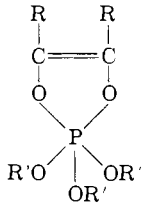
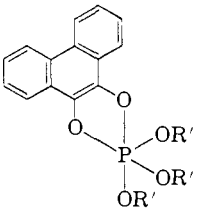
nuclear magnetic resonance spectra, dipole moments and infrared spectra is consistent only with cyclic structures containing pentacovalent phosphorus, such as I, II, III, IV and V.

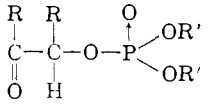


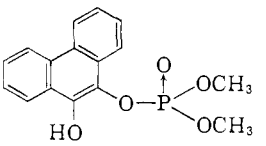


I, R = C_6H_5; R′ = CH_3; m.p. 49°
II, R = R′ = C_6H_5; m.p. 99°
III, R = R′ = CH_3; $n^{20}D$ 1.4391
IV, R′ = CH_3; m.p. 74°
V, R′ = C_6H_5; m.p. 147°

(2) Evidently, the alkyl group translocation which takes place during the reaction of *p*-quinones with certain trialkyl phosphites[2e,f] and which gives rise to alkyl ethers of *p*-quinol monophosphates, *p*-$ROC_6H_4OP(O)(OR)_2$, does not occur with *o*-quinones. Contrary to a recent report,[3b] no alkyl group translocation has been observed in the reaction of α-diketones.

(3) The adducts react readily with *anhydrous hydrogen chloride* in ether solution. The trimethyl phosphite adducts, I, III and IV give α-ketol-monophosphates or *o*-quinol-monophosphates, VI, VII and VIII, respectively. This represents a convenient preparation of a type of compound of interest in biological chemistry.

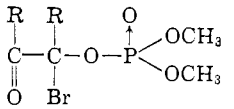


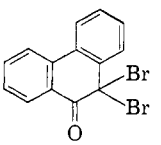


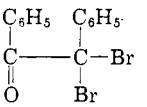
VI, R = C_6H_5; R′ = CH_3 (m.p. 78°) VIII, m.p. 132°
VII, R = R′ = CH_3 ($n^{22}D$ 1.4215)

One of the *triphenyl* phosphite adducts (V) did not react with hydrogen chloride, while the other, II, afforded desyl chloride $C_6H_5COCHCl\text{-}C_6H_5$ and triphenyl phosphate $(C_6H_5O)_3PO$.

(4) The adducts react instantaneously with *bromine in carbon tetrachloride solution* at low temperatures. Two of the trimethyl phosphite adducts, I and III, gave α-bromo-α-ketol-monophosphates, IX and X, respectively. The third, IV, was converted into a dibromo ketone, XI. The triphenyl phosphite adducts, II and V, gave dibromoketones, XII and XI, respectively.

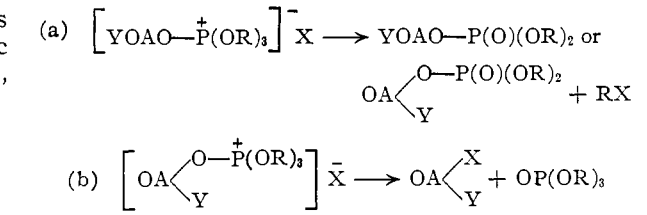






IX, R = C_6H_5; m.p. 118°
X, R = CH_3 XI, m.p. 106° XII, m.p. 112°

These reactions could be interpreted as attacks of a reagent YX (HCl or Br_2) on the open, dipolar form of the adducts, $\overset{-}{O}AO\text{—}\overset{+}{P}(OR)_3$, or on the cyclic forms themselves. In any event, the reactions conform to the general expressions



Step (a) achieves the *reductive phosphorylation* of the carbonyl compound OAO, while step (b) effects its partial deoxygenation.

The trimethyl phosphite adducts were obtained in benzene solution at room temperature. The triphenyl phosphite adducts were prepared at 100° in the absence of solvent. Yields are nearly quantitative and the crystalline substances can be recrystallized from hexane. Some of the adducts (I and III) could be distilled unchanged, at reduced pressures.

