charge rearrangement in this state may give a much larger singlet–triplet splitting than expected (*i.e.*, large electron correlation effects).

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Selective Phosphorylation of the 5′-Hydroxy Groups of Thymidine and Uridine

Sir:

An obvious disadvantage in the use of protected nucleosides for the syntheses of nucleotides and oligonucleotides is that undesirable side reactions take place in some instances during the conditions necessary for phosphorylation or for the removal of the protecting groups.[1-4] It would therefore be desirable to develop selective phosphorylation of unprotected nucleosides in connection with the specific synthesis of oligonucleotides. We wish to report the selective phosphorylation of the 5′-hydroxy groups of thymidine and uridine.

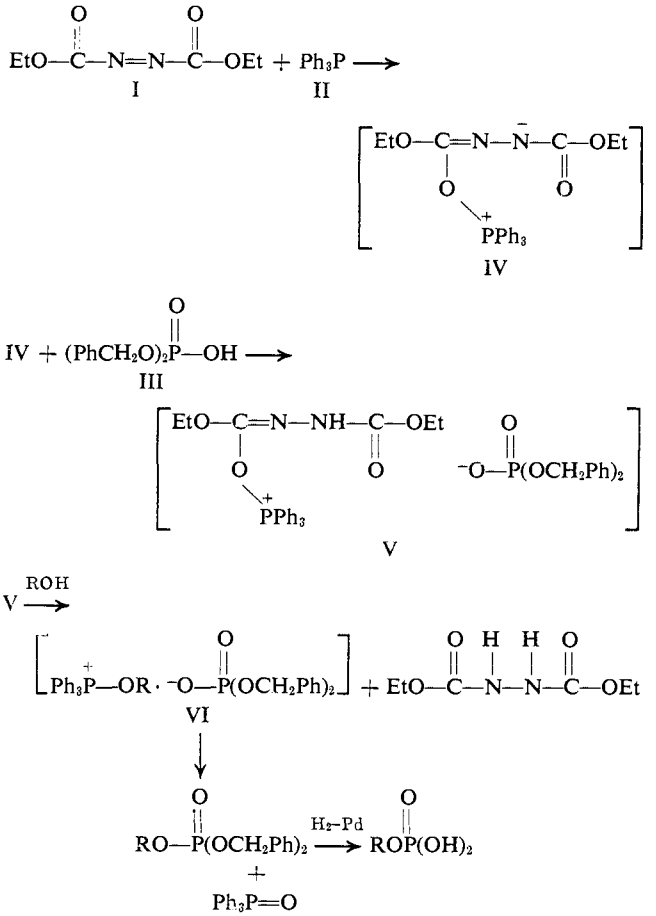
A number of other laboratories have reported the direct phosphorylation of unprotected nucleosides and nucleotides.[5-9] Thus, Tener[5] has reported that the phosphorylation of unprotected deoxyribonucleosides by the use of dicyclohexylcarbodiimide and 2-cyanoethyl dihydrogen phosphate resulted in the formation of the corresponding 5′-phosphates as major products. Similar results have been obtained in the ribonucleotide series.[6,7] Weimann and Khorana[10] have demonstrated that the rate of phosphorylation of the secondary hydroxy group of 5′-O-tritylthymidine is somewhat slower than that of the primary hydroxy group of 3′-O-acetylthymidine. Recently, the selective phosphorylations of thymidine and ribonucleosides by means of dibenzyl phosphorochloridate[8] and pyrophosphoryl chloride,[9] respectively, have been reported. Further, the 5′-hydroxy group of nucleoside is sulfonylated more readily than the 3′-hydroxy group.[8,11]

We have studied the phosphorylation of alcohols with diethyl azodicarboxylate (I), triphenylphosphine (II), and dibenzyl hydrogen phosphate (III), giving corresponding alkyl dibenzyl phosphates. A mechanistic pathway for this reaction involving quaternary phosphonium salts (IV–VI) is presumed to be as in Scheme I.[12]

It is well established that trityl chloride mainly attacks the 5′-hydroxy group of nucleosides to give corresponding 5′-O-trityl nucleosides. The selectivity of the tritylation of the 5′-hydroxy group of nucleosides is explained by the steric hindrance of the triphenylmethyl group. Since the phosphorus cation in the intermediate V similarly has three bulky phenyl groups, nucleophilic attack at the 3′-hydroxy group of the nucleoside would be expected to be much more hindered than that at the 5′-hydroxy group, the corresponding nucleoside 5′-phosphate being predominantly formed.[13]

