The combined yield of dimethylacetamide was 3.60 g. (83%), and the yield of diethylacetamide was 0.65 g. (11%).

*B. In Nitromethane Solution.* The decrease in amine content was followed in a solution of 1.1365 g. of diethylaminomethanethiol acetate and 0.300 g. of diethylamine in 100 ml. of nitromethane solution. The concentrations were determined as described above under Catalysis by Pyridine. The reaction followed second-order kinetics (first with respect to ester and to amine) in the early part of the reaction, and the rate constant was 0.0707 l./mole min.

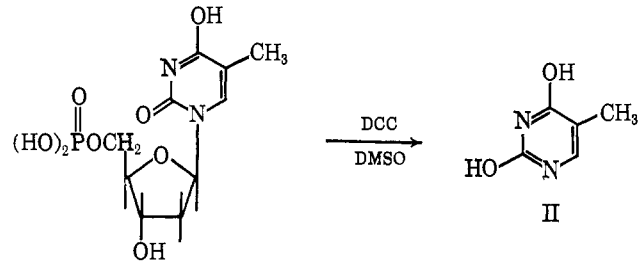
# Sulfoxide–Carbodiimide Reactions. I. A Facile Oxidation of Alcohols

**K. E. Pfitzner[1] and J. G. Moffatt**

*Contribution No. 27 from the Syntex Institute of Molecular Biology, Stanford Industrial Park, Palo Alto, California.* *Received August 18, 1965*

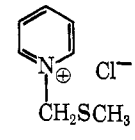
*The reaction of thymidine 5′-phosphate with dicyclohexylcarbodiimide (DCC) in dimethyl sulfoxide (DMSO) leads to rapid cleavage of the N-glycosidic bond and release of thymine. This glycosidic cleavage is found to be typical of nucleotide derivatives containing a free 3′-hydroxyl group and also occurs with free 3′-hydroxyl-containing nucleosides in the presence of anhydrous phosphoric acid. Such a reaction applied to 3′-O-acetylthymidine leads to the formation of 3′-O-acetylthymidine-5′-aldehyde which was isolated as its crystalline dinitrophenylhydrazone. The cleavage of the N-glycosidic bond in nucleosides is explained by oxidation of the 3′-hydroxyl group to a ketone followed by spontaneous β-elimination of the heterocyclic base. The general reaction of alcohols with DMSO and DCC in the presence of certain acids has been found to lead to efficient oxidation to the corresponding aldehydes or ketones under extremely mild conditions. Optimal conditions for the oxidation of testosterone to Δ⁴-androstene-3,17-dione have been determined, and it is concluded that this reaction is most advantageously carried out using 0.5 molar equiv. of pyridinium trifluoroacetate and 3 molar equiv. of DCC in DMSO or mixed solvents containing DMSO at room temperature. A mechanism for the reaction is proposed.*

Recently, we have had occasion to investigate the feasibility of phosphorylating an alcohol by the cyanoethyl phosphate–dicyclohexylcarbodiimide method,[2] using dimethyl sulfoxide (DMSO) as the solvent rather than pyridine. As a test we attempted the phosphorylation of 2′,3′-O-isopropylideneuridine and were surprised to find that the presence of even 10% anhydrous DMSO in pyridine completely prevented the formation of any phosphorylated nucleoside as demonstrated by paper electrophoresis. As a further test we attempted the carbodiimide-promoted polymerization of thymidine 5′-phosphate (I),[3] once again using anhydrous DMSO rather than pyridine as the reaction medium. The reaction in DMSO, unlike that in pyridine, rapidly became colored and emitted a foul, sulfide-like smell. Chromatographic examination after various times showed that within 15 min. the nucleotide had completely degraded to thymine (II) with cleavage of the N-glycosidic bond. The crystalline thymine was isolated almost quantitatively from the reaction by ion-exchange chromatography and identified by ultraviolet and infrared spectroscopy as well as by paper and thin layer chromatography.




I II

A second product, in much lower yield, was also formed if excess pyridine was present in the reaction. This was found by ultraviolet spectroscopy to be an N-substituted pyridinium compound, this structure being supported by paper electrophoresis which showed it to have a single positive charge both at pH 3 and 8. A small amount of this material was isolated chromatographically from a pyridine-containing reaction and shown to have the structure III by nuclear magnetic resonance spectroscopy and comparison with an authentic sample prepared from pyridine and chloromethyl methyl sulfide. The possible implications of presence of this by-product will be discussed later.




III

Cleavage of thymidine 5′-phosphate to thymine was also observed in similar reactions using the free acid and tributylammonium salt forms of the nucleotide. Using the free acid, quantitative cleavage to thymine occurred very rapidly but with the trialkylamine salts the reaction was slow and after 18 hr. consider-

(1) Syntex Postdoctoral Fellow, 1961–1963.
(2) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).
(3) G. M. Tener, H. G. Khorana, R. Markham, and E. H. Pol, *ibid.*, **80**, 6223 (1958).

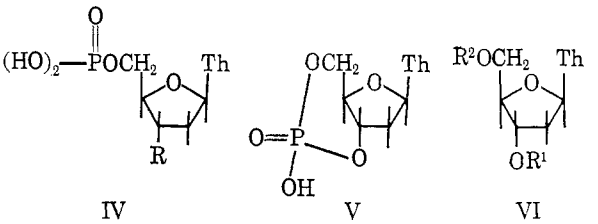
able starting material and some dithymidine pyrophosphate were still present. The pyridinium and tributylammonium salts of uridine 5′-phosphate behaved similarly, being cleaved to uracil at rates comparable to the analogous thymidine compounds. Free acid and pyridinium adenosine 5′-phosphates were also quantitatively converted to adenine within 2 hr. at room temperature, but the tributylammonium salt gave only traces of adenine after several days. The principal products in this case were unreacted starting material and diadenosine pyrophosphate. Tributylammonium-2′-deoxyadenosine 5′-phosphate behaved similarly.

Paper chromatographic examination of aliquots removed during degradation of thymidine 5′-phosphate showed that within the first 15 min. of reaction there was extensive release of orthophosphate which was subsequently converted into principally trimetaphosphate by the excess carbodiimide. A control reaction between tributylammonium orthophosphate and dicyclohexylcarbodiimide (DCC) in anhydrous DMSO also led predominantly to trimetaphosphate. A similar result has been obtained using pyridine as thes olvent[4].

It is thus clear that the reaction between a 5′-nucleotide and DCC in anhydrous DMSO leads to extensive degradation of the molecule with cleavage of both the heterocyclic base and the phosphate group. We have unsuccessfully attempted to identify the products from the sugar moiety. No distinct products could be detected chromatographically using spray reagents for either reducing compounds (*p*-anisidine or aniline phthalate) or glycols (periodate–benzidine). Attempted stabilization of any carbonyl-containing intermediate by reduction with sodium borohydride also failed to give any distinct product. The addition of 2,4-dinitrophenylhydrazine hydrochloride to the crude petroleum ether extracted reaction mixtures resulted in the separation of a mixture of partially crystalline dinitrophenylhydrazones in low yield. If the reaction mixture from treatment of uridine 5′-phosphate was steam distilled, the crystalline dinitrophenylhydrazone of formaldehyde could be isolated from the distillate. It is not certain whether this formaldehyde originated from the sugar or the DMSO. The nonsteam-distillable fraction also gave a small, insoluble precipitate with dinitrophenylhydrazine showing $\lambda_{max}$ 575 mμ in alkali which strongly suggests the presence of an α-dicarbonyl system.[5] Similar treatment of a reaction from thymidine 5′-phosphate gave a very small amount of the formaldehyde derivative and an amorphous dinitrophenylhydrazone with $\lambda_{max}$ 440 mμ in alkali typical of unconjugated carbonyl compounds.

In a final effort to locate the degradation products of the sugar fragment, uniformly $C^{14}$-labeled uridine 5′-phosphate was reacted with DCC in DMSO and the products were examined by paper chromatography. In addition to a radioactive spot of uracil there was a general distribution of isotope over much of the chromatogram which suggested a complex degradation of the sugar into many products. This aspect of the problem was not further studied.