Satisfactory elemental analyses were obtained for all new compounds. The molecular weight of the adducts corresponds to the monomeric formulas. The proton n.m.r. spectrum[4] of the biacetyl adduct, III, had one peak for the methyl group protons and one peak for the methoxyl group protons disclosing considerable symmetry. The phosphorus n.m.r. spectra[5] showed these chemical shifts (± 2 p.p.m.) relative to 85% H_3PO_4: I, +53, III, +53 and IV, +49. (The solids were examined in benzene solution.) The dipole moments[4] of the five adducts measured in benzene at 25° were very similar and close to 2 Debye units.

The characteristic feature of the infrared spectra[6] of the trimethyl phosphite adducts, I, III and IV, was a set of six bands in the 8.5–10.5μ region (P-O-C) with a very strong band at 9.40–9.45μ. The triphenyl phosphite adducts, II and V, had very strong bands at 8.25–8.30μ and 10.40–10.42μ.

(4) We are grateful to Professor M. T. Rogers of Michigan State University for this determination. The dipole moments measured by Prof. Rogers will be described in detail elsewhere.

(5) Obtained through the courtesy of Drs. N. McKelvie and J. Lancaster of the American Cyanamid Co., Stamford, Conn. A detailed discussion will appear elsewhere.

(6) The adducts were examined as mineral oil mulls, as liquid films and in solutions in carbon tetrachloride and carbon disulfide. No significant differences were observed in the various media.

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RECEIVED MARCH 23, 1960

THE USE OF METABOLIC INHIBITORS IN THE STUDY OF MICROBIAL TRANSFORMATIONS OF ORGANIC COMPOUNDS

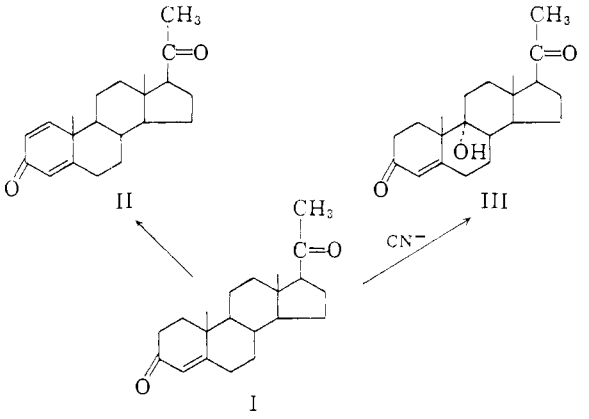
Sir:

In the study of microbiological transformations of organic compounds such as steroids, alkaloids, vitamins, amino acids, etc., one often observes that some microörganisms are capable of oxidizing organic compounds readily to carbon dioxide and water without the apparent accumulation of any intermediates. These intermediates are highly desirable not only because they are often biologically active (*e.g.*, hydrocortisone) but also because

they aid in resolving the sequence of microbial degradation. Several methods have been used to study these intermediate transformations: (a) a genetic mutant which has a metabolic block is employed; (b) the nutrition of the organism is carefully studied in order to limit a particular factor required for a metabolic process during degradation; (c) the environment (*p*H, temperature, aeration) is changed.

The present communication deals with an alternate method using a metabolic inhibitor. Since the organism can oxidize an organic compound to carbon dioxide and water, it was thought that by slowing down the electron transport system of the organism, the rate of oxidative processes would also be decreased with the possible accumulation of new intermediate compounds. Evidence for this mode of inhibition was obtained by the following observations:

Cells of a *Nocardia sp.* were grown in a medium previously described.[1] After 48 hours of growth, progesterone (I) dissolved in dimethylformamide was added to the fermentation medium to give a concentration of 500 λ/ml. of medium; a total of one gram was fermented. After six hours, the culture broth was filtered, and the filtrate (2500 ml.) extracted three times with 500 ml. portions of chloroform. The combined chloroform extracts were concentrated to dryness. An aliquot of the extract was chromatographed on Whatman No. 1 paper and developed in a toluene–propylene glycol system[2] for 3 hours. A major product was observed having a R_f of 0.90 of progesterone which corresponds to the mobility of 1-dehydroprogesterone (II). By fractional crystallization, 200 mg. of a compound with the constants m.p. 150–151°, λ_{max}^{EtOH} 244 mμ (ε = 16,500), was obtained. The infrared spectrum in Nujol was identical to that of an authentic sample of 1-dehydroprogesterone.