A mixture of thymidine (242 mg, 1×10^{-3} mol), triphenylphosphine (393 mg, 1.5×10^{-3} mol), and dibenzyl hydrogen phosphate (417 mg, 1.5×10^{-3} mol) in dry tetrahydrofuran (THF, 1 ml) was stirred, and diethyl azodicarboxylate (261 mg, 1.5×10^{-3} mol) in dry THF (1 ml) was added at room temperature. Stirring was continued for 3 hr, and the solution was then kept standing overnight at room temperature. After the solution was concentrated, the residue was dissolved in 75% ethanol and hydrogenated on palladium. After absorption of hydrogen had ceased, the catalyst was removed by filtration. Paper chromatography (1-propanol–2 *N* HCl, 3:1) of the filtrate revealed two uv-absorbing components; one was the unreacted thymidine (R_f 0.86) and the other was thymidine 5′-phosphate (R_f 0.81). The filtrate was concentrated to a small bulk and adjusted to pH 7.5 by adding 1 *N* barium hydroxide solution. After removal of a precipitate by centrifugation,

Scheme I



(1) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, London, 1963.
(2) (a) D. H. Rammler and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 3112 (1962); (b) B. E. Griffin and C. B. Reese, *Tetrahedron Letters*, 2925 (1964); (c) C. B. Reese and D. R. Trentham, *ibid.*, 2459 (1965).
(3) D. Söll and H. G. Khorana, *J. Am. Chem. Soc.*, **87**, 360 (1965).
(4) C. B. Reese and D. R. Trentham, *Tetrahedron Letters*, 2467 (1965).
(5) G. M. Tener, *J. Am. Chem. Soc.*, 83, 159 (1961).
(6) H. Follmann, *Tetrahedron Letters*, 2113 (1967).
(7) M. Yoshikawa, T. Kato, and T. Takenishi, *ibid.*, 5065 (1967).
(8) K. L. Agarwal and M. M. Dhar, *Experientia*, **21**, 432 (1965); *Chem. Abstr.*, **63**, 13379 (1965).
(9) M. Honjo, T. Masuda, K. Imai, and S. Fujii, 7th Meeting of the International Congress of Biochemistry, Tokyo, Aug 1967, Abstracts, IV, B-16.
(10) G. W. Weimann and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 4329 (1962).
(11) T. M. Jacob and H. G. Khorana, *ibid.*, 86, 1630 (1964).
(12) O. Mitsunobu and M. Yamada, *Bull. Chem. Soc. Japan*, **40**, 2380 (1967).
(13) When *l*-menthol was treated with I, II, and III at room temperature, more than 90% of the *l*-menthol was recovered unchanged. This result is best accounted for by steric hindrance to the approach of an attacking nucleophile by the three phenyl groups on the phosphorus cation of V. Because *l*-menthol has a bulky isopropyl group on the α carbon, nucleophilic attack to form VI may be virtually impossible.

two volumes of ethanol was added to the clear supernatant. The precipitated barium thymidine 5′-phosphate was successively washed with ethanol, acetone, and ether (yield 47.0%). The product was chromatographically homogeneous.

Similarly, the reaction of uridine with I, II, and III resulted in the formation of uridine 5′-phosphate in 27.8% yield. The product was isolated as its barium salt and identified by paper chromatography. When the reaction was carried out in dioxane at 60°, the yield of uridine 5′-phosphate was increased to 63%. The ultraviolet absorption characteristics of the products and the R_f's of different compounds are listed in Tables I and II, respectively.

Table I. Ultraviolet Absorption Characteristics

	OD_{290}/OD_{260}	OD_{280}/OD_{260}	OD_{250}/OD_{260}
Thymidine 5′-phosphate (pH 7.0)	0.23	0.71	0.65
Uridine 5′-phosphate (pH 4.8)	0.017	0.38	0.73

Table II. Paper Chromatography of Different Compounds[a]

Compounds	R_f Solvent I	R_f Solvent II
Thymidine 5′-phosphate	0.70	
Thymidine 3′-phosphate	0.83	
Uridine 5′-phosphate		0.22
Uridine 3′(2′)-phosphate		0.31

[a] Paper chromatography was performed by the ascending technique using Toyo Roshi No. 51A paper. The solvent systems used were solvent I, 1-propanol–2 *N* HCl (5:1); solvent II, 1-propanol–concentrated NH_4OH–H_2O (6:3:1).

Although the yields of thymidine 5′- and uridine 5′-phosphates were not high, no isomeric 3′- and/or 2′-phosphates could be detected in the debenzylated products by paper chromatography. As expected, this result and other work in the related fields show that steric factors are of importance for the selective phosphorylation. Work is continuing to find optimum conditions of the method as well as further development of the present system.

