at δ 8.63, respectively. Irradiation at δ 8.63 results in the collapse of the C_5 proton into a doublet ($J_{5,6}$ = 8 cps) and irradiation at δ 5.11 reduces N_3H to a singlet. The nonequivalence of the 5′-methylene protons is also striking, these protons appearing as a pair of quartets centered at δ 3.24 (J_{gem} = 10 cps, $J_{4',5'a}$ = 2.2 cps) and δ 3.51 (J_{gem} = 10 cps; $J_{4',5'b}$ = 5.2 cps). The $C_{4'}H$ occurs as a quartet at δ 4.30 and irradiation at this point reduces the 5′-methylene protons to a pair of geminally coupled (J = 10 cps) doublets. Similarly, irradiation at δ 3.4 collapses $C_{4'}H$ to a singlet at δ 4.30. The $C_{1'}$ and $C_{2'}$ protons occur as simple doublets at δ 5.69 and 4.78 ($J_{1',2'}$ = 6 cps) and irradiation of ether collapses the other to a singlet. Addition of D_2O to the sample caused

(9) (a) N. C. Yung and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 3060 (1961); (b) J. Žemlička, *Collection Czech. Chem. Commun.*, **29**, 1734 (1964).

(10) H. Halpaap, *Chem. Ing. Tech.*, **35**, 488 (1963).

(11) Carbonyl absorptions in the 1770-cm^{-1} region are known for other ketofuranosides. See, *e.g.*, K. Onodera, S. Hirano, and N. Kashimura, *J. Am. Chem. Soc.*, **87**, 465 (1965).

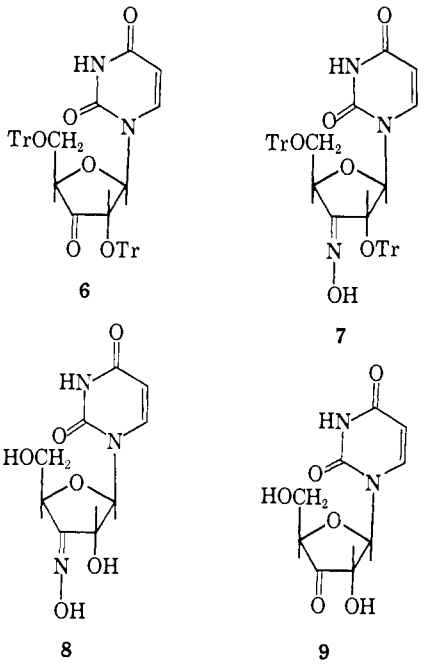
exchange of the N_3H for deuterium and eliminated the coupling with C_5H which then became a doublet.

We have also examined the oxidation of **3** by the closely related DMSO–phosphorus pentoxide[11] and DMSO–acetic anhydride[12] methods and found them to be equally suitable. These methods offer the distinct advantage of giving only water-soluble by-products, thus eliminating the necessity of removing dicyclohexylurea.[13] Using the phosphorus pentoxide method at 60° the starting alcohol **3** essentially disappeared and a 42% yield of the ketone **6** could be obtained by direct crystallization. A further 15% yield could be obtained by preparative thin layer chromatography of the mother liquors and the remaining material appeared to have largely degraded to uracil which was identified in the aqueous extracts. A similar situation obtained with the DMSO–acetic anhydride reaction which gave quite a clean reaction mixture from which a 45% yield of pure **6** could be obtained by crystallization.

While we were unable to isolate either a 2,4-dinitrophenylhydrazone or a tosylhydrazone derivative of **6** a crystalline oxime **7** could be obtained readily in 65% yield. Repeated attempts to reduce this oxime to a 3′-amino-3′-deoxynucleoside with hydrogen and palladium have, as yet, been unsuccessful and treatment with lithium aluminum hydride led to extensive degradation of the molecule. Treatment of **7** with 2 equiv of hydrogen chloride in chloroform at 0° successfully removed the trityl groups giving the free 3′-oximinouridine (**8**) which gave the expected nmr spectrum but appeared to be unstable, becoming discolored on exposure to air and decomposing upon attempted purification. Palladium-catalyzed hydrogenation of freshly prepared **8** gave only unchanged starting material.

Treatment of the ditrityl ketone **6** with 2 equiv of anhydrous hydrogen chloride in chloroform at 0° led to smooth detritylation and precipitation of free 3′-ketouridine (**9**) in quantitative yield. Elemental analysis of this material showed it to be the pure keto form rather than the hydrate. On paper electrophoresis in borate buffer at pH 6.0,[14] however, the ketone had a mobility of 1.2 times that of uridine itself and roughly comparable to that of lyxofuranosyl uracil. This suggests hydration of the ketone in aqueous solution leading to a product that can form both 2′,3′- and 3′,5′-borate complexes. Paper chromatography on bisulfite impregnated paper markedly reduced the mobility of the ketone relative to uridine, the R_f being comparable to that of uridine 5′-aldehyde.[3a,5]

Both the ditrityl ketone **6** and the free ketone **9**, being β-ketoglycosylamines, are extremely sensitive to alkaline conditions and readily undergo β elimination of the uracil moiety. Free 3′-ketouridine is especially sensitive and is instantaneously cleaved to uracil in pH 10.8 buffer at room temperature during attempted measurement of its ultraviolet spectra at that pH. The spectrum obtained had λ_{max} 286 mμ typical of uracil and, in addition, showed a broad maximum at 337 mμ which is not present in uracil. The latter peak is presumably due to an unsaturated sugar fragment such as **10**, resulting from the elimination reaction. Attempts to prepare a quinoxaline derivative of **10** by addition of *o*-phenylenediamine failed, but uracil was identified in the alkaline mixture by paper chromatography. Rapid elimination of uracil also seemed to occur at pH 9.8, but was more difficult to follow since uracil and uridine have rather similar spectra below pH 10.5. The ditrityl 3′-ketone **6** was much more stable toward alkali than was **9** and the rate of release of uracil could be readily followed in 0.1 *N* methanolic sodium hydroxide by the increase in ultraviolet absorption at 285 mμ relative to that at 260 mμ. In this case only a very weak absorption at 330 mμ resulted and by plotting the ratio of optical densities at 285 and 260 mμ a half-time of 7 min was determined for **6** under these conditions. This increased stability due to the presence of the 2′- and 5′-trityl groups is perhaps a consequence of steric distortion of the furanose ring into a conformation less amenable to β elimination. It may be noted that attempted oxidation of 5′-O-tritylthymidine (**1**, R = thymine, R′ = trityl) with either DMSO–DCC in the presence of pyridinium trifluoroacetate or with DMSO–acetic anhydride led only to thymine and to considerable amounts of triphenylcarbinol without detectable accumulation of the intermediate ketone (**2**, R = thymine, R′ = trityl). The presence of bulky substituents at both $C_{2'}$ and $C_{5'}$ thus appears to be a prerequisite for successful oxidation.



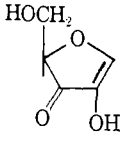
Reduction of the ditrityl ketone **6** with sodium borohydride in ethanol was rapid and separation of the products by preparative thin layer chromatography and crystallization gave a 59% yield of 1-(2,5-di-O-trityl-β-D-xylofuranosyl)uracil (**11**) and 31% of 2′,5′-di-O-trityluridine (**3**) in crystalline form. Both products had physical constants identical to those described by Yung and Fox.[9a] Detritylation of the crude reduction mixture by treatment with hot 80% acetic acid gave a mixture of uridine and 1-(β-D-xylofuranosyl)-uracil (**12**) which were cleanly separated by borate

(12) J. D. Albright and L. Goldman, *J. Am. Chem. Soc.*, 87, 4215 (1965).