A number of compounds related to thymidine 5′-phosphate were also examined in order to ascertain the structural features necessary for cleavage of the N-glycosidic or phosphate ester bonds. In summary, it was found that there was no release of thymine upon treatment of the pyridine salts of 3′-O-acetylthymidine 5′-phosphate (IV, R = OAc),[6] 3′-O-tetrahydropyranylthymidine 5′-phosphate (IV, R = tetrahydropyranyl-2-oxy), 3′-deoxythymidine 5′-phosphate (IV, R = H),[7] or thymidine-3′,5′-cyclic phosphate (V),[3] with 3–4 equiv. of DCC in anhydrous DMSO at room temperature for up to 24 hr. The only ultraviolet absorbing products from these reactions were either unchanged starting material or the corresponding $P^1$–$P^2$-dinucleoside pyrophosphates which could be independently prepared by reaction of the appropriate tributylammonium nucleotide with DCC in anhydrous pyridine.[8] Surprisingly, di(thymidine-5′) pyrophosphate, which would normally be expected to be the initial product from thymidine 5′-phosphate and DCC, was also almost completely inert. The reaction with thymidine 3′-phosphate[2] was more complex and gave a number of products, including thymine, which have not been further investigated.




IV V VI

Thymidine itself remained completely unchanged after prolonged treatment with DMSO and DCC, but upon addition of 1 molar equiv. of anhydrous orthophosphoric acid an immediate reaction took place and thymine was released. A similar, but considerably slower, release of thymine occurred when an equivalent amount of trifluoroacetic acid was used rather than phosphoric acid, but no glycosidic cleavage was observed using acetic or formic acids. With the knowledge that the reaction was definitely acid catalyzed it was possible to examine a number of simple substituted nucleosides. In such studies it was shown that no release of thymine accompanied the reaction of 3′-O-acetylthymidine (Vl, $R^1$ = acetyl, $R^2$ = H), 3′-O-*p*-nitrobenzoylthymidine (VI, $R^1$ = *p*-nitrobenzoyl; $R^2$ = H) or 3′,5′-di-O-*p*-nitrobenzoylthymidine (VI, $R^1$ = $R^2$ = *p*-nitrobenzoyl) in DMSO containing 3 molar equiv. of DCC and 0.5–1 molar equiv. of anhydrous orthophosphoric acid. On the other hand, compounds containing a free 3′-hydroxyl group such as 5′-O-acetylthymidine (VI, $R^1$ = H; $R^2$ = Ac)[6] and 5′-O-*p*-nitrobenzoylthymidine (VI, $R^1$ = H; $R^2$ = *p*-nitrobenzoyl) underwent rapid and complete cleavage of both the N-glycosidic and ester bonds.

While the reaction of 3′-O-acetylthymidine did not lead to glycosidic cleavage the product of the reaction moved slightly faster than the starting material when examined by thin layer chromatography on Silica G or paper chromatography. Most significantly this product, unlike the starting material, gave a positive

(4) G. Weimann and H. G. Khorana, *J. Am. Chem. Soc.*, 84, 4329 (1962).

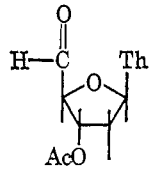
(5) (a) L. A. Jones and C. K. Hancock, *ibid.*, 82, 105 (1960); (b) L. A. Jones, J. C. Holmes, and R. B. Seligman, *Anal. Chem.*, 28, 191 (1956).

(6) P. T. Gilham and H. G. Khorana, *J. Am. Chem. Soc.*, 80, 6212 (1958).

(7) K. E. Pfitzner and J. G. Moffatt, *J. Org. Chem.*, 29, 1508 (1964).

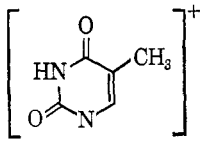
(8) M. Smith, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, 80, 6204 (1958).

test for carbonyl groups on spraying the chromatogram with acidic dinitrophenylhydrazine. The crude reaction mixture also gave an intense diphenylamine test[9] with $\lambda_{max}$ 530 mμ.[10] Following evaporation of the solvent it was possible to isolate a crystalline dinitrophenylhydrazone directly from the reaction mixture in 61% yield. This material showed ultraviolet maxima at 261 mμ (ε 19,300) and at 350 mμ (ε 21,650) which is quite consistent with the superimposition of the chromophores of thymidine ($\lambda_{max}$ 267 mμ (ε 9600)) and an aldehyde dinitrophenylhydrazone ($\lambda_{max}$ 260 mμ (ε ~10,000); $\lambda_{max}$ 350 mμ (ε ~20,000)).[5b] Elemental analysis confirmed that this compound was the 2,4-dinitrophenylhydrazone of 3′-O-acetylthymidine-5′-aldehyde (VII).

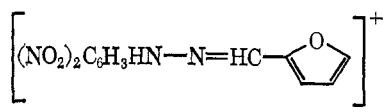



VII

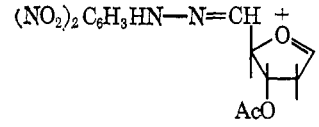
The mass spectrum of the dinitrophenylhydrazone of VII[11] showed no molecular ion (*m/e* 462), and its most significant peaks were at *m/e* 125, 276, and 337 corresponding to the species VIII–X, respectively.




VIII, *m/e* 125



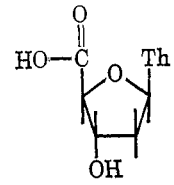

IX, *m/e* 276



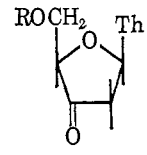

X, *m/e* 337

The free compound VII proved to be very difficult to isolate in pure form since some residual 3′-O-acetylthymidine remained and the two could not be separated chromatographically on a preparative scale. Also, its solubility in water makes it impossible to remove the DMSO by extraction, and complete removal of the solvent by evaporation necessitates prolonged warming under high vacuum which leads to some destruction of VII. Repeated efforts to obtain VII in crystalline form from approximately 90% pure preparations have been unsuccessful. It has, however, been possible to confirm the structure of VII in several ways. First, VII was quantitatively reduced by sodium borohydride to 3′-O-acetylthymidine and thymidine due to concomitant hydrolysis of the acetate. Treatment of crude VII with alkaline sodium hypoiodite resulted in rapid oxidation to thymidine 5′-carboxylate (XI, R = H) which was chromatographically and electrophoretically identical with an authentic specimen prepared by platinum-catalyzed oxidation of thymidine.[12] Similar treatment of thymidine or 3′-O-acetylthymidine resulted in no oxidation to the 5′-carboxylate. Of unique importance is the fact that no thymidine carboxylate derivatives whatsoever were formed in the DMSO–DCC oxidation of 3′-O-acetylthymidine, the reaction being entirely specific for oxidation to the aldehyde level. All other oxidative techniques that have been applied to the carbohydrate moiety of nucleosides have led exclusively to the carboxylic acids.[12,13]

It would appear that the initially observed release of thymine from thymidine 5′-phosphate, or from thymidine derivatives possessing a free 3′-hydroxyl group, is a consequence of oxidation of this group to a ketone (XII), followed by spontaneous β-elimination of both the heterocyclic base and the 5′-phosphate if one is present. Previous attempts to oxidize a free 3′-hydroxyl group in deoxynucleoside derivatives with manganese dioxide[13] or with platinum and oxygen under forcing conditions[14] have led to glycosidic cleavage without accumulation of detectable carbonyl-containing intermediates.