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Micelle Formation in Pure Ethylene Glycol[1]

Sir:

Ethylene glycol plays an important role in protein conformation studies[2-6] because it is a weak protein denaturant compared to urea or other organic solvents such as ethanol, dioxane, etc. However, at low or intermediate concentrations, it does weaken hydrophobic bonding in model systems[7-12] including those containing ionic[8-11] and nonionic[10,12] detergent micelles in water.

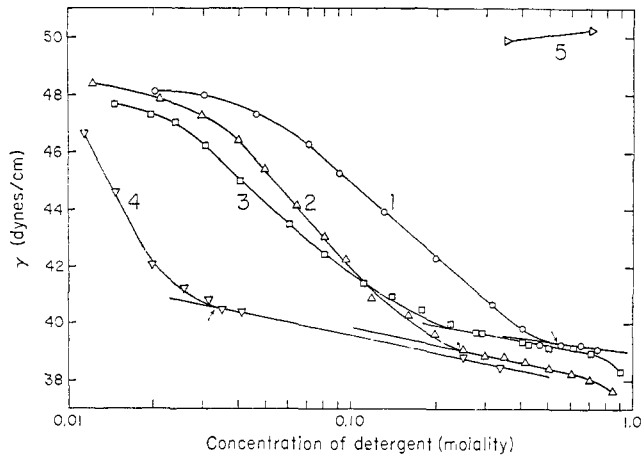

Figure 1. The variation of surface tension, γ, with the logarithm of concentration: (○) DPB, (△) MTAB, (□) CPC, and (▷) EPB, in pure ethylene glycol; (▽) DPB in 40 vol % ethylene glycol.

Although the limited solubility of hydrocarbons in pure ethylene glycol in contrast to their complete miscibility with ethanol or dioxane[13] and the close resemblances between ethylene glycol and water in many of their properties would indicate that hydrophobic or, more correctly, lyophobic bonding may still persist in pure ethylene glycol, no report seems to have appeared so far demonstrating the existence of micelles in this solvent. As is well known, the formation of detergent micelles in water provides an excellent example of hydrophobic bond formation.

The present work reports the determination of what appear to be the critical micelle concentrations (cmc's) of three cationic detergents of three different chain lengths, *viz.* dodecylpyridinium bromide (DPB), myristyltrimethylammonium bromide (MTAB), and cetylpyridinium chloride (CPC) in pure ethylene glycol. No such determination was possible in the case of another detergent, cetyltrimethylammonium bromide (CTAB), a higher homolog of MTAB, because of the surprisingly low solubility of CTAB in ethylene glycol at room temperature.

Chromatoquality reagent grade ethylene glycol (EG), purchased from Matheson Coleman and Bell, was dried with anhydrous Na_2SO_4 and distilled under reduced pressure before use.

The method used involved the measurements of surface tensions of detergent solutions using a Rosano surface tensiometer. About 10 ml of solution was used for each measurement, and the time allowed for equilibration varied between 5 and 20 min, during which the solutions were kept covered with aluminum foil. The measurements were carried out at 27.5 ± 0.5°.

Figure 1 shows plots of surface tension (γ) *vs.* logarithm of concentrations for the three detergents and

(1) This work was supported by the National Science Foundation under Grant No. GB-8410, held by Professor G. Némethy.
(2) C. Tanford, C. E. Buckley, III, P. K. De, and E. P. Lively, *J. Biol. Chem.*, 237, 1168 (1962).
(3) H. J. Sage and S. J. Singer, *Biochemistry*, 1, 305 (1962).
(4) T. T. Herskovits and M. Laskowski, Jr., *J. Biol. Chem.*, 237, 2481, 3418 (1962).
(5) P. H. von Hippel and K. Y. Wong, *ibid.*, 240, 3909 (1965).
(6) S. N. Timasheff and H. Inoue, *Biochemistry*, 7, 2501 (1968).
(7) Y. Nozaki and C. Tanford, *J. Biol. Chem.*, 240, 3568 (1965).
(8) M. L. Corrin and W. D. Harkins, *J. Chem. Phys.*, 14, 640 (1946).
(9) M. F. Emerson and A. Holtzer, *J. Phys. Chem.*, 71, 3320 (1967).
(10) G. Némethy and A. Ray, Symposium on Water Structure and Hydrophobic Bonding, 157th National Meeting of the American Chemical Society, Minneapolis, Minn., April 1969.
(11) A. Ray, unpublished work.
(12) W. B. Gratzer and G. H. Beaven, *J. Phys. Chem.*, 73, 2270 (1969).
(13) S. J. Singer, *Advan. Protein Chem.*, 17, 1 (1962).