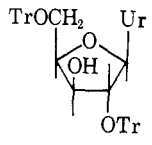
(13) This problem can also be circumvented by using diethylcarbodiimide and DMSO, the resulting diethylurea being water soluble: G. H. Jones and J. G. Moffatt, unpublished observations.

(14) J. F. Codington, R. Fecher, and J. J. Fox, *J. Am. Chem. Soc.*, 82, 2794 (1960).

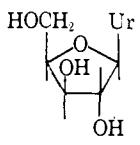
electrophoresis.[14] Quantitative estimation of the eluted spots by ultraviolet absorption showed that borohydride reduction of **6** gave 66% of the xylosyl derivative (**11**) and 34% of the ribose epimer **3**. This result indicates that the trityl ether at $C_{2'}$ offers less steric hindrance to approach by the reducing agent than does the uracil ring and the 5′-trityl group on the β face of the ribose ring. Borohydride reduction of free 3′-ketouridine (**9**) in aqueous ethanol was also studied and quantitative estimation of the two products by borate electrophoresis showed 1-(β-D-xylofuranosyl)uracil (**12**) and uridine to be present in 69 and 31% yields, respectively. The similarity of these results to those with the ditrityl ketone once again emphasizes the predominant directive influence of the uracil ring in the reduction process.



10



11

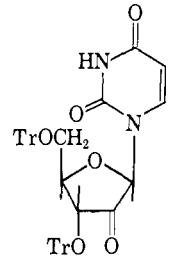


12

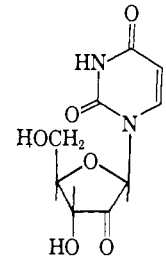
The oxidation of 3′,5′-di-O-trityluridine (**4**) has also been accomplished. Thus treatment with DMSO–DCC in the presence of pyridinium trifluoroacetate gave some unreacted **4** together with the major product, 3′,5′-di-O-trityl-2′-ketouridine (**13**), which was isolated in pure form in 63% yield by preparative thin layer chromatography. In spite of repeated efforts we have been unable to obtain this product in crystalline form. Its structure, however, is certain from its elemental analysis and nmr spectrum, the latter clearly showing the $C_{1'}$ proton as a sharp singlet at δ 5.79, and the $C_{3'}$–H as a doublet ($J_{3',4'}$ = 4 cps) at δ 4.40. Coupling between C_5H and N_3H ($J_{3,5}$ = 2 cps) was again apparent but, unlike **6**, the 5′-methylene protons were equivalent and appeared as a doublet ($J_{5',4'}$ = 4 cps) at δ 2.91.

Oxidation of **4** with DMSO–phosphorus pentoxide at 65° was also successful and gave a 52% yield of only slightly impure **13** by precipitation from the crude, extracted reaction mixture without chromatography. Once again an appreciable amount of uracil was found in the aqueous extracts. In a similar way oxidation of **4** by DMSO–acetic anhydride gave a 56% yield of slightly impure **13** by precipitation without chromatography.

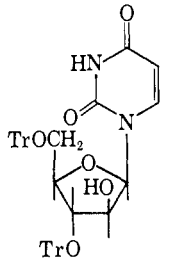
Treatment of the ditrityl ketone **13** with 2 equiv of anhydrous hydrogen chloride in chloroform at 0° led to the precipitation of chromatographically and analytically pure 2′-ketouridine (**14**) in 72% yield. The nmr spectrum of **14** was completely compatible with the assigned structure, the $C_{1'}$–H once again appearing as a sharp singlet at δ 5.42, and the $C_{3'}$–H as a doublet (J = 9 cps) at δ 7.30, both confirming the absence of a proton at $C_{2'}$. For convenience a tabulation of 100-Mc nmr assignments for a number of the uridine nucleosides described in this paper is given in Table I and all have been confirmed by spin-decoupling studies. A crystalline 2,4-dinitrophenylhydrazone of 2′-ketouridine was readily prepared using the extremely useful method of Parrick and Rasburn[15] with DMSO as solvent. The reduction of both the ditrityl 2′-ketone **13** and the free 2′-ketone **14** has been examined using sodium borohydride in ethanol and gave results quite similar to those with the 3′-keto compounds. Thus reduction of **13** gave predominantly 1-(3,5-di-O-trityl-β-D-arabinofuranosyl)uracil[16] (**15**) and lesser amounts of 3′,5′-di-O-trityluridine (**4**) which were isolated in yields of 57 and 11% by preparative thin layer chromatography. Removal of the trityl groups from these compounds gave 1-(β-D-arabinofuranosyl)uracil (**16**) and uridine, which were identified by comparison with authentic samples using borate electrophoresis and paper chromatography. Direct acid hydrolysis of the borohydride reduction mixture followed by borate electrophoresis showed that the products were the arabinosyl and the ribosyl derivatives in yields of 82 and 18%, respectively. In a similar way, reduction of free 2′-ketouridine (**14**) followed by quantitative borate electrophoresis showed the products to be arabinosyluracil (**16**) and uridine in yields of 90 and 10%. The predominant directive influence of the uracil ring on the reduction process is once again evident, the greater stereoselectivity being a consequence of the closer proximity of the heterocyclic base and the keto group. The reduction of 2′- and 3′-ketouridines with tritiated sodium borohydride provides a convenient synthesis of the specifically 2′-tritiated arabinosyluracil and uridine and 3′-tritiated xylosyluracil and uridine, and these results will be reported elsewhere.[17]



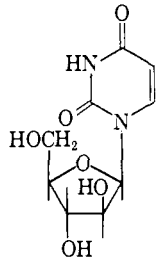
13



14



15



16

The action of alkali on the 2′-ketouridines has also been studied and, as with 3′-ketouridine, it was shown that essentially instantaneous elimination of uracil took place in either 0.01 *N* sodium hydroxide or in pH 10.8 buffer. Thus while **14** had the typical ultraviolet spectrum of uridine under neutral conditions the spectrum of a freshly prepared solution at pH 10.8 showed a maximum at 286 mμ typical of uracil and a second maximum at 337 mμ similar to that found with 3′-ketouridine. Uracil could be identified by paper chromatography and the other elimination product has not been studied further. A similar alkaline instability of the ditrityl 2′-ketone **13** was also observed and uracil could be readily identified by thin layer and

(15) J. Parrick and J. W. Rasburn, *Can. J. Chem.*, 43, 3453 (1965).

(16) J. F. Codington, I. L. Doerr, and J. J. Fox, *J. Org. Chem.*, **29**, 564 (1964).

(17) A. F. Cook, G. H. Jones, and J. G. Moffatt, in preparation.