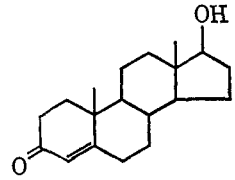

XI



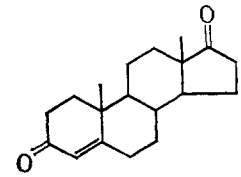

XII

The very facile and selective oxidation of both primary and secondary hydroxyl groups described above has led us to explore the utility of this reaction as a synthetic tool. In the accompanying paper[15] a number of examples are described which adequately outline the scope of the reaction. In addition, the synthesis of several nucleoside 5′-aldehydes using this method will be described separately.[16] Preliminary accounts of some of this work have already appeared.[17]

In this paper we wish to describe experiments designed to determine the optimal conditions for the oxidation reaction. These studies were carried out using the oxidation of testosterone (XIII) to Δ⁴-androstene-3,17-dione (XIV) as the test system. Testosterone was chosen for this purpose because of its facile separation from its oxidation product XIV by thin layer chromatography and its strong ultraviolet absorption ($\lambda_{max}^{MeOH}$ 241 mμ (ε 16,400)) which permits quantitative estimation of the products. Details of this oxidation and characterization of the product are given in the accompanying paper.[15]




XIII




XIV

(9) F. B. Seibert, *J. Biol. Chem.*, **133**, 593 (1940).
(10) *Cf.* thymidine which gives a weak color with $\lambda_{max}$ 610 mμ.
(11) Obtained by Dr. John Wilson of Stanford University using a CEC Model 21-103C spectrometer with a heated (200°) glass inlet system. We are very grateful to Dr. Wilson for his help.
(12) (a) G. P. Moss, C. B. Reese, K. Schofield, R. Shapiro, and A. R. Todd, *J. Chem. Soc.*, 1149 (1963); (b) J. P. Vizsolyi and G. M. Tener, *Chem. Ind.* (London), 263 (1962).
(13) A. S. Jones, R. T. Walker, and A. R. Williamson, *J. Chem. Soc.*, 6033 (1963).
(14) Personal communication from Dr. G. M. Tener of the University of British Columbia, Jan. 1963.
(15) K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, 87, 5670 (1965).
(16) J. G. Moffatt, to be published.
(17) K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, **85**, 3027 (1963).

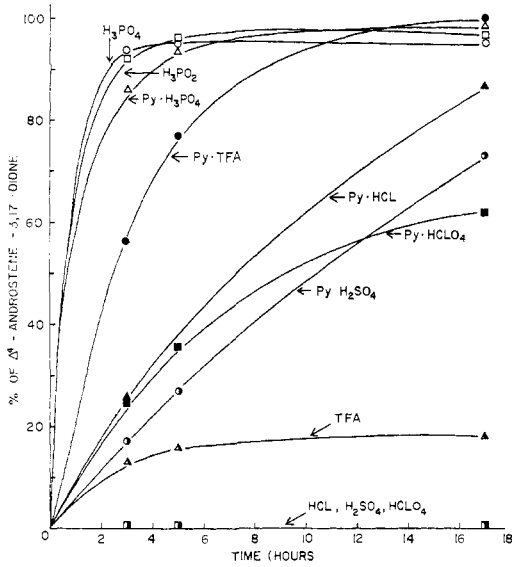



Figure 1. The effect of different acids on the oxidation of testosterone. Each reaction contained testosterone (0.1 mmole), DCC (0.3 mmole), and the appropriate acid (0.05 mmole) in DMSO (0.5 ml.). See the Experimental Section for details.

First it was shown that the oxidation of XIII to XIV proceeded efficiently in the presence of DMSO, anhydrous phosphoric acid, and DCC. The omission of one of these reagents, however, completely blocked the reaction. The same absolute requirement for all these reagents was also apparent during oxidation of a primary alcohol, *p*-nitrobenzyl alcohol, to *p*-nitrobenzaldehyde.[15] Gas chromatographic examination of the crude reaction mixtures showed the release of roughly 1 molar equiv. of dimethyl sulfide per equivalent of alcohol oxidized. The DMSO is, therefore, the oxidizing agent. We then attempted to ascertain what acids are most advantageously used in conjunction with DMSO and DCC. A number of identical reactions were set up containing chromatographically homogeneous testosterone and 3 molar equiv. of DCC in anhydrous DMSO. A number of different acids (0.5 molar equiv.) were then added and aliquots were removed after 3, 5, and 17 hr. These were examined by thin layer chromatography on Silica G, and the products were quantitatively determined by ultraviolet measurement of the eluted spots. We have separately shown that this method is both reproducible and accurate. The only ultraviolet absorbing products were XIII, XIV, and a trace amount (up to 2%) of a spot moving faster than XIV. The nature of this minor impurity will be discussed separately.[15] The results using different acids are shown in Figure 1, from which it can be seen that anhydrous phosphoric and phosphorous acids lead to greater than 90% yields of the dione XIV within 3 hr. It was separately shown that these reactions were actually complete within 1 hr. Stronger acids such as trifluoroacetic acid were less effective and gave no more than 20% oxidation while mineral acids (HCl, $H_2SO_4$, and $HClO_4$) gave no product whatsoever. As their pyridine salts, however, all the above acids were effective to varying degrees. On the other hand, the triethylamine salts of hydrochloric and sulfuric acids led to no oxidation at all and triethylammonium phosphate resulted in only minimal formation of XIV. Since the reaction proceeds well with free acids of moderate strength and with weak base (pyridine) salts of strong acids but fails completely with strong acids and with trialkylamine salts of various acids, it appears that both acid- and base-catalyzed steps are involved. Inhibition of various acid-catalyzed carbodiimide reactions in the presence of trialkylamines is well known.[8]

From a practical point of view the most efficient oxidations were obtained using either anhydrous, free acid phosphoric acid or the pyridine salts of either phosphoric or trifluoroacetic acids. All these reactions produced at least 95% of the desired dione XIV and in the case of pyridinium trifluoroacetate no trace of any other ultraviolet-absorbing product could be detected at all. Both phosphoric acid and pyridinium phosphate gave 1% of the fast-moving impurity previously mentioned and in the case of phosphoric acid 4% testosterone remained unreacted. We have also examined a number of other acids and, in general, the conclusion can be drawn that free acids of intermediate strength are required. Thus, for example, under comparable conditions acetic acid ($pK = 4.76$)[18] and trichloroacetic acid ($pK = 0.66$)[18] fail to promote the oxidation of testosterone and monochloroacetic acid ($pK = 2.86$)[18] leads to slow and incomplete reaction (about 15% in 15 min. and largely unchanged after 1 hr.). Dichloroacetic acid ($pK = 1.25$),[18] however, leads to quantitative oxidation of XIII within 10 min. Less than 1% of the fast-moving spot contaminates the product in this reaction. A comparison of the efficacies of orthophosphate, monophenyl phosphate, and diphenyl phosphate has also been made and is summarized in Table I.

**Table I.** Use of Phosphoric Acids in the Oxidation of Testosterone

| Acid source[a] | Δ⁴-Androstene-3,17-dione formed, % 15 min. | 30 min. | 1 hr. | 2 hr. |
|---|---|---|---|---|
| Orthophosphate | 79 | 86 | 92 | 92 |
| Monophenyl phosphate | 90 | 90 | 89 | 91 |
| Diphenyl phosphate[b] | 1 | 3 | 3 | 3 |

[a] All reactions were carried out under identical conditions, using 0.5 molar equiv. of the anhydrous acid and 3 molar equiv. of DCC relative to testosterone. [b] After 5 days of reaction, 37% of the dione was present.

From Table I it can be seen that monophenyl phosphate leads to a slightly faster reaction than orthophosphate itself, while diphenyl phosphate is very unreactive. While diesters of phosphoric acid are somewhat stronger acids than monoesters (the $pK$ values of mono- and diphenyl phosphates are 2.61 and 2.28, respectively, in 50% ethanol),[19] and both of these are stronger than orthophosphoric acid itself,[18] these differences would appear to be too small to explain the widely divergent behavior described above. Also, since diphenyl phosphate and dichloroacetic acid must have quite similar $pK$ values in water or in DMSO, it is difficult to rationalize these results in terms of acid strength alone. Another example of the poor reactivity of phosphate diesters is shown in the slow release of thymine from the

(18) A. Albert and E. P. Serjeant in "Ionization Constants of Acids and Bases," John Wiley and Sons, Inc., New York, N. Y., 1963, p. 124.
(19) T. A. Mastryukova, T. A. Melent'eva, A. E. Shipov, and M. A. Kabachnik, *Zh. Obshch. Khim.*, **29**, 2178 (1959).