When the steroid was left in contact with the organism for 24 hours, the substrate completely disappeared without any apparent detectable products. When potassium cyanide at a $10^{-3}M$ concentration was added at the time of steroid addition, however, a new product (30% yield), having a mobility of 0.55 of progesterone in the toluene–propylene glycol system, was observed. It is important that the cyanide be added at the same time as the steroid or no conversion takes place. Also, the inhibitor must be added to a culture that is about 48 to 72 hours old.

A 1-g. preparative fermentation was carried out under the conditions described above. The product was isolated by chromatographing a portion of the steroid mixture on a cellulose powder column (1.5 × 12 cm.) using propylene glycol as the stationary phase; the mobile phase consisted of a gradient system of methylcyclohexane and toluene. Three and one-half milliliter fractions were collected every three minutes. Fractions 8–55 were combined and the solvent removed *in vacuo*. The residue was taken up in 100 ml. of chloroform and washed twice with 50-ml. portions of water to remove any propylene glycol. The chloroform solution was dried over sodium sulfate, concentrated to dryness, and the residue recrystallized from acetone–hexane yielding 149 mg. of 9α-hydroxyprogesterone[3] (III), m.p. 190–191°, $[\alpha]D$ +188° (chloroform), λ_{max}^{EtOH} 242 mμ (ε = 15,100), λ_{max}^{Nujol} 2.97 μ, 5.88 μ, 6.10 μ, 6.22μ.

One might suspect that cyanide ion inhibits the 1-dehydrogenation reaction. Using partially purified Δ^1-dehydrogenase[4] from this organism, however, we observed no inhibition by cyanide. A more plausible explanation is that, under normal conditions, the organism carries out both of the reactions required for degradation of the steroid, but the 1-dehydrogenation reaction operates at a faster rate than the hydroxylation reaction. On addition of cyanide, a known inhibitor of hematin cytochrome oxidases, the terminal electron transport systems are slowed down so that more reduced pyridine nucleotide, which is essential for hydroxylation reactions,[5] is available. Thus, in the presence of cyanide, the hydroxylation reaction proceeds at a faster rate than the 1-dehydrogenation reaction with the accumulation of 9α-hydroxyprogesterone.

(1) C. J. Sih and R. E. Bennett, *Biochim. et Biophys. Acta*, **38**, 378 (1960).

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(3) D. Perlman, J. D. Dutcher, J. Fried and E. O. Titus, U. S. Patent 2,840,578 (1958).

(4) C. J. Sih and R. E. Bennett, to be published.

(5) (a) J. K. Grant, *Biochem. J.*, **64**, 559 (1956); (b) G. M. Tomkins, J. F. Curran and P. M. Michael, *Biochim. et Biophys. Acta*, **28**, 449 (1958).

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RECEIVED APRIL 8, 1960

A NEW SYNTHESIS OF CHLOROPURINE RIBONUCLEOSIDES[1]

Sir:

Chloropurine nucleosides have served as valuable synthetic intermediates for the preparation of a number of important purine nucleoside derivatives.[2–9]

(1) This investigation has been supported by Contract No. SA-43-ph-1928 with the Cancer Chemotherapy National Service Center of the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

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(3) L. Goldman, J. W. Marsico and R. B. Angier, THIS JOURNAL, **78**, 4173 (1956).

(4) H. M. Kissman and M. J. Weiss, *J. Org. Chem.*, **21**, 1053 (1956).