Table I. Nmr Spectra of Uridine Nucleosides at 100 Mc[a,b]

Compd	C_5H	C_6H	N_3H	$C_1'H$	$C_2'H$	$C_3'H$	$C_4'H$	$C_5'H$
2′,5′-Ditrityluridine (3)	5.10 (q) $J_{5,6}$ = 8 cps $J_{5,3}$ = 2 cps	7.66 (d) $J_{5,6}$ = 8 cps	9.60 (d) $J_{3,5}$ = 2 cps	6.57 (d) $J_{1',2'}$ = 7.5 cps	4.49 (q) $J_{1',2'}$ = 7.5 cps $J_{2',3'}$ = 4.5 cps	2.77 (d) $J_{2',3'}$ = 4.5 cps	3.96 (s)	3.13 (s)
3′,5′-Ditrityluridine (4)	5.25 (q) $J_{5,6}$ = 8 cps $J_{5,3}$ = 2 cps	7.56 (d) J = 8 cps	8.93 (d) $J_{3,5}$ = 2 cps	6.00 (d) $J_{1',2'}$ = 5 cps	3.70 (m)	4.30 (q) $J_{2',3'}$ = 4 (5) cps $J_{3',4'}$ = 5 (4) cps	3.70 (m)	3.35 (q) J_{gem} = 10 cps $J_{4',5'b}$ = 10 cps 3.00 (q) J_{gem} = 10 cps $J_{4',5'a}$ = 3 cps
2′,3′,5′-Tritrityluridine (5)	4.85 (q) $J_{5,6}$ = 8 cps $J_{5,3}$ = 2 cps	...[d]	8.38 (d) $J_{3,5}$ = 2 cps	...[d]	4.63 (q) $J_{1',2'}$ = 8 cps $J_{2',3'}$ = 4 cps	3.52 (d) $J_{2',3'}$ = 4 cps $J_{3',4'}$ = <1 cps	3.96 (broad s)	2.61 (q) J_{gem} = 11 cps $J_{4',5'b}$ = 1.5 cps 2.27 (q) J_{gem} = 11 cps $J_{4',5'a}$ = <1 cps
2′,5′-Ditrityl-3′-ketouridine (6)	5.11 (q) $J_{5,6}$ = 8 cps $J_{5,3}$ = 2 cps	6.77 (d) $J_{5,6}$ = 8 cps	8.63 (d) $J_{3,5}$ = 2 cps	5.69 (d) $J_{1',2'}$ = 6 cps	4.78 (d) $J_{1',2'}$ = 6 cps	...	4.30 (q) $J_{4',5'a}$ = 2.2 cps $J_{4',5'b}$ = 4.2 cps	3.51 (q) J_{gem} = 10 cps $J_{4',5'b}$ = 4.2 cps 3.24 (q) J_{gem} = 10 cps $J_{4',5'a}$ = 2.2 cps
3′,5′-Ditrityl-2′-ketouridine (13)	5.34 (q) $J_{5,6}$ = 8 cps $J_{5,3}$ = 2 cps	...[d]	8.88 (d)[e] $J_{3,5}$ = 2 cps	7.57 (s)	...	4.40 (d) $J_{3',4'}$ = 4 cps	4.04 (q) $J_{3',4'}$ = 4 cps $J_{4',5'}$ = 4 cps	2.91 (d) $J_{4',5'}$ = 4 cps
3′-Ketouridine (9)[c]	5.76 (q) $J_{5,6}$ = 8 cps $J_{5,3}$ = 0.5 cps	7.89 (d) $J_{5,6}$ = 8 cps	11.42 (broad s)	6.05 (d) $J_{1',2'}$ = 8 cps	4.24 (d) $J_{1',2'}$ = 8 cps	...	4.22 (t) $J_{4',5'}$ = 3 cps	3.63 (d) $J_{4',5'}$ = 3 cps
2′-Ketouridine (14)[c]	5.64 (q) $J_{5,6}$ = 8 cps $J_{5,3}$ = 2 cps	7.72 (d) $J_{5,6}$ = 2 cps	11.49 (d) $J_{3,5}$ = 2 cps	5.42 (s)	...	7.30 (d) $J_{3',4'}$ = 9 cps	3.40–3.90 (m)	3.40–3.90 (m)
2′,5′-Ditrityl-xylosyluracil (11)	5.52 (q) $J_{5,6}$ = 8 cps $J_{5,3}$ = 2 cps	...[d]	8.91 (d)[e] $J_{3,5}$ = 2 cps	6.10 (d) $J_{1',2'}$ = 2 cps	4.11 (d) $J_{1',2'}$ = 2 cps	3.18 (d) $J_{3',4'}$ = 3 cps	4.05 (m)	3.46 (q) J_{gem} = 11 cps $J_{4',5'b}$ = 4.5 cps 3.32 (q) J_{gem} = 11 cps $J_{4',5'a}$ = 5 cps
3′,5′-Ditrityl-arabinosyluracil (15)	5.44 (q) $J_{5,6}$ = 8 cps $J_{5,3}$ = 2 cps	7.49 (d) $J_{5,6}$ = 8 cps	9.02 (d)[e] $J_{3,5}$ = 2 cps	6.12 (d) $J_{1',2'}$ = 3 cps	3.70 (m)	3.96 (m)	3.90 (m)	3.29 (q) J_{gem} = 10 cps $J_{4',5'b}$ = 2 cps 3.01 (q) J_{gem} = 10 cps $J_{4',5'a}$ = 4 cps
N_3-Me-2′,5′-ditritylxylosyluracil	5.59 (d) $J_{5,6}$ = 8 cps	...[d]	N_3-Me at 3.31 (s)	6.09 (d) $J_{1',2'}$ = 2 cps	4.12 (d) $J_{1',2'}$ = 2 cps	3.27 (m)	4.06 (m)	3.42 (m)
N_3-Me-3′-5′-ditrityluridine	5.12 (d) $J_{5,6}$ = 8 cps	5.62 (d) $J_{5,6}$ = 8 cps	N_3-Me at 3.40 (s)	6.59 (d) $J_{1',2'}$ = 8 cps	4.49 (q) $J_{1',2'}$ = 8 cps $J_{2',3'}$ = 5 cps	2.84 (d) $J_{2',3'}$ = 5 cps $J_{3',4'}$ = 0	3.96 (broad s)	3.10 (d) $J_{4',5'}$ = 2 cps

[a] All spectra in deuteriochloroform unless otherwise noted. [b] (s) = singlet, (d) = doublet, (t) = triplet, (q) = quartet (m) = multiplet. [c] In deuteriodimethyl sulfoxide. No hydroxyl coupling was evident. [d] In trityl envelope. [e] Some further undefined coupling (J = 0.5 cps) is also present.

paper chromatography. Determination of the rate of elimination by examination of ultraviolet spectral changes was, however, rendered impossible by the rapid formation of an orange color and broad absorption below 270 mμ. When the alkaline treatment was done on a larger scale the appearance of the orange color was accompanied by the separation of a cream-colored solid. From this material it was possible to isolate a crystalline compound identified as bis(triphenylmethyl) peroxide[18] by physical constants, elemental analyses, and spectral data. The mechanism by which this peroxide is formed remains oscure but its formation can be markedly reduced by conducting the alkaline treatment under nitrogen. In addition to uracil, triphenylcarbinol was the only other major product identified in the methanol-soluble fractions from the alkaline reaction mixture.

A number of attempts have been made to alkylate the carbonyl group of 2′,5′-di-O-trityl-3′-ketouridine so as to prepare branched chain nucleosides such as 3′-C-methyluridine (17).[19] In general, however, reactions of **6** with methyl Grignard reagents in ether, tetrahydrofuran, monoglyme, or tetrahydrothiophene[20] under a variety of conditions led to no observable products. In most cases some precipitation resulted upon addition of the Grignard reagent but the lack of

(18) M. Gomberg, *Ber.*, **33**, 3155 (1900).