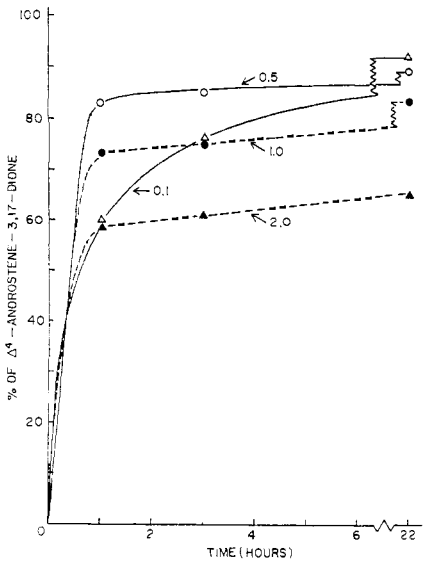



Figure 2. The effect of different amounts of acid. Each reaction contained testosterone (0.1 mmole) and DCC (0.5 mmole) in DMSO (0.125 ml.) and benzene (0.375 mmole), together with 0.1, 0.5, 1.0, or 2.0 molar equiv. of anhydrous orthophosphoric acid.

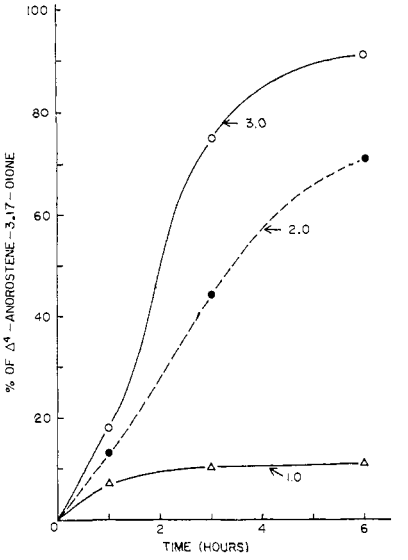



Figure 3. The effect of different amounts of DCC. Each reaction contained testosterone (0.1 mmole) and anhydrous orthophosphoric acid (0.025 mmole) in DMSO (0.5 ml.) together with 0.1, 0.2, or 0.3 mmole of DCC.

monomethyl ester of thymidine 5′-phosphate. While, as above, thymidine 5′-phosphate is completely degraded to thymine within 15 min., only 25% cleavage of the glycosidic bond is obtained in 1 hr. with the methyl ester.

We next turned our attention to the question of how much acid it is necessary to use in the oxidation reaction. The results of identical experiments using testosterone in a mixture of DMSO and benzene containing 3 molar equiv. of DCC and from 0.1 to 2 molar equiv. of anhydrous phosphoric acid are shown in Figure 2. From this it can be seen that effective oxidation was achieved using 0.1, 0.5, and 1.0 equiv. of phosphoric acid relative to the testosterone. A distinctly lower yield resulted from the use of 2.0 equiv. of the acid however. This can perhaps be explained by depletion of the carbodiimide through side reaction with the larger excess of acid.

The amount of DCC was investigated through a series of reactions using testosterone and 0.5 molar equiv. of phosphoric acid in DMSO containing 1, 3, 5, and 8 equiv. of DCC. This experiment showed virtually no difference between the use of 3, 5, and 8 molar equiv. of carbodiimide, the three reactions giving 87, 88, and 82% yields within 1 hr. With 1 equiv. of DCC, however, the yield was only 6%. A more clear-cut distinction was possible using only 0.25 equiv. of phosphoric acid together with 1, 2, and 3 equiv. of DCC, the results being given in Figure 3. Here, clearly, 3 molar equiv. of DCC is superior to 2 and once again 1 equiv. is insufficient. At least in part, the poor results with 1 equiv. of DCC may be a consequence of competitive acid-catalyzed hydration of the carbodiimide brought about by the phosphoric acid.[8] Figure 3 also shows a rather typical S-shaped curve which is observed during the early stages of oxidations using phosphoric acid but not with pyridinium trifluoroacetate.

The amount of DMSO required for efficient oxidation was examined using various mixtures of benzene and the sulfoxide as the solvent for oxidations using 0.5 equiv. of phosphoric acid and 3 equiv. of DCC. These experiments showed that only minor differences could be observed between mixtures containing 100, 50, 25, or 10% DMSO, these reactions giving 91, 95, 88, and 85% oxidation, respectively, within 1 hr. The mixture containing 10% DMSO corresponds to 6 molar equiv. of the sulfoxide relative to testosterone.

Finally we have shown that sulfoxides other than DMSO and carbodiimides (and related substances) other than DCC may be used in the oxidation reaction. Thus, tetramethylene sulfoxide supports the oxidation of both testosterone and *p*-nitrobenzyl alcohol at rates roughly comparable to those with DMSO. Also, diisopropyl carbodiimide behaves similarly to DCC, although a little slower due probably to increased steric hindrance.[20] Other reagents such as trichloroacetonitrile,[21] N-ethyl 5-phenylisoxazolium 3′-sulfonate,[22] and ethoxyacetylene,[23] which resemble carbodiimides in some of their reactions, do not lead to appreciable oxidation at room temperature. At 100° trichloroacetonitrile does lead to oxidation of testosterone in DMSO containing phosphoric acid, but the reaction mixture becomes dark and gives only 40–50% of the product in 2 hr. (by thin layer chromatography).

The results obtained above show that optimal oxidation conditions consist of the reaction of an alcohol in DMSO (or in a mixture of an inert solvent and DMSO) in the presence of 3 equiv. of DCC and 0.5 equiv. of anhydrous phosphoric acid or pyridinium trifluoroacetate. Phosphoric acid provides a very rapid oxidation but pyridinium trifluoroacetate, while being somewhat slower and requiring an overnight reaction, offers the advantages of minimal by-products and an essentially neutral reaction medium. The over-all results of

(20) *Cf.* the low reactivity of di-*t*-butyl carbodiimide as compared with DCC in reactions with acids.[8]

(21) F. Cramer and G. Weimann, *Chem. Ber.*, 94, 996 (1961).

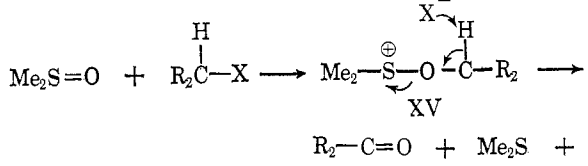
(22) R. B. Woodward and R. A. Olofson, *J. Am. Chem. Soc.*, 83, 1007 (1961).

(23) J. F. Arens, *Advan. Org. Chem.*, 2, 117 (1960).

many oxidations[15] lead us to generally prefer pyridinium trifluoroacetate as the proton source.

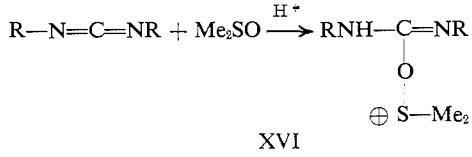
Many examples of dimethyl sulfoxide acting as an oxidizing agent have appeared in recent years. Thus, the oxidation of $\alpha$-halo ketones,[24] $\alpha$-halo esters,[25] alkyl halides,[26] alkyl tosylates,[26a,27] benzylic alcohols,[28] and epoxides[29] have been reported upon heating these various substances with DMSO, frequently in the presence of an acid acceptor such as sodium bicarbonate. With primary iodides and tosylates oxidation is often quite efficient, but with less reactive compounds the formation of olefins and other by-products frequently predominates.[24b,27c]

The mechanism generally accepted for these reactions involves nucleophilic displacement of the halide or tosylate by DMSO giving an intermediate sulfoxonium compound (XV) which then collapses to the carbonyl compound by concerted elimination of a proton and dimethyl sulfide.[25,27a] Recently Barton, *et al.*,[30] have succeeded in preparing the intermediate XV under much milder conditions through the reaction of alkyl chloroformates with DMSO. Neutralization of the released hydrogen chloride with triethylamine then gave the carbonyl compounds in quite good yields.



$$R_2C{=}O + Me_2S + HX$$

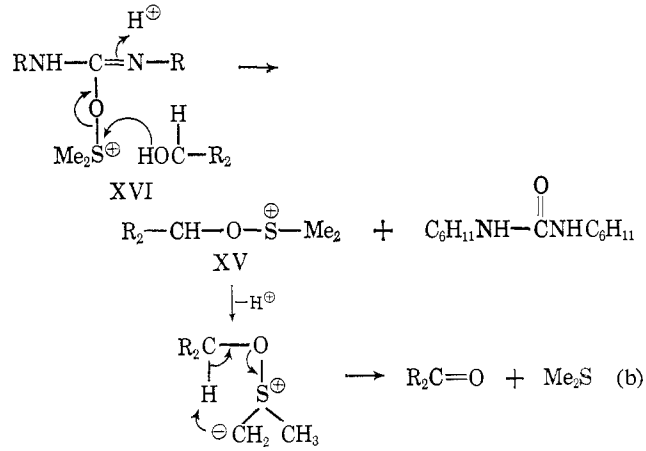
We consider that the mechanism of the present reaction involves nucleophilic attack of DMSO upon the protonated carbodiimide to give an ionic intermediate XVI which is then attacked by the alcohol to once again form XV and dicyclohexylurea. Collapse of XV would then give the aldehyde or ketone and dimethyl sulfide.



