the chloro sugar XX, prepared as described above (*cf.* preparation of XXI) from 5.29 g. (10 mmoles) of 1-*O*-acetyl-2,3-di-*O*-benzoyl-5-deoxy-5-phthalimido-D-ribofuranose, in 50 cc. of xylene was added to an azeotropically dried suspension of 1.75 g. (5 mmoles) of acetylcytosinemercury (III)[11] in 180 cc. of xylene. The stirred mixture was allowed to reflux for three hours and was then evaporated *in vacuo*. The residual brown gum was dissolved in chloroform (200 cc.) and the solution was washed with two 20-cc. portions of 30% aqueous potassium iodide solution and with 40 cc. of water. The organic phase was dried and partially decolorized over magnesium sulfate and Norit. The filtered solution was evaporated *in vacuo* and the residual gum (6.05 g.) was dissolved in 10 cc. of chloroform and 10 cc. of benzene. The solution was chromatographed on a column prepared from 120 g. of silica gel in benzene–chloroform (1:1). The column (3.5 × 29.5 cm.) was eluted with 400 cc. of this solvent mixture and the 0.22 g. of gum obtained from the pooled fractions was discarded. Elution was continued with 200 cc. of chloroform, 200 cc. of chloroform–acetone (98:2) and 150 cc. of chloroform–acetone (97:3). Solid material was isolated from the following fractions which were eluted with 100 cc. of chloroform–acetone (97:3) and 100 cc. of chloroform–acetone (95:5). By crystallization and one recrystallization from ethanol–ethyl acetate there was obtained 1.03 g. (29% based on 10 mmoles of sugar derivative XX), m.p. 145–146°, undepressed by admixture of the $C_{20}H_{15}NO_5$ compound isolated in the preparation of XXI. Continued elution of the column with the last mentioned solvent mixture (150 cc.), with 350 cc. of chloroform–acetone (9:1) and with 100 cc. of chloroform–acetone (85:15) afforded gummy material (1.06 g.) which was discarded. Washing the column with 450 cc. of chloroform–acetone (65:35) yielded a solid which was crystallized and recrystallized from acetone to afford 1.69 g. (27% based on 10 mmoles of XX; 54% based on III), m.p. 147–150°. For analysis the substance was recrystallized twice more from acetone; m.p. 146–148°. Material dried at 74° *in vacuo* still contained acetone of crystallization. The compound showed $[\alpha]^{25}D$ +37.8° (*c* 0.98 in $CHCl_3$); λ_{max}^{MeOH} 283 mμ (ε 10,880 in acid), 298 mμ (ε 7,480 in methanol) and 272 mμ (ε 11,700 in base).[31]

Anal. Calcd. for $C_{33}H_{26}N_4O_9 \cdot C_3H_6O$: C, 63.52; H, 4.74; N, 8.23; C–CH_3, 4.42. Found: C, 63.24; H, 4.83; N, 8.01; C–CH_3, 3.91.

5′-Amino-5′-deoxycytidine [1-(5-Amino-5-deoxy-β-D-ribofuranosyl)-cytosine (XXIV)].—A mixture of 1.86 g. (3 mmoles) of the 4-acetamidopyrimidinone derivative XXIII, 40 cc. of absolute methanol and 7 cc. of butylamine was heated under reflux for 16 hours. The reaction mixture was worked up as described in the preparation of VII and the residue (0.74 g.) obtained by evaporation of the combined water layers was dissolved in 30 cc. of hot methanol and filtered through Norit. The filtrate was evaporated to a small volume and decanted from a small amount of yellow gum. Ethanol was added at the b.p. until the solution became cloudy. The solid which precipitated on standing was collected, washed with ethanol and ether. The vacuum-dried material (0.37 g.) was amorphous and melted around 145°; ultraviolet analysis indicated a purity of 84%[49]; the 0.37 g. of product, therefore, represented a 74% yield. The compound as such could not be purified further and was characterized through the **picrate** prepared in methanol and recrystallized from methanol–ethanol; m.p. 218–220° dec., $[\alpha]^{25}D$ +21.5° (*c* 1.02 in methyl Cellosolve).

Anal. Calcd. for $C_{15}H_{17}N_7O_{11} \cdot H_2O$: C, 36.81; H, 3.91; N, 20.04. Found: C, 36.51; H, 3.23; N, 19.37.

PEARL RