facial, [1,5] hydrogen shift in the transformation of *cis*-1-methyl-2-vinylcyclopropane to *cis*-hexa-1,4-diene, which occurs very readily at 160° [$E^* \sim 31$ kcal.],[15] exemplifies this process.

It is also worthy of note that orbital symmetry arguments are applicable to sigmatropic changes within ionic species. Thus, the suprafacial [1,2] shift within a carbonium ion is symmetry-allowed and is very well known. The as yet undetected [1,4] migration within a but-2-en-1-yl cation must proceed through an antarafacial transition state, which may be difficult of access because of serious uncoupling within the framework π-system. By contrast, it may be predicted that the [1,6] shift within a hexa-2,4-dien-1-yl cation should take place through a readily accessible suprafacial transition state.

(15) R. J. Ellis and H. M. Frey, *Proc. Chem. Soc.*, 221 (1964); *cf.* also W. Grimme, *Chem. Ber.*, **98**, 756 (1965).
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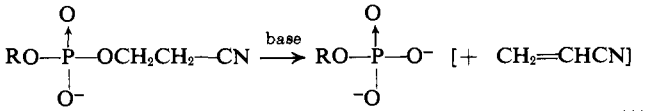
A New Phosphorylation Procedure

Sir:

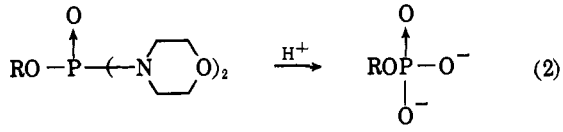
Monoesters of phosphoric acid, a class of compounds of considerable interest in biology, are frequently synthesized chemically by condensation of a properly activated derivative of phosphoric acid with an alcohol.[1] Because of the nature of phosphoric acid, means had to be found to prevent the formation of di- or triesters as well as anhydrides by further reaction of the desired condensation product. This is customarily accomplished by blockade of at least one, and sometimes two, of the substituents on the central phosphorus atom with a masking group which can be selectively removed subsequent to the desired condensation.[2] We wish to report on the use of the alkylthio substituent on phosphorus as a shielding group which can be replaced under mild conditions appropriate for the treatment of acid- or base-sensitive molecules of biological interest.[5]

(1) See H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," John Wiley and Sons, Inc., New York, N. Y., 1961.
(2) This "unmasking" is typically brought about by rather drastic hydrolytic or hydrogenolytic procedures: thus, in the widely useful phosphorylation method of Tener,[3] a cyanoethyl ester blocking group is split off *via* β-elimination by means of alkaline reagents (1). On the other hand, the method of Montgomery and Turnbull[4] uses acidic



(1)

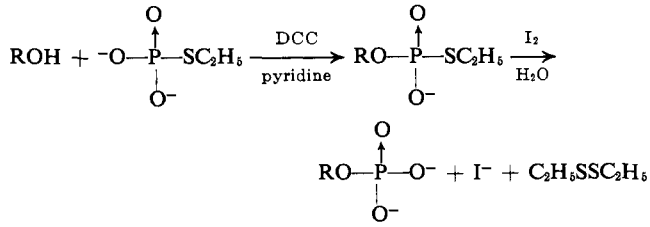
hydrolysis (2) to remove blocking groups in the alkyl phosphorodimorpholidates formed in the primary condensation.



(2)

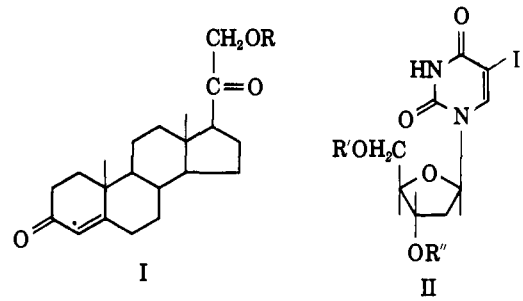
(3) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).
(4) H. A. C. Montgomery and J. H. Turnbull, *J. Chem. Soc.*, 1963 (1958).