(a)

XVI

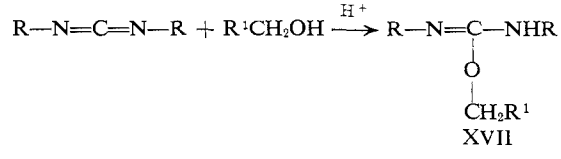
There is ample evidence for the addition of nucleophiles to protonated carbodiimides[31] and the acid-catalyzed addition of DMSO to ketenes and ketenimines has recently been demonstrated by Lillien.[32] The attack of the alcohol on XVI with expulsion of highly insoluble dicyclohexylurea and formation of XV



(b)

is similar to the SN2 displacements which have been demonstrated with other alkoxysulfonium salts.[33] Finally, loss of a proton from XV would be expected to occur very readily at one of the carbons adjacent to the positively charged sulfur giving a d-orbital-stabilized carbanion[34] which can collapse *via* a cyclic mechanism to the observed carbonyl compound and dimethyl sulfide. This proton loss is very similar to that which has been proposed in the Pummerer reaction of sulfoxides with acid anhydrides[35] and can be brought about by bases as weak as acetate anion. In the oxidation reaction this proton could be removed by either pyridine (which is usually used in 100% excess over the trifluoroacetic or other strong acid) or by the anion of the secondary dissociation of phosphoric acid. The failure of the oxidation in the presence of free strong acids, or of phosphate diesters which lack the secondary dissociation, can probably be attributed to inhibition of this proton loss.

We have also considered another mechanism involving initial, acid-catalyzed addition of the alcohol to DCC giving an isourea ether (XVII) which could then undergo nucleophilic attack by DMSO giving the alkoxysulfonium salt (XV). To check this possibility we have independently prepared O-*p*-nitrobenzyl-N,N′-dicyclohexylisourea (XVII, R = $C_6H_{11}$; $R^1$ = *p*-nitrophenyl) through the cupric ion catalyzed addition of *p*-nitrobenzyl alcohol to DCC.[36] As the free base, this compound was reacted in anhydrous DMSO in the presence of phosphoric acid, DCC, pyridine, and various combinations of these reagents. While the formation of some *p*-nitrobenzyl phosphate was observed, no *p*-nitrobenzaldehyde resulted and, hence, isourea ethers such as XVII cannot be involved in the oxidation reaction. Attempts to prove our proposed mechanism through studies with isotopically labeled compounds will be described shortly.



XVII

(24) (a) N. Kornblum, J. W. Powers, G. J. Anderson, W. J. Jones, H. O. Larson, O. Levand, and W. M. Weaver, *J. Am. Chem. Soc.*, 79, 6562 (1957); (b) R. N. Iacona, A. T. Rowland, and H. R. Nace, *J. Org. Chem.*, 29, 3495 (1964).

(25) I. M. Hunsberger and J. M. Tien, *Chem. Ind.* (London), 88 (1959).

(26) (a) W. Kornblum, W. J. Jones, and G. J. Anderson, *J. Am. Chem. Soc.*, 81, 4113 (1959); (b) A. P. Johnson and A. Pelter, *J. Chem. Soc.*, 520 (1964).

(27) (a) H. R. Nace and J. J. Monagle, *J. Org. Chem.*, 24, 1792 (1959); (b) M. M. Baizer, *ibid.*, 25, 670 (1960); (c) D. N. Jones and M. A. Saeed, *J. Chem. Soc.*, 4657 (1963).

(28) V. J. Traynelis and W. N. Hergenrother, *J. Am. Chem. Soc.*, 86, 298 (1964).

(29) T. Cohen and T. Tsuji, *J. Org. Chem.*, 26, 1681 (1961).

(30) D. H. R. Barton, B. J. Garner, and R. H. Wightman, *J. Chem. Soc.*, 1855 (1964).

(31) H. G. Khorana in "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," John Wiley and Sons, Inc., New York, N. Y., 1961, Chapter 6.

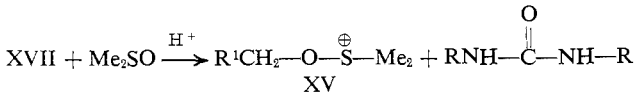
(32) I. Lillien, *J. Org. Chem.*, 29, 1631 (1964).

(33) C. R. Johnson, *J. Am. Chem. Soc.*, 85, 1020 (1963).

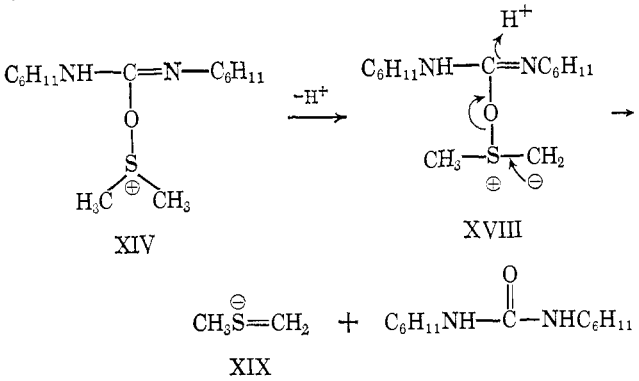
(34) G. Cilento, *Chem. Rev.*, 60, 147 (1960).

(35) (a) W. E. Parham and M. D. Bhavsar, *J. Org. Chem.*, 28, 2686 (1963); (b) W. E. Parham and S. H. Groen, *ibid.*, 30, 728 (1965); (c) S. Oae, T. Kitao, S. Kawamura, and Y. Kitaoka, *Tetrahedron*, 19, 817 (1963).

(36) (a) E. Schmidt and F. Moosmuller, *Ann.*, 597, 235 (1955); (b) E. Schmidt and W. Carl, *ibid.*, 639, 24 (1961).



The formation of small amounts of N-thiomethoxymethylpyridinium compounds (III) during oxidation of thymidine 5′-phosphate in the presence of excess pyridine deserves some comment. Paper electrophoresis of evaporated reaction mixtures from the oxidation of testosterone using phosphoric acid in the presence of excess pyridine (4 equiv. relative to phosphoric acid) also shows the presence of traces (perhaps 1%) of III. During the normal work-up of the reaction mixtures this compound is removed during aqueous extraction of DMSO. Its formation seems to be best explained by the presence of minor amounts of an activated species such as XIX. This same species has been proposed by Parham[35a,b] as a possible intermediate in the Pummerer reaction of sulfoxides with acid anhydrides and could arise as follows.



Once formed, XIX should be susceptible to nucleophilic attack on carbon, such an attack by pyridine giving the observed product III. The formation of trace amounts of O-thiomethoxymethyl ethers during oxidation of alcohols is described in the accompanying paper[15] and could arise *via* a similar intermediate.

## Experimental Section

*General Methods.* Thin layer chromatography was carried out using Silica G from Brinkmann Instruments containing 0.05% of extracted Radelin GS-115 (Type P-1) phosphor as previously described.[37] For quantitative measurements, the ultraviolet-absorbing spots were scraped off the plate, and the silica was eluted three times with 1-ml. portions of methanol in a microcentrifuge from Microchemical Specialities, Berkeley, Calif. Samples were read against a silica blank from a comparable plate. Ultraviolet measurements were made on Zeiss Model PMQ-II and Cary Model 15 spectrophotometers, and infrared spectra were obtained from potassium bromide pellets on a Perkin-Elmer Model 237 instrument. Nuclear magnetic resonance spectra were determined with a Varian A-60 instrument, peaks being measured in c.p.s. downfield from a standard of tetramethylsilane. Vapor phase chromatography was done on a Barber Coleman, Series 5000, instrument. Elemental analyses were obtained from Dr. A. Bernhardt, Mülheim, Germany, and from Midwest Microlabs, Indianapolis, Ind.