(19) The analogous 2′-C-methyladenosine and 3′-C-methyladenosine have been recently described by other routes. See E. Walton, S. R. Jenkins, R. F. Nutt, M. Zimmerman, and F. W. Holly, *J. Am. Chem. Soc.*, **88**, 4524 (1966), and E. Walton, F. W. Holly, and R. F. Nutt, Abstract 37C of the Winter Meeting of the American Chemical Society, Phoenix, Ariz., Jan 1966.

(20) A. A. Scala and E. I. Becker, *J. Org. Chem.*, **30**, 3491 (1965).

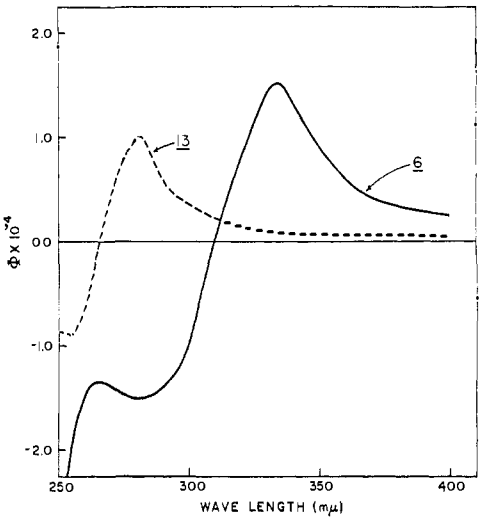
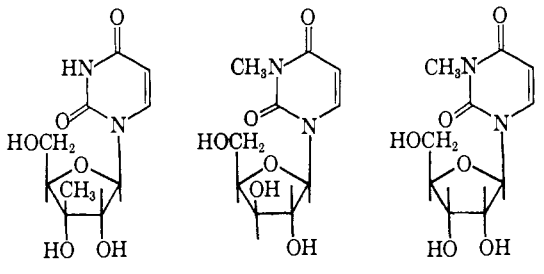


Figure 2. Optical rotatory dispersion spectra of 2′,5′-di-O-trityl-3′-ketouridine (**6**) ———, and of 3′,5′-di-O-trityl-2′-ketouridine (**13**) - - - -, in methanol.

any alkylation was confirmed by thin layer chromatographic identification of unreacted starting material and by borohydride reduction to the previously described xyloside **11** and riboside **3**. As yet no successful methylation has been achieved using methyllithium in ether, tetrahydrofuran, or monoglyme. Under these conditions extensive decomposition of the ketone, and of 2′,5′-ditrityluridine itself, was apparent.

An attempt was also made to react **6** and **13** with diazomethane, a reaction that has been successfully applied to alkylation of protected keto sugars.[21] Methylation at N_3 of the uracil ring was, of course, an expected side reaction.[22] After storage of **6** with an excess of diazomethane in ether thin layer chromatography indicated the presence of one major product and several minor ones. The major product was isolated, treated with sodium borohydride, and then detritylated with acetic acid giving two products. These were identified by both paper chromatography and borate electrophoresis as N_3-methyl-1-(β-D-xylofuranosyl)uracil (**18**) and N_3-methyluridine (**19**), authentic samples of which were prepared by the action of diazomethane on 2′,5′-di-O-trityl-1-(β-D-xylofuranosyl)uracil (**11**) and 2′,5′-di-O-trityluridine (**3**), respectively, followed by acid hydrolysis. Thus while N-methylation readily occurred, alkylation of the keto group cannot have taken place to more than a minor degree.



17 **18** **19**

Further work will, however, be necessary in order to fully explore the possibility of using 2′- and 3′-ketonucleosides as synthetic intermediates.

A final point of interest concerns a comparison of the optical rotatory disperion (ORD) spectra of the 2′- and 3′-ketouridine derivatives **6** and **13** which are shown in Figure 2. It can be seen from Figure **2** that the 2′-ketone (**13**) has a positive Cotton effect (molecular amplitude 191) with a zero rotation at 265 mμ similar to that of uridine[23] or 2′,5′-ditrityluridine. The 3′-ketone **6**, however, shows a positive Cotton effect (amplitude 307) displaced toward longer wavelengths and showing zero rotation at 310 mμ. A second maximum is also present at lower wavelengths and is more pronounced when dioxane is used as the solvent. The position of this Cotton effect indicates its relationship to the n–π* transition of the carbonyl group rather than to the uracil ring. A similar effect is noted in the optical rotatory dispersion curves of the detritylated 2′- and 3′-ketouridines (**14** and **9**) which showed zero rotations at 265 and 302 mμ, respectively.

Further work on the synthesis of other ketonucleosides is in progress and will be reported at a later date.

Experimental Section

Methods. Thin layer chromatography was carried out on 0.25-mm layers of Merck silica gel GF, and the products were visualized by ultraviolet absorption or by spraying with a 5% solution of ammonium molybdate in 10% sulfuric acid followed by brief heating at 150°. Preparative thin layer chromatography was done on 20 × 100 cm glass plates coated with a 1.3-mm layer of Merck silica gel HF and column chromatography on Merck silica with 0.05–0.20-mm particles. Nuclear magnetic resonance spectra were obtained using solutions in deuteriochloroform (unless otherwise stated) and either a Varian A-60 or HA-100 spectrometer. Mass spectra were determined using an Atlas CH-4 spectrometer with a direct inlet system. Ultraviolet spectra were determined using a Cary Model 15 instrument and infrared spectra were obtained from potassium bromide pellets on a Perkin-Elmer Model 237 instrument. Optical rotatory dispersion spectra were obtained using a Jasco Model ORD/UV-5 instrument. Instrumental analyses were performed by the staff of the Analytical Laboratory of Syntex Research. We are particularly grateful to Mr. J. Murphy and to Drs. T. Toube and L. Tokes for their painstaking assistance with nmr and mass spectrometry respectively. Elemental analyses were obtained from Dr. A. Bernhardt, Mulheim, Germany.

Tritylation of Uridine. Uridine (15 g, 61.5 mmoles) and triphenylmethyl chloride (51.4 g, 183 mmoles) were stored overnight in pyridine (150 ml) and then heated at 100° for 4 hr. The light brown solution was poured into vigorously stirred ice water, and the gummy precipitate was dissolved in chloroform, washed with cadium chloride solution and then with water, dried over sodium sulfate, and evaporated to a yellow syrup. Crystallization from benzene–ether gave 13.6 g (30%) of 2′,5′-di-O-trityluridine (**3**) which was homogeneous by thin layer chromatography using chloroform–ethyl acetate (1:1) and had mp 217–220° (lit. mp 224–225°[9a] and 215–220°[9b]); $\lambda_{max}^{dioxane}$ 261 mμ (ε 9100). The nmr spectrum in deuteriochloroform is recorded in Table I.

Chromatography of the combined mother liquors[24] on 1200 g of silicic acid using chloroform–ethyl acetate (1:1) gave a small amount (2%) of 2′,3,′5′-tri-O-trityluridine (**5**) of mp 286–288° from ethyl acetate after final purification by preparative thin layer chromatography using chloroform–ethyl acetate (10:1); $\lambda_{max}^{dioxane}$ 261 mμ (ε 9990); $[\alpha]^{22}D$ −37.5° (*c* 0.1, chloroform). The nmr spectrum is recorded in Table I.

Anal. Calcd for $C_{66}H_{54}N_2O_6$: C, 81.65; H, 5.57; N, 2.89; O, 9.90. Found: C, 81.53; H, 5.74; N, 3.06; O, 10.15.