It has been observed that S-alkylphosphorothioic acids are converted to organic phosphate at pH 7 in the presence of iodine.[6] We therefore studied the monophosphorylation of alcohols with S-ethylphosphorothioic acid[7] according to the scheme



DCC = dicyclohexylcarbodiimide

The feasibility of this procedure was tested by studying the phosphorylation of a steroidal alcohol, 11-desoxycorticosterone (I, R = H). Condensation of the latter with pyridinium S-ethylphosphorothioate by means of DCC in pyridine gave a crude phosphate which was directly treated with iodine in aqueous acetone. Fractionation by ion-exchange chromatography on DEAE[8]-cellulose, using gradient elution with



I II

a bicarbonate buffer, gave 11-desoxycorticosterone 21-monophosphate (I, R = PO_3^{3-}), eluted with buffer strength 0.04 *M*, in 35% yield. This material traveled as a single spot in paper electrophoresis (16 cm. toward the cathode during 1 hr., 20 v./cm., pH 7.3 with 0.05 *M* ammonium acetate), absorbed maximally at 243 mμ, and was split completely to the starting steroidal alcohol with bacterial alkaline phosphatase.[9] A comparison sample prepared by Tener's procedure[3,10] had the same properties. The material was lyophilized to give the bistriethylammonium salt; it contained 1.06 μmoles of organically bound phosphate per 16 optical density units (theory: 1.0 μmole). This demonstrates the capability of the alkylthio substituent to function as a blocking group in DCC condensations.[11]

(5) The use of an enzyme at pH 8.8 (rattle snake venom phosphodiesterase) to strip a *p*-nitrophenyl substituent has been described: see R. W. Chambers, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3741 (1957). Aqueous acetic acid has been used to unmask nucleoside phosphates blocked with *t*-butyl groups: F. Cramer, H. Neunhoeffer, K. H. Scheit, G. Schneider, and J. Tennigkeit, *Angew Chem.*, **74**, 387 (1962).
(6) S. Åkerfeldt, *Svensk. Kem. Tidskr.*, **75**, 4 (1963).
(7) S. Akerfeldt, *Acta Chem. Scand.*, **16**, 1897 (1962).
(8) DEAE = diethylaminoethyl.
(9) E. C. 3.13.1, Worthington Biochemical Corp., Freehold, N. J.
(10) A number of steroidal phosphates have been prepared by this latter procedure: see R. B. Brownfield and W. Shultz, *Steroids*, **2**, 597 (1963).
(11) The possibility that the alkylthio substituent acts as an activating group in its own right has been investigated by T. Wieland and R. Lambert, *Chem. Ber.*, **89**, 2476 (1956). These authors reported that when S-alkylthiophosphates are alcoholized in the presence of iodine, the corresponding phosphomonoesters can be demonstrated. It would seem, however, that a large excess of the alcohol is required: an at-

Because of our interest in antiviral substances, we decided to investigate this reaction in some detail as applied to the pyrimidine antagonist 5-iododeoxyuridine.[12] Dilithium ethyl phosphorothioate[7] (46 mg., 0.3 μmole) was converted to the pyridinium salt by passage over Dowex 50 (pyridinium form) and dried by concentrating its solution in pyridine. The residue was again dissolved in 1 ml. of dry pyridine. 5-Iododeoxyuridine 3′-acetate[13] (II, R′ = H, R″ = $COCH_3$), 40 mg. (0.1 mmole), and DCC, 300 mg. (1.5 mmoles), were added and the well-stoppered reaction mixture was shaken in the dark for 48 hr. Water (2 ml.) was added, and the resulting suspension was kept in the refrigerator for 18 hr. The solution was filtered, the precipitate was washed well with water, and the combined filtrate was concentrated to dryness *in vacuo*. The residue was treated for 2 hr. at room temperature with 10 ml. of a 50% aqueous acetone solution containing 85 mg. of iodine and then again concentrated to dryness. Concentrated ammonia, 20 ml., was added; exposure was maintained at room temperature overnight, and the ammonia was removed *in vacuo*. The residue was dissolved in 15 ml. of water, the pH adjusted to 8.5, and the solution charged to a column (1 × 10 cm.) of DEAE–Sephadex A-25 (bicarbonate cycle). A linear gradient consisting of 1 l. of 0.1 *M* triethylammonium bicarbonate continuously diluting 1 l. of 0.005 *M* buffer was applied, 15-ml. fractions of eluate being collected. The product, 5-iodo-5′-deoxyuridylic acid[14] (II, R′ = PO_3^{2-}, R″ = H), emerged as a single peak at 0.06 *M* buffer, as monitored by ultraviolet absorption. The fractions were combined, concentrated, and lyophilized to give a homogeneous material, 450 optical density units, λ_{pH2}^{max} 286 mμ. (A parallel condensation, using Tener's reagent,[3] gave a 50% yield of the same material.) The material had an organic phosphate content of 0.96 μmole per 5.7 optical density units (theory: 1.0 μmole).