*The Reaction of Thymidine 5′-Phosphate with DMSO and DCC. A. Without Excess Pyridine.* The ammonium salt of thymidine 5′-phosphate (392 mg., 1 mmole) was passed through a 1 × 10 cm. column of Dowex-50 ($H^+$) resin, and the effluent was mixed with pyridine (1 ml.) and evaporated to dryness *in vacuo*. The residue was dissolved in pyridine (10 ml.) and evaporated to dryness. It was then rendered anhydrous by two further evaporations with pyridine. The final residue was evacuated on an oil pump for several hours and then dissolved in anhydrous dimethyl sulfoxide (DMSO)[38] (5 ml.). Dicyclohexylcarbodiimide (618 mg., 3 mmoles) was added and within several minutes the mixture became warm and turned brownish green. After 15, 30, and 60 min., 10-μl. aliquots were removed, diluted with water (0.2 ml.), and extracted twice with pentane (0.2 ml.). The aqueous solution was then examined by paper electrophoresis on Schleicher and Schuell No. 589 orange ribbon paper impregnated with 0.05 *M* ammonium bicarbonate (pH 7.5). After 15 min. only a trace (1–2%) of thymidine 5′-phosphate remained and after 30 min. only neutral, ultraviolet-absorbing materials could be seen. Paper chromatography in several solvents showed only a spot with the $R_f$ and ultraviolet spectrum in acid and alkali of thymine. After 2 hr. water (25 ml.) was added and the mixture was shaken with pentane (25 ml.). Dicyclohexylurea was removed by filtration, and the aqueous phase was extracted twice more with pentane. The aqueous layer was then made 0.5 *M* with ammonium hydroxide and passed through a 1.5 × 10 cm. column of Dowex-2 ($Cl^-$) resin. After a thorough water wash the column was eluted with 0.1 *M* acetic acid giving a slightly yellowish effluent showing $\lambda_{max}$ 264 mμ (total of 9700 optical density units, 1.0 mmole based on thymine). This was evaporated to dryness giving a brownish solid that was crystallized from 5 ml. of hot water giving 115 mg. (91%) of thymine, m.p. 318–319°, which had ultraviolet and infrared spectra identical with an authentic sample.

*B. With Excess Pyridine.* An experiment was carried out exactly as above except that after the pyridine evaporations, the residue was not evacuated exhaustively on the oil pump, an additional 0.24 ml. (3 mmoles) of pyridine was added prior to the DCC. Paper chromatography and electrophoresis showed the nucleotide to have disappeared in less than 1 hr. Once again, thymine was the major product but an appreciable amount of a compound moving towards the negative pole on electrophoresis at pH 7.5 was also present. Following addition of water, pentane extraction, and filtration as above, the aqueous layer was passed through a 2 × 10 cm. column of Amberlite IRC-50 ($H^+$) resin, and the column was washed well with water. Elution with 0.02 *N* hydrochloric acid and evaporation to dryness left a mixture of a pyridinium compound and pyridine hydrochloride as a brown syrup (180 mg.). Part of the pyridine hydrochloride was removed by adjusting an aqueous solution of this mixture to pH 5 with free base Amberlite IR 4B resin and evaporation to dryness. The residue (100 mg.) still contained a lot of pyridine hydrochloride and was purified by preparative thin layer chromatography on a 20 × 20 cm. glass plate carrying a 1.3-mm. layer of microcrystalline

(37) K. E. Pfitzner and J. G. Moffatt, *J. Org. Chem.*, 29, 1508 (1964).

(38) Obtained through the kindness of the Crown Zellerbach Corp., Camas, Wash., and carefully dried by distillation under reduced pressure followed by storage over Linde Molecular Sieve, Type 4A, from the Linde Co., Los Angeles, Calif.

cellulose (Avicel) and Celite (3:1)[39] using isopropyl alcohol–ammonium hydroxide–water (7:1:2) as solvent. In addition to a diffuse, brown area near the origin an intense, ultraviolet-absorbing band occurred at $R_f$ 0.7. This was eluted with methanol and evaporated to dryness giving 32 mg. of an oil which crystallized upon addition of a few drops of acetone but was extremely hygroscopic and difficult to crystallize from a solvent. Its infrared, ultraviolet, and nuclear magnetic resonance spectra and its chromatographic and electrophoretic behavior in several systems were, however, identical with those of a pure sample of III prepared independently as below.

*N-(Thiomethoxymethyl)pyridinium Chloride (III).* Chloromethyl methyl sulfide[40] (1 ml.) and pyridine (2 ml.) were refluxed in acetone (20 ml.) for 1 hr. An oil which had separated crystallized upon cooling and was rinsed several times in the flask with acetone. The residue was then dissolved in isopropyl alcohol (5 ml.) and diluted with acetone (30 ml.), giving beautiful, white platelets which were collected by centrifugation, washed with acetone, and dried immedately *in vacuo*, giving 250 mg. of N-(thiomethoxymethyl)pyridinium chloride (m.p. 158–160°) which was extremely hygroscopic, and even under the most careful conditions absorbed roughly 0.3 equiv. of water during analysis, $\lambda_{max}^{MeOH}$ 258 mμ (ε 3800). *Anal.* Calcd. for $C_7H_{10}NSCl \cdot 0.3H_2O$: C, 46.40; H, 5.90; N, 7.73. Found: C, 46.77; H, 5.82; N, 7.20. Its n.m.r. spectrum in $D_2O$ showed a three-proton singlet (SMe) at 128, a two-proton singlet ($NCH_2S$) at 342, and five aromatic protons between 485 and 545 c.p.s.

*3′-O-Tetrahydropyranylthymidine 5′-Phosphate.* An aqueous solution of ammonium thymidine 5′-phosphate (2 mmoles) was converted to the free acid with Dowex-50 ($H^+$) resin and evaporated to dryness. The residue was evaporated to dryness three times with dioxane (10 ml. each) and dissolved in anhydrous dimethyl sulfoxide (3 ml.). Two additional 10-ml. portions of dioxane were added and removed by evaporation to roughly half-volume. Dihydropyran (3 ml.) and trifluoroacetic acid (0.1 ml.) were added, and the mixture was stored overnight at room temperature. Paper chromatography in isopropyl alcohol–ammonium hydroxide–water (7:1:2) showed the reaction to be complete. Triethylamine (1 ml.) was added and the solution was concentrated to about 3 ml. *in vacuo*. Ethanol (50 ml.) was added followed by a 1 *M* solution of calcium chloride in ethanol (5 ml.), giving a white precipitate which was collected and washed with ethanol–acetone (1:1). The final precipitate was dried *in vacuo*, giving 712 mg. (74%) of the calcium salt of 3′-O-tetrahydropyranylthymidine 5′-phosphate as the dihydrate. *Anal.* Calcd. for $C_{15}H_{21}CaN_2O_9 \cdot H_2O$: C, 37.45; H, 5.14. Found: C, 37.04; H, 4.89.

It was also observed that, if the reaction did not proceed to completion, it is possible to completely selectively extract the tributylamine salt of the desired product into chloroform leaving any unreacted thymidine 5′-phosphate in the aqueous layer. Evaporation of the chloroform and precipitation of the calcium salt then gives a product identical with that above.

(39) Avicel was obtained from the FMC Corp., Newark, Del. The addition of Celite is a modification of the method of Wolfrom, *et al.*, *J. Chromatog.*, 17, 488 (1965), developed by Dr. J. P. H. Verheyden in this laboratory and results in a much more rapid development.

(40) Aldrich Chemical Co., Milwaukee, Wis.