(21) (a) W. G. Overend and N. R. Williams, *J. Chem. Soc.*, 3446 (1965); (b) J. J. K. Novák and F. Šorm, *Collection Czech. Chem. Commun.*, **30**, 3303 (1965).

(22) J. A. Haines, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 1406 (1964).

(23) T. L. V. Ulbricht, J. P. Jennings, M. M. Scopes, and W. Klyne, *Tetrahedron Letters*, 695 (1964).

(24) The tritrityluridine was actually found almost exclusively in the mother liquors from recrystallization of the second and third crops of 2′,5′-ditrityluridine.

Detritylation with 80% acetic acid at 100° for 1 hr gave crystalline uridine. Continued elution gave a further 3.8 g of crystalline 2′,5′-ditrityluridine (total yield 39%) followed by 8.4 g (19%) of chromatographically homogeneous but apparently amorphous 3′,5′-di-O-trityluridine (**4**) with $\lambda_{max}^{dioxane}$ 261 mμ (ε 9540) and $[\alpha]^{22}D$ −5.6° (*c* 0.1, chloroform). See Table I for the nmr spectral results.

2′,5′-Di-O-trityl-3′-ketouridine (6). (a) By the DMSO–DCC Method. 2′,5′-Di-O-trityluridine (3.02 g, 4 mmoles) was dissolved in a mixture of anhydrous DMSO[25] (15 ml) and benzene (15 ml) containing dicyclohexylcarbodiimide (2.48 g, 12 mmoles) and pyridine (0.32 ml, 4 mmoles). Trifluoroacetic acid (0.16 ml, 2 mmoles) was then added, and the mixture was stored overnight at room temperature. Oxalic acid (1.3 g, 12 mmoles) was then added to destroy excess DCC and after 30 min, chloroform (50 ml) and water (50 ml) were added and dicyclohexylurea was removed by filtration. The chloroform layer was rapidly extracted twice with 1 *N* sodium bicarbonate and then with water. It was then dried over sodium sulfate and evaporated leaving a residual froth which was dissolved in hot methanol, seeded,[26] and allowed to slowly cool giving 1.40 g (46%) of 2′,5′-di-O-trityl-3′-ketouridine (**6**) of mp 146–148° unchanged upon recrystallization, λ_{max}^{MeOH} 261 mμ (ε 9600); $[\alpha]^{22}D$ +62.3° (*c* 0.1, chloroform). The nmr and optical rotatory dispersion spectra are shown as Figures 1 and 2 and are discussed in the text.

Anal. Calcd for $C_{47}H_{38}N_2O_6$: C, 77.67; H, 5.27; N, 3.85. O, 13.21. Found: C, 77.54; H, 5.17; N, 4.02; O, 13.01.

The mother liquors were evaporated leaving 1.8 g of a syrup that was chromatographed on four 20 × 100 cm preparative thin layer plates using chloroform–ethyl acetate (10:1) which separated unreacted 2′,5′-ditrityluridine from the faster moving ketone. The faster band was eluted wth actone giving 890 mg of the homogeneous ketone which was crystallized from methanol giving 620 mg (total yield 2.02 g, 66%) of pure material.

(b) Using DMSO–Phosphorus Pentoxide.[11] Phosphorus pentoxide (1.74 g, 12 mmoles) was added slowly to anhydrous dimethyl sulfoxide (75 ml). After the mixture cooled 2′,5′-di-O-trityluridine (7.28 g, 10 mmoles) was added, and the mixture was stirred at 60° for 2 hr. Ether (200 ml) was added, and the solution was extracted three times with 5% aqueous sodium bicarbonate and then with water. After drying with sodium sulfate and evaporating the solvent, 6.33 g of a solid froth remained and was directly crystallized from methanol with slow cooling giving 3.08 g (42%) of the pure ketone **6** in two crops. The mother liquors were then chromatographed on 200 g of silicic acid using chloroform–ethyl acetate (21:1) giving a further 1.08 g of pure product (total yield 57%) after crystallization from methanol. The product was identical with that from the DMSO–DCC reaction.

(c) Using DMSO–Acetic Anhydride.[12] 2′,5′-Ditrityluridine (7.25 g, 10 mmoles) was dissolved in DMSO (65 ml) and acetic anhydride (20 ml) and stored overnight at room temperature. Most of the acetic anhydride was then evaporated *in vacuo* leaving a residue which was dissolved in ether, extracted three times with aqueous sodium bicarbonate, then with water, and dried over sodium sulfate. After evaporation of the solvent the residue was crystallized from methanol giving 3.18 g (44%) of the pure ketone **6**, mp 145–147°.

2′,5′-Di-O-trityl-3′-oximinouridine (7). 2′,5′-Di-O-trityl-3′-ketouridine (**6**, 1.01 g) was dissolved in pyridine (20 ml) together with hydroxylamine hydrochloride (1.02 g), and the mixture was kept at 60° for 2.5 hr at which time thin layer chromatography using chloroform–ethyl acetate (6:1) showed the disappearance of the starting material and formation of a somewhat slower product. The solvent was then evaporated to dryness, and the residue was dissolved in chloroform. After extraction with aqueous cadmium chloride and then water, the solution was dried with sodium sulfate and evaporated leaving 1.32 g of a white froth which was crystallized twice from ethanol giving 0.67 g (65%) of the pure oxime **7**, mp 224–227°; $\lambda_{max}^{dioxane}$ 260 mμ (ε 9900); $[\alpha]^{22}D$ +38.2° (*c* 0.1, chloroform).

Anal. Calcd for $C_{47}H_{39}N_3O_6$: C, 76.11; H, 5.26; N, 5.67. Found: C, 75.94; H, 5.26; N, 5.80.

The ORD spectrum in dioxane showed a positive Cotton effect with a maximum at 282 mμ (molecular rotation +27,000°), zero rotation at 260 mμ, and a shoulder at 245 mμ (molecular rotation −23,500°). The nmr spectrum in DMSO-d_6 showed the oximino hydrogen as a singlet at δ 11.77.

Attempted reduction of **7** with hydrogen and palladium on barium sulfate[27] in ethyl acetate was unsuccessful and using lithium aluminum hydride in tetrahydrofuran there was extensive loss of ultraviolet-absorbing materials.

3′-Ketouridine (9). 2′,5′-Ditrityl-3′-ketouridine (1.228 g, 1.69 mmoles) was dissolved in dry chloroform (20 ml) and cooled to 0°. A freshly standardized solution of hydrogen chloride in chloroform (9.3 ml of 0.40 *N*, 3.72 mmoles) was then added dropwise with stirring over 20 min, and the mixture was stirred for 1 hr more. The resulting white precipitate was then collected by centrifugation, washed six times with 25-ml portions of ether, and dried *in vacuo* over potassium hydroxide giving 404 mg (99%) of 3′-ketouridine as an amorphous white solid that was not obtained crystalline but melted at 130–138°.

Anal. Calcd for $C_9H_{10}N_2O_6$: C, 44.63; H, 4.16; N, 11.57. Found: C, 44.76; H, 4.32; N, 11.51.