The advantage of the present method was demonstrated by omitting the final hydrolytic step: the corresponding 3′-acetate (II, R′ = PO_3^{2-}, R″ = $COCH_3$), retaining the base-labile ester group, was isolated in 36% yield. Structure of the latter was proved by dephosphorylation with bacterial alkaline phosphatase[9] to IUdR 3′-acetate (II, R′ = H, R″ = $COCH_3$). Finally, by omitting both iodine treatment and alkaline hydrolysis, the intermediate S-alkylphosphorothioate 3′-acetate (II, R′ = P-$(SC_2H_5)O_2^{2-}$, R″ = $COCH_3$) was isolated in 91% yield, its structure following from its diminished electrophoretic mobility (7.0 cm.) as compared to the corresponding phosphomonoester and from conversion to the 3′-acetate phosphate (II, R′ = PO_3^{2-}, R″ = $COCH_3$) by treatment with iodine.

The method is not restricted to the 5-halogenated pyrimidine nucleosides: phosphorylation of thymidine 3′-acetate to 5′-thymidylic acid was carried out in high yield. Its application to the monophosphorylation of other types of molecules is under current study.

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Flash Photolysis of Cobalt(III) Acidopentaammine and of $PtBr_6^{-2}$ and PtI_6^{-2} Complexes

Sir:

We wish to report the results of some current flash photolytic work which serve to distinguish between alternative mechanisms in two cases of interest.

A flash of about 150 joules of 30-μsec. decay time was used to induce about 10% reaction in 10^{-4} *M* solutions. Intermediates were observed by following their absorption as a function of time after the flash, using a monochromatized scanning light.

The first group of complexes studied consisted of $[Co(NH_3)_5I]Cl_2$, $[Co(NH_3)_5N_3]Cl_2$, and $[Co(NH_3)_5Br]Br_2$, prepared by previously described methods.[1,2] Steady light illumination of pH 4–5 aqueous solutions of the above compounds led to photo oxidation–reduction decomposition with conversion of Co(III) to Co(II), and to I_2 and N_2, but not Br_2 production, respectively. It was postulated that the primary chemical act was a homolytic bond fission to produce I, N_3, and Br radicals and that the absence of bromine in the last case was due to reaction of Br with the released ammonia. An alternative possibility would be that an ammonia ligand was oxidized directly in the primary step, as appears to be suggested in a study of the pyrolysis of $[Co(NH_3)_5Br]Cl_2$.[3]

For aqueous systems we confirm the original mechanism in that aqueous $Co(NH_3)_5Br^{+2}$ and $Co(NH_3)_5I^{+2}$ both give strongly the respective halogen atom transients on flashing in either a quartz or a Pyrex cell.[4] Thus the ammonia oxidation in the over-all photolytic reaction of $Co(NH_3)_5Br^{+2}$ must indeed be due to a secondary reaction of Br atoms produced in the primary step. Interestingly, the decay time of the iodine transient is distinctly less than expected[4]; it may be that the reaction of iodine atoms with unphotolyzed complex is being observed rather than the recombination.[5]

No transient absorption could be located in the case of the $Co(NH_3)_5N_3^{+2}$ ion, but the presence of azide

tempt to apply this reaction to the phosphorylation of 11-desoxycorticosterone gave yields of at most 0.3% based on steroid. The same principle, the activation of only one of the substituents of phosphorus, underlies the procedure recently published by A. J. Kirby, *Chem. Ind.* (London), 1877 (1963); the latter appears to be subject to the same limitation. Furthermore, the relative inefficiency of the S-alkylthio substituent as an activating group is demonstrated by the absence of disteroid monophosphate in our DCC condensation reactions.

(12) E. S. Perkins, R. M. Wood, M. L. Sears, W. H. Prusoff, and A. D. Welch, *Nature*, **194**, 985 (1962).

(13) Prepared from 5-iodouracil 2′-deoxyriboside (II, R′ = R″ = H) (W. H. Prusoff, *Biochim. Biophys. Acta*, **32**, 299 (1959)) by the standard procedure of 5′-tritylation, acetylation at 3′, and subsequent detritylation. All new compounds here described display satisfactory analytical data.

(14) A. Hampton, E. Hampton, and M. L. Eidinoff, *Biochem. Pharmacol.*, 11, 155 (1962).

(1) A. W. Adamson and A. H. Sporer, *J. Am. Chem. Soc.*, **80**, 3865 (1958).

(2) A. W. Adamson, *Discussions Faraday Soc.*, **29**, 163 (1960).

(3) W. H. Wendland and J. P. Smith, *J. Inorg. Nucl. Chem.*, **25**, 843 (1963).

(4) T. A. Gover and G. Porter, *Proc. Roy. Soc.* (London), **A262**, 476 (1961).

(5) A. Haim and H. Taube, *J. Am. Chem. Soc.*, **85**, 495 (1963).