*5′-O-p-Nitrobenzoylthymidine and 3′,5′-Di-O-p-nitrobenzoylthymidine.* Recrystallized *p*-nitrobenzoyl chloride (1.05 g., 5.5 mmoles) was added portionwise over 30 min. to a solution of thymidine (1.21 g., 5 mmoles) in pyridine (10 ml.), and the yellow solution was allowed to stand overnight at room temperature. The mixture was then added to 400 ml. of ice water and the precipitate was collected, washed with water, and dried. It was then dissolved in 20 ml. of chloroform from which 5′-O-*p*-nitrobenzoylthymidine (940 mg., 48%) immediately crystallized. This material (m.p. 180–181°) was homogeneous by thin layer chromatography using ethyl acetate–methanol (4:1). The mother liquors were chromatographed on 25 g. of silicic acid (Davidson) giving 3′,5′-di(*p*-nitrobenzoyl)thymidine (480 mg., m.p. 169–170°, see below) with acetone–chloroform (1:19), a small amount of 3′-O-*p*-nitrobenzoylthymidine (see below) with acetone–chloroform (1:3), and an additional 140 mg. of the desired product with acetone. The total yield of 5′-O-*p*-nitrobenzoylthymidine was 1.08 g. (55%), and the melting point remained unchanged (180–181°) upon recrystallization from ethyl acetate.

*Anal.* Calcd. for $C_{17}H_{17}N_3O_8$: C, 52.17; H, 4.38; N, 10.74. Found: C, 52.25; H, 4.66; N, 10.80.

*3′-O-p-Nitrobenzoylthymidine.* 5′-O-Tritylthymidine[41] (2 mmoles) was dissolved in pyridine (10 ml.) containing *p*-nitrobenzoyl chloride (407 mg., 2.2 mmoles). After 4 hr., thin layer chromatography (ethyl acetate) showed the reaction to be complete, and the mixture was poured into 250 ml. of ice water. The precipitate was collected, washed with water, and dried *in vacuo*, giving 1.27 g. of crude product containing only a trace of tritanol by thin layer chromatography. In a separate reaction this material was crystallized from ethanol giving 3′-O-*p*-nitrobenzoyl-5′-O-tritylthymidine which contained 1 mole of ethanol and sintered at 129–134° and finally melted at 188°. Fox[42] has reported m.p. 131–133° for this compound prepared by a different route. *Anal.* Calcd. for $C_{36}H_{31}N_3O_8 \cdot C_2H_5OH$: C, 67.15; H, 5.49; N, 6.18. Found: C, 67.14; H, 5.31; N, 6.02.

The crude product from above was dissolved in 80% acetic acid (20 ml.) and heated at 100° for 30 min. After thorough removal of the solvent by evaporation and evacuation, the residue was washed with chloroform leaving 400 mg. of chromatographically pure, crystalline product. The chloroform-soluble portion was chromatographed on 50 g. of Merck silica giving tritanol with chloroform and a further 250 mg. of the desired product with chloroform–acetone (1:1). The total yield of 3′-O-*p*-nitrobenzoyl thymidine was 650 mg. (83%), m.p. 209–210°, unchanged upon recrystallization from methanol, $\lambda_{max}^{MeOH}$ 263 mμ (ε 23,000). *Anal.* Calcd. for $C_{17}H_{17}N_3O_8$: C, 52.17; H, 4.38; N, 10.74. Found: C, 52.05; H, 4.35; N, 10.91.

*Reactions of Thymidine Derivatives with DMSO and DCC.* Portions (0.1 mmole) of 5′-O-*p*-nitrobenzoylthymidine, 3-O-*p*-nitrobenzoylthymidine, and 3′,5′-di-O-*p*-nitrobenzoylthymidine were separately dissolved in anhydrous DMSO (0.5 ml.), and DCC (63 mg., 0.3 mmole) and crystalline anhydrous phosphoric acid[43]

(41) J. P. Horwitz, J. A. Urbanski, and J. Chua, *J. Org. Chem.*, 27, 3300 (1962).

(42) J. J. Fox and N. C. Miller, *ibid.*, 28, 936 (1963).

(0.05 ml. of a 1 *M* solution in anhydrous DMSO) were added. After 2 hr., additional 20-mg. portions of DCC were added and after 24 and 48 hr., aliquots were removed and directly spotted on paper chromatograms, which were developed in four solvents. Aliquots were also evaporated to dryness on the oil pump and examined by thin layer chromatography using ethyl acetate–methanol (4:1). 3′,5′-Di-O-*p*-nitrobenzoylthymidine remained completely unchanged. 3′-O-*p*-Nitrobenzoylthymidine showed a trace of unreacted starting material and a major spot moving just ahead of the starting compound. This spot gave a positive carbonyl test upon spraying with 0.2% dinitrophenylhydrazine in 1 *N* hydrochloric acid. 5′-O-*p*-Nitrobenzoylthymidine was completely converted to thymine and *p*-nitrobenzoic acid. Completely analogous results were obtained with 3′-, 5′-, and 3′-,5′-acetylthymidine.

*3′-O-Acetylthymidine-5′-aldehyde (VII).* 3′-O-Acetylthymidine (284 mg., 1 mmole) was dissolved in anhydrous DMSO (5 ml.) containing DCC (1.03 g., 5 mmoles) and anhydrous phosphoric acid (0.5 ml. of 1 *M* solution in DMSO). After 20 hr. at room temperature the solvent was evaporated to dryness on an oil diffusion pump using a Dry Ice trap very close to the reaction flask and a bath temperature of 40°. The residue was partitioned between water (25 ml.) and petroleum ether (25 ml., b.p. 30–60°), and dicyclohexylurea (1.03 g.) was removed by filtration. After one further petroleum ether extraction the aqueous solution (which chromatographically contained only a very small amount of starting material in addition to a strong carbonyl-containing compound) was added to an excess of 2,4-dinitrophenylhydrazine in 1 *N* sulfuric acid. After chilling in ice 280 mg. (61%) of the yellow 2,4-dinitrophenylhydrazone of 3′-O-acetylthymidine-5′-aldehyde (VII) was removed by filtration. Thin layer chromatography of this derivative using toluene–ethyl acetate (1:1) as solvent showed it to be contaminated by only a faint trace of dinitrophenylhydrazine, which was removed by crystallization from methanol, giving yellow needles, m.p. 233–234°, $\lambda_{max}^{MeOH}$ 261 mμ (ε 19,300) and 350 mμ (ε 21,650). *Anal.* Calcd. for $C_{18}H_{18}N_6O_9$: C, 46.76; H, 3.92; N, 18.18; acetyl, 9.31. Found: C, 46.99; H, 4.16; N, 18.37; acetyl, 9.61.

In spite of many attempts the free aldehyde could not be obtained crystalline and was contaminated by about 5% 3′-O-acetylthymidine. Treatment of 20 μmoles of the crude product with excess sodium borohydride in water for 30 min. at room temperature (the reaction becomes alkaline) followed by acidification with Dowex-50 ($H^+$) resin, and repeated evaporation with methanol led to complete conversion to thymidine and a little 3′-O-acetylthymidine, as demonstrated chromatographically.

Separately 10 μmoles of the crude aldehyde was treated at pH 9 ($Na_2CO_3$) with 0.1 ml. of an aqueous solution 1 *N* in $I_2$ and KI for 30 min. at room temperature. The mixture was acidified with Dowex-50 ($H^+$) resin and decolorized with sodium thiosulfate. The nucleoside was then adsorbed on charcoal, washed free of iodide and iodate with water, and eluted with a 2% solution of ammonium hydroxide in 50% aqueous ethanol. The eluates were concentrated and examined by paper chromatography and electrophoresis. The aldehyde was completely converted to thymidine 5′-carboxylate (XI) and its 3′-O-acetyl derivative. A brief hydrolysis with ammonium hydroxide led to XI as the sole product.

*Determination of Optimal Conditions for the Oxidation of Testosterone. A. Selection of the Acid.* A number of reactions was set up with each containing testosterone (29 mg., 0.1 mmole) and dicyclohexylcarbodiimide (62 mg., 0.3 mmole) in 0.5 ml. of anhydrous DMSO. To each reaction was added 0.05 mmole of one of the following acids: anhydrous phosphoric acid, phosphorous acid, trifluoroacetic acid, anhydrous hydrogen chloride in ether, sulfuric acid, 72% perchloric acid, and the pyridine salts (100% excess pyridine) of trifluoroacetic, phosphoric, hydrochloric, sulfuric, and perchloric acids. After 2 hr., an additional 0.1 mmole of DCC was added and after 3, 5, and 17 hr., aliquots of approximately 50 μl. were removed and quickly evaporated to dryness on a high vacuum pump using a bath of about 45°. The residues were dissolved in a few drops of methanol and examined by thin layer chromatography using chloroform–ethyl acetate (4:1) as solvent. The ultraviolet-absorbing spots were scraped off, eluted three times with 1-ml. portions of methanol, and quantitatively measured at 240 mμ. The results are shown in Figure 1.