The product was homogeneous by thin layer chromatography with chloroform–methanol (4:1) having an R_f identical with that of uracil, and on paper using *n*-butyl alcohol–acetic acid–water (5:2:3) where it had R_f 0.41, just faster than uridine. Using the same solvent system with paper previously impregnated with sodium bisulfite it had a very low R_f while uracil moved normally. On paper electrophoresis[14] in 1 *M* boric acid adjusted to pH 6.0 using 1000 v for 4 hr **9** had a mobility of 1.2 relative to uridine while uracil remained on the origin; $\lambda_{max}^{H_2O}$ 261 mμ (ε 9400); $[\alpha]^{22}D$ +71.2° (*c* 0.1, water).

The ORD spectrum in water showed a positive Cotton effect with a maximum at 316 mμ (molecular rotation +4400°), zero rotation at 292 mμ, and a minimum at 251 mμ (molecular rotation −8420°).

Borohydride Reduction of 6 and 9. (a) 2′,5′-Di-O-trityl-3′-ketouridine was dissolved in ethanol (15 ml) and sodium borohydride (286 mg) was added. After 1 hr at room temperature the solution was partitioned between chloroform and water, and the chloroform solution was washed three times with water, dried over sodium sulfate, and evaporated to dryness. Crystallization from benzene–ether gave a first crop (55 mg) of pure 2′,5′-di-O-trityluridine, mp 219–221°, undepressed on admixture with an authentic sample. The infrared spectra were also identical. The mother liquors were then chromatographed on three 20 × 100 cm preparative thin layer plates using carbon tetrachloride–acetone (4:1) which clearly resolved two bands. Elution of the faster band with acetone gave a further 35 mg of 2′,5′-ditrityluridine after crystallization from ether (total yield 31%). The slower band gave, after elution with acetone, 210 mg of a crystalline residue which was recrystallized from ethanol giving 170 mg of pure 1-(2,5-di-O-trityl-β-D-xylofuranosyl)uracil, mp 150–154° (lit.[9a] mp 151.5–153.5°). The nmr spectrum indicated the presence of 1 mole of ethanol of crystallization rather than one-half mole as described previously;[9a] $\lambda_{max}^{dioxane}$ 258 mμ (ε 11,760); $[\alpha]^{22}D$ +49.6° (*c* 0.1, chloroform). The ORD spectrum in methanol showed a positive Cotton effect with a maximum at 276 mμ (molecular rotation +20,100°), zero rotation at 272 mμ, and a minimum at 242 mμ (molecular rotation −20,100°).

A small portion (11 mg) of the crude reduction mixture was also treated with 80% acetic acid (1 ml) at 100° for 1 hr, evaporated to dryness, and examined by electrophoresis in pH 6.0 borate buffer at 1000 v for 4 hr. Elution of the two resulting spots which had mobilities of 1.0 and 0.67 relative to uridine showed the reduction to give 34% uridine and 66% 1-β-D-xylofuranosyluracil (**12**).[28]

(b) 3′-Ketouridine (7.2 mg) was dissolved in water (1 ml) together with sodium borohydride (8 mg). After 30 min the solution was neutralized with Dowex 50 (H^+) resin and directly examined by borate electrophoresis. Quantitative elution of the resulting spots with 0.1 *N* hydrochloric acid showed the products to be 31% uridine and 69% xylosyluracil.

Action of Alkali on 6 and 9. (a) 2′,5′-Di-O-trityl-3′-ketouridine was dissolved in methanol at a concentration of 106.3 μg/ml and diluted with an equal volume of 0.2 *N* methanolic sodium hydroxide. The ultraviolet spectrum of the solution was recorded at intervals over 2 hr, and the course of the reaction was followed by the ratio of absorbance at 285 and 260 mμ. The initial 285:260 ratio was 0.17 and the final value was 1.40. The half-time of the reaction was 7 min, and the product was identified as uracil by paper and thin layer chromatography.

(25) Distilled and stored over Linde Molecular Sieve Type 4A.

(26) Initial seeds were obtained by crystallization of material from another reaction that was purified by preparative thin layer chromatography using chloroform–ethyl acetate (8:1).

(27) R. Kuhn and H. J. Hass, *Angew. Chem.*, **67**, 785 (1955).

(28) We are grateful to Dr. J. J. Fox for an authentic sample of **12**.

(b) An aqueous solution of 3′-ketouridine in water at a concentration of 20.20 μg/ml (λ_{max} 262 mμ) was diluted with an equal volume of 0.05 *M* carbonate buffer (pH 10.8). Within 2 min the spectrum showed the complete disappearance of the 262-mμ peak with formation of two new maxima at 286 and 336 mμ of roughly equal intensities. Paper chromatographic examination of a more concentrated solution showed a strong spot identical with uracil.

3′,5′-Di-O-trityl-2′-ketouridine (13). (a) Using DMSO–DCC. 3′,5′-Di-O-trityluridine (728 mg, 1 mmole) was dissolved in a mixture of DMSO (10 ml) and benzene (10 ml) containing DCC (0.62 g, 3 mmoles) and pyridine (0.075 ml, 1 mmole). Trifluoroacetic acid (0.04 ml, 0.5 mmole) was added and after 16 hr at room temperature oxalic acid (0.38 g, 3 mmoles) was added. After 30 min the mixture was diluted with chloroform (50 ml), filtered, and extracted with aqueous sodium bicarbonate, followed by water. The chloroform solution was dried over sodium sulfate and evaporated *in vacuo* leaving 1.02 g of a white froth that was separated by preparative thin layer chromatography on three 20 × 100 cm plates using two consecutive developments with chloroform–ethyl acetate (9:1). The slower band was eluted with acetone giving 217 mg (30%) of unreacted 3′,5′-ditrityluridine while the faster band gave 455 mg (63%) of chromatographically homogeneous 3′,5′-di-O-trityl-2′-ketouridine which we have been unable to crystallize but which melted at 130–135°; λ_{max}^{MeOH} 260 mμ (ε 10,900); $[\alpha]^{22}D$ +30.8° (*c* 0.1, chloroform).

Anal. Calcd for $C_{47}H_{38}N_2O_6$: C, 77.67; H, 5.27; N, 3.85. Found: C, 77.77; H, 5.50; N, 3.91.

The ORD spectrum is shown in Figure 2, and significant features of the nmr spectrum are discussed in the text. The carbonyl group appeared in the infrared spectrum at 1785 cm^{-1} (KBr) and at 1790 cm^{-1} (CCl_4).

(b) Using DMSO–Phosphorus Pentoxide. 3′,5′-Di-O-trityluridine (3.85 g, 5.3 mmoles) was added to a solution of phosphorus pentoxide (0.90 g, 6.3 mmoles as P_2O_5) in DMSO (25 ml), and the solution was heated for 1 hr at 65°. It was then diluted with ether, washed with aqueous sodium bicarbonate, dried, and evaporated leaving an off-white froth (2.77 g) which contained several minor by-products in addition to **13**. This was dissolved in ether and precipitated with hexane giving 1.99 g (52%) of **13** of sufficient purity for most purposes. Completely pure product could be obtained by preparative thin layer chromatography.

(c) Using DMSO–Acetic Anhydride. 3′,5′-Di-O-trityluridine (1.46 g, 2 mmoles) was treated overnight at room temperature with a mixture of DMSO (10 ml) and acetic anhydride (4 ml). The acetic anhydride was largely evaporated *in vacuo*, and the residue was dissolved in ether and extracted twice with aqueous sodium bicarbonate and then with water. Evaporation of the solvent left 1.39 g of a froth which was dissolved in ether and precipitated with hexane giving 858 mg (56%) of **13** containing only minor impurities. Detritylation of the nonprecipitated fraction gave a complex mixture of products that was not studied further.

2′-Ketouridine (14). 3′,5′-Di-O-trityl-2′-ketouridine (597 mg, 0.82 mmole) was dissolved in dry chloroform (20 ml) and cooled to 0°. A solution of hydrogen chloride in chloroform (8 ml of 0.23 *N*, 1.84 mmoles) was added dropwise over 15 min and stirring was continued for a further 30 min. The precipitated solid was collected by centrifugation and washed six times with 25-ml portions of ether. The white residue was then dried *in vacuo* over potassium hydroxide giving 144 mg (72%) of 2′-ketouridine (mp 186–189°) which ran as a single spot just ahead of uridine on borate electrophoresis and as a somewhat elongated spot between uridine and uracil on thin layer chromatography using chloroform–methanol (4:1); $\lambda_{max}^{H_2O}$ 261 mμ (ε 9600); $[\alpha]^{22}D$ +43.7° (*c* 0.1, water).

Anal. Calcd for $C_9H_{10}N_2O_6$: C, 44.63; H, 4.16; N, 11.57. Found: C, 44.48; H, 4.31; N, 11.51.

The ORD spectrum in water showed a positive Cotton effect with a maximum at 274 mμ (molecular rotation +11,200°), zero rotation at 248 mμ, and a minimum at 234 mμ (molecular rotation −3900°).

Reaction[15] of **14** (27 mg) with 2,4-dinitrophenylhydrazine (20 mg) and concentrated hydrochloric acid (10 μl) in DMSO (1 ml) at room temperature for 2 hr gave the crystalline 2,4-dinitrophenylhydrazone, mp 174–177° from ethanol; λ_{max}^{MeOH} 355 mμ (ε 21,400), 254 mμ (ε 18,700); λ_{max}^{1NNaOH} 437 mμ (ε 18,400), 250 mμ (ε 16,300), if taken immediately. On standing the 437-mμ peak slowly disappears and is replaced by peaks at 336 and 285 mμ (uracil?).

Anal. Calcd for $C_{15}H_{14}N_6O_9$: C, 42.64; H, 3.34; N, 19.90. Found: C, 42.68; H, 3.33; N, 19.00.

Borohydride Reduction of 13 and 14. (a) 3′,5′-Di-O-trityl-2′-ketouridine (**13**, 339 mg) was treated at room temperature for 1 hr with sodium borohydride (300 mg) in ethanol (10 ml). The mixture was then partitioned between chloroform and water, and the organic phase was washed with water, dried, and evaporated leaving 340 mg of a white froth. Most of this (300 mg) was chromatographed on three 20 × 100 cm silica plates using seven consecutive developments with carbon tetrachloride–acetone (5:1) which cleanly separated two very close bands. The faster band was eluted with acetone giving 33 mg (11%) of 3′,5′-ditrityluridine that was identical with an authentic sample by thin layer chromatography and by its infrared spectrum. Elution of the slower band gave 172 mg (57%) of chromatographically homogeneous 1-(3,5-di-O-trityl-β-D-arabinofuranosyl)uracil (**15**)[16] which melted at 120–130° but could not be obtained crystalline; λ_{max}^{MeOH} 261 mμ (ε 10,700); $[\alpha]^{22}D$ +11.1° (*c* 0.1, chloroform).

Anal. Calcd for $C_{47}H_{40}N_2O_6$: C, 77.47; H, 5.49; N, 3.85. Found: C, 76.73; H, 5.82; N, 3.93.

The ORD spectrum in dioxane showed a positive Cotton effect with a maximum at 280 mμ (molecular rotation +9900°), zero rotation at 272 mμ, and a minimum at 252 mμ (molecular rotation −18,400°). The nmr spectrum is recorded in Table I.

Detritylation of a small portion of the crude reduction mixture was effected by treatment with 80% acetic acid at 100° for 4 hr and, after removal of triphenylmethanol by chloroform extraction, the products were separated by borate electrophoresis. Quantitative elution of the spots showed the products to be 18% uridine and 82% 1-(β-D-arabinofuranosyl)uracil (**12**).

(b) 2′-Ketouridine (6 mg) was treated for 30 min at room temperature with 7 mg of sodium borohydride in 80% ethanol (2 ml). The material was then passed through a small column of Dowex 50 (H^+) resin, evaporated to dryness, and evaporated four times with small portions of methanol. Borate electrophoresis and quantitative elution of the spots showed the products to be 10% uridine and 90% 1-(β-D-arabinofuranosyl)uracil.

Action of Alkali on 13. 3′,5′-Di-O-trityl-2′-ketouridine (100 mg) was dissolved in methanol (3.6 ml) and 1 *N* methanolic sodium hydroxide (0.4 ml) was added. Within a minute the solution turned yellow and then orange and a precipitate separated. After 20 min the mixture was cooled in ice, and the solid was collected by centrifugation. The precipitate was washed four times with cold methanol and dried *in vacuo* leaving a cream solid (23 mg) which contained about 4 mg of uracil (ultraviolet and chromatography) and no triphenylmethanol. The solid was washed with chloroform, and the soluble portion was evaporated leaving 12 mg of crystalline bis(triphenylmethyl) peroxide that was recrystallized from chloroform–methanol and had mp 186–188° (lit.[18] mp 186°). The nmr spectrum showed only aromatic protons and the mass spectrum (at 15 ev) showed intense peaks at *m/e* 259 (Ph_3CO^+) (relative intensity 100%) and *m/e* 243 (relative intensity 73%, Ph_3C^+).

Anal. Calcd for $C_{38}H_{30}O_2$: C, 88.00; H, 5.83. Found: C, 88.28; H, 5.62.

The methanol-soluble fraction from the alkaline reactions contained tritanol and uracil as shown by thin layer and paper chromatography.

Action of Diazomethane on 13. 2′,5′-Di-O-trityl-3′-ketouridine (400 mg) was treated overnight in ether (10 ml) with an excess of diazomethane. Thin layer chromatography showed the presence of one major product and several minor ones. The major product was isolated by preparative thin layer chromatography using two developments with chloroform–benzene (1:1) giving a chromatographically homogeneous product that contained some aliphatic impurities by nmr spectroscopy. This product had λ_{max}^{MeOH} 262 mμ which gradually changed to a broad maximum at 265–280 mμ in alkali during 10–15 min. Treatment of this product with sodium borohydride followed by detritylation with 80% acetic acid at 100° for 30 min gave a mixture of 1-(β-D-xylofuranosyl)-N_3-methyluracil (see below) and N_3-methyluridine (roughly 4:1) which were readily separated by paper chromatography in *n*-propyl alcohol–concentrated ammonium hydroxide–water (6:3:1) (R_f's 0.75 and 0.70, respectively), in *n*-butyl alcohol–acetic acid–water (5:2:3) (R_f's 0.64 and 0.59), or by borate electrophoresis (mobilities of 1.03 and 0.80 relative to uridine). In all cases the products behaved identically with authentic samples.

2′,5′-Di-O-trityl-N_3-methyluridine. 2′,5′-Di-O-trityluridine (209 mg) was stored overnight in a mixture of chloroform (2 ml) and ether (4 ml) containing an excess of diazomethane. The solvent was then evaporated leaving a crystalline residue that was recrystallized from ether giving 2′,5′-di-O-trityl-N_3-methyluridine (109 mg), mp 243–245°; λ_{max}^{MeOH} 262 mμ (ε 7940), unchanged in alkali; $[\alpha]^{22}D$ +90° (*c* 0.1, chloroform).