In addition to the above acids similar studies were done with triethylammonium phosphate, monophenyl and diphenyl phosphoric acids, and mono-, di-, and trichloroacetic acids. These results are described in the text.

*B. The Amount of Acid.* Four reactions were set up, each containing testosterone (29 mg., 0.1 mmole) and DCC (103 mg., 0.5 mmole) in a mixture of DMSO (0.125 ml.) and benzene (0.375 ml.). Anhydrous phosphoric acid (0.01, 0.05, 0.1, and 0.2 mmole of a 1 *M* solution in dry DMSO) was added, and aliquots were removed and quantitatively measured as is A above. The results are shown in Figure 2.

*C. The Amount of DCC.* Three reactions were set up, each containing testosterone (29 mg., 0.1 mmole) and anhydrous phosphoric acid (25 μl. of a 1 *M* solution in DMSO, 0.025 mmole) in anhydrous DMSO (0.5 ml.). Dicyclohexylcarbodiimide (0.1, 0.2, and 0.3 mmole) was added, and aliquots were removed and analyzed as in A above. The results are shown in Figure 3. Similar reactions were also run using 0.5 molar equiv. of phosphoric acid together with 1, 3, 5, and 8 molar equiv. of DCC relative to testosterone. In this case the reactions with 3, 5, and 8 equiv. of DCC gave 87, 88, and 82% $\Delta^4$-androstene-3,17-dione within 1 hr., while the reaction with 1 equiv. gave only 5% in 3 hr.

*D. The Amount of DMSO.* Four reactions were set up, each containing testosterone (29 mg., 0.1 mmole), anhydrous phosphoric acid (0.05 mmole), and DCC (103 mg., 0.5 mmole) in 0.5 ml. of a mixture of benzene and DMSO containing 10, 25, 50, and 100% DMSO. After 1 and 3 hr., aliquots were removed and quantitatively analyzed as in A above. Little differences were observed, the four reactions giving 85, 88, 99, and 91% of the dione within 1 hr.

*O-p-Nitrobenzyl-N,N′-dicyclohexylisouronium Chloride (XVII, $R = C_6H_{11}$; $R^1$ = p-Nitrophenyl).* Di-

(43) International Chemical and Nuclear Corp., City of Industry, Calif.

cyclohexylcarbodiimide (2.06 g., 0 mmoles) and *p*-nitrobenzyl alcohol (1.56 g., 10 mmoles) were dissolved in dry acetone (5 ml.) containing anhydrous cupric chloride (10 mg.). After 3 hr. at room temperature paper electrophoresis in 1 *M* acetic acid showed only a trace of neutral ultraviolet-absorbing material remaining, while a heavy spot moved towards the negative pole. The mixture was evaporated to dryness leaving a yellowish-green oil which was partitioned between ether and water. The ether layer was then shaken with 25 ml. of water containing 1 ml. of concentrated hydrochloric acid, whereupon a heavy white precipitate separated. This was removed by filtration, washed with water and ether, and dried giving 3.32 g. of chromatographically and electrophoretically pure product. This was readily crystallized from methanol–ether, giving 3.07 g. (85%) of O-*p*-nitrobenzyl-N,N′-dicyclohexylisouronium chloride which changed crystal structure at about 120° and melted at 170–190°, depending upon the rate of heating. *Anal.* Calcd. for $C_{20}H_{30}ClN_3O_3$: C, 60.71; H, 7.64; N, 10.62. Found: C, 60.62; H, 7.77; N, 10.91.

*Attempted Oxidation of the Isourea XVII* ($R$ = $C_6H_{11}$; $R^1$ = $NO_2C_6H_5$). The isourea hydrochloride (396 mg., 1 mmole) was dissolved in methanol (5 ml.), and lithium hydroxide (2 ml. of 1 *N*) was added. The resulting oil was extracted with ether, dried with sodium sulfate, and evaporated to dryness leaving the free base as a viscous oil which crystallized very slowly. Seven portions (36 mg., 0.1 mmole each) were weighed out and dissolved in DMSO (0.25 ml.) containing the following: (a) 10 μl. of pyridine; (b) 0.05 ml. of 1 *M* anhydrous phosphoric acid in DMSO; (c) 0.05 ml. of 1 *M* phosphoric acid in DMSO plus 10 μl. of pyridine; (d) 63 mg. of DCC; (e) 63 mg. of DCC plus 10 μl. of pyridine; (f) 63 mg. of DCC plus 0.05 ml. of 1 *M* anhydrous phosphoric acid in DMSO; (g) no additions.

After 3 hr. at room temperature aliquots of each reaction were removed, evaporated to dryness on an oil pump, and examined by paper electrophoresis in 1 *M* acetic acid and by thin layer chromatography. In no case was there more than a trace of neutral, ultraviolet-absorbing material behaving like *p*-nitrobenzaldehyde. The reactions containing phosphoric acid showed the presence of some *p*-nitrobenzyl phosphate,[44] and the reaction with both phosphoric acid and pyridine contained quite a strong spot of N-*p*-nitrobenzylpyridinium ion (independently prepared from pyridine and *p*-nitrobenzyl chloride), but in general the isouronium compound remained unchanged.

(44) D. L. M. Verheyden, W. E. Wehrli, and J. G. Moffatt, *J. Am. Chem. Soc.*, 87, 2257 (1965).

# Sulfoxide–Carbodiimide Reactions. II. Scope of the Oxidation Reaction[1]

**K. E. Pfitzner[2] and J. G. Moffatt**

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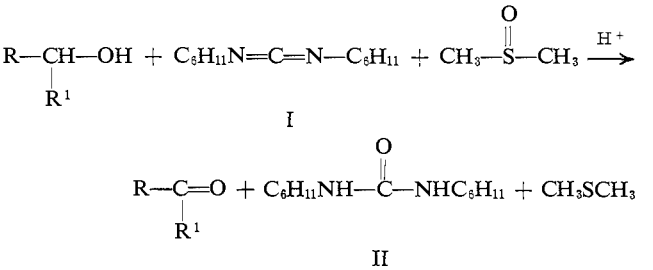
*Oxidation of many different types of hydroxyl function, particularly in the steroid area, has been carried out through reaction with dimethyl sulfoxide and dicyclohexylcarbodiimide in the presence of an appropriate acid. Relatively minor differences in rate were apparent during oxidation of epimeric pairs of 3- and 17-hydroxy steroids. On the other hand, the equatorial 11α-hydroxyl group in several steroids was readily oxidized under conditions where the axial epimer was inert. The particular utility of the method for the oxidation of primary alcohols to aldehydes and of sensitive molecules such as homoallylic alcohols is emphasized. In addition, the method has been successfully applied to alkaloids, and an unusual dehydration of a hemiacetal to a vinyl ether is described.*

In the accompanying paper[1] we have described the development of a new oxidative reaction which promises to be of considerable synthetic utility. In this reaction an alcohol is treated with dicyclohexylcarbodiimide (DCC, I) and dimethyl sulfoxide (DMSO) in the presence of a suitable acid according to the following scheme.



Using the oxidation of testosterone (IIIa) to androst-4-ene-3,17-dione (IIIb) as a model, we have shown that optimal results are obtained upon treatment of the alcohol at room temperature with 0.5 molar equiv. of pyridinium trifluoroacetate (usually in the presence of excess pyridine) and 3 molar equiv. of DCC in anhydrous DMSO or mixtures of DMSO and a suitable inert solvent such as benzene. Under these conditions the reaction remains essentially neutral and should be applicable to the oxidation of both acid- and base-sensitive compounds. Of particular significance is the fact that the oxidation of primary alcohols leads only to aldehydes with no trace of the corresponding acids

(1) For part I see K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, 87, 5661 (1965), accompanying paper.
(2) Syntex Postdoctoral Fellow, 1961–1963.