Anal. Calcd for $C_{48}H_{42}N_2O_6$: C, 77.60; H, 5.70; N, 3.77. Found: C, 77.84; H, 5.62; N, 3.91.

The ORD spectrum showed a positive Cotton effect with a maximum at 276 mμ (molecular rotation +28,500°), zero rotation at 260 mμ, and a minimum at 232 mμ (molecular rotation −40,500°). See Table I for the nmr spectrum.

Hydrolysis with 80% acetic acid at 100° for 30 min gave N_3-methyluridine (**19**) that was chromatographically identical with the major product from the action of diazomethane on uridine.

1-(2,5-Di-O-trityl-β-D-xylofuranosyl)-N_3-methyluracil. 1-(2,5-Di-O-trityl-β-D-xylofuranosyl)uracil (**11**, 57 mg) was treated overnight in a mixture of chloroform (1 ml), methanol (1 ml), and ether (2 ml) with an excess of diazomethane. Evaporation of the solvent left a solid residue (63 mg) which was homogeneous by thin layer chromatography using chloroform–ethyl acetate (20:1). Crystallization from ether gave 43 mg of 1-(2,5-di-O-trityl-β-D-xylofuranosyl)-N_3-methyluracil, mp 244–246°; λ_{max}^{MeOH} 260 mμ (ε 9900), unchanged in alkali, $[\alpha]^{22}D$ +51.9° (*c* 0.1, chloroform).

Anal. Calcd for $C_{45}H_{42}N_2O_6$: C, 77.60; H, 5.70; N, 3.77. Found: C, 77.39; H, 5.64; N, 3.73.

The ORD spectrum in methanol showed a positive Cotton effect with a maximum at 273 mμ (molecular rotation +14,900°), zero rotation at 256 mμ, and a minimum at 236 mμ (molecular rotation −17,200°). The mass spectrum (15 ev) showed an intense peak at *m/e* 126 corresponding to N-methyluracil.

Detritylation with 80% acetic acid at 100° for 30 min gave 1-(β-D-xylofuranosyl)-N_3-methyluracil which ran as a single spot on paper chromatograms and on borate electrophoresis but was not isolated in crystalline form.

An Electron Spin Resonance Study of the Radical Cations of Some *p*-Dialkoxybenzenes

W. F. Forbes, P. D. Sullivan, and H. M. Wang

Contribution from the Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada. *Received October 31, 1966*

Abstract: The electron spin resonance spectra of some *p*-dialkoxybenzene cation radicals in aluminum chloride–nitromethane have been investigated. The spectra can be interpreted by assuming *cis–trans* isomerism. The temperature dependence of the β-proton splitting constants, observed for the larger alkoxy compounds (ethoxy upwards), is explained by assuming hindered rotation of the alkoxy side chain.

As a continuation of earlier work,[1,2] the cation radicals of some *p*-dialkoxybenzenes have been studied. The aim was to investigate how larger alkyl groups would affect properties such as *g* values, spin densities, conformations, and the free energy difference between conformations. These systems also illustrate some of the advantages of the aluminum chloride–nitromethane system for producing cation radicals.

Experimental Section

Hydroquinone, *p*-dimethoxybenzene, *p*-diethoxybenzene, and *p*-di-*n*-butoxybenzene were commercially available samples, which were recrystallized several times. The other *p*-dialkoxybenzenes were prepared by the Williamson reaction[3,4] and purified by standard methods.

The sulfuric acid was best grade commercially available 98% sulfuric acid. Dideuteriosulfuric acid was a commercially available sample (Fluka). Sulfuric acid spectra were determined in *ca.* 0.2 *M* solution, *ca.* 0.04 ml of which was placed in a glass capillary tube which was then inserted in the spectrometer cavity.

Nitromethane and aluminum chloride (anhydrous) were Fisher reagent grade. Nitromethane was dried (CaH_2) and deoxygenated by passing dried (H_2SO_4, Al_2O_3) nitrogen gas through the solution, followed by degassing under vacuum. Radical formation was carried out (*cf.* ref 5) in an inverted U tube (diameter *ca.* 8 mm), one arm of which was filled with aluminum chloride and the appropriate benzene (*ca.* 20 and 5 mg, respectively). The U tube was connected to a vacuum line (*ca.* 0.5 mm) and nitromethane (*ca* 1 ml) was distilled into the mixture. The reaction proceeded at room temperature, and the solution was subsequently transferred under vacuum to a capillary tube (diameter *ca.* 1 mm) which was sealed directly to one arm of the U tube. The capillary tube was then placed in the esr cavity. If necessary, concentrations were varied by distilling solvent from one arm to the other.

Esr spectra were determined on a JES-3BX spectrometer at 100-kc/sec modulation using a field-selector unit. *g* Values were obtained in a dual cavity with reference to Fremy's salt which was used as a secondary standard being first calibrated against the spectrum of anthracene (positive ion) for which an accurate *g* value (2.002565 ± 0.000006) is available.[6] The magnetic field was calibrated with both Fremy's salt (a_N = 13.07 gauss)[7] and an nmr probe, and values of the splitting constants are believed accurate to ±0.5% (for splitting constants >1 gauss). The magnetic field was swept in both directions, and the first derivative spectra of the energy absorption were recorded in the relevant figures. Radical concentrations were estimated by overmodulating the signal and comparing it, under similar conditions, with a standard diphenylpicrylhydrazyl (DPPH) sample.

Spectra were simulated on an IBM 7040 computer, with a modified program kindly supplied by Dr. Lawrence C. Snyder of the Bell Telephone Laboratories.[8] The output data from the IBM 7040, in the form of punched cards, were plotted *via* an IBM 1710 computer. Approximate splitting constants were obtained directly from the observed spectra, usually by careful inspection of the wings. Sometimes, however, such direct measurement is not possible, and it is necessary to estimate splitting constants by measuring the total width of the spectrum. It is these values which are then slightly altered, within the experimental error, until a good simulated spectrum is obtained. Molecular orbital calculations were carried out by the self-consistent-field method of McLachlan[9] on an IBM 7040 computer, with a program kindly supplied by Dr. J. M. Fritsch[10] of the University of Kansas.

Results and Discussion

***p*-Dihydroxybenzene.** The spectrum of the cation radical of hydroquinone in aluminum chloride–nitro-

(1) W. F. Forbes and P. D. Sullivan, *Can. J. Chem.*, **44**, 1501 (1966).
(2) A. B. Barabas, W. F. Forbes, and P. D. Sullivan, *ibid.*, 45, 267 (1967).
(3) G. S. Hartley, *J. Chem. Soc.*, *1828 (1939)*.
(4) J. B. Gallent, *J. Org. Chem.*, 23, 75 (1958).
(5) W. F. Forbes and P. D. Sullivan, *J. Am. Chem. Soc.*, 88, 2862 (1966).
(6) B. G. Segal, M. Kaplan, and G. K. Fraenkel, *J. Chem. Phys.*, **43**, 4191 (1965).
(7) G. Vincow and P. M. Johnson, *ibid.*, **39**, 1143 (1963).
(8) S. H. Glarum and L. C. Snyder, *J. Chem. Phys.*, **36**, 2989 (1962).
(9) A. D. McLachlan, *Mol. Phys.*, 3, 233 (1960).
(10) J. M. Fritsch, T. P. Layloff, and R. N. Adams, *J. Am. Chem. Soc.*, 87, 1724 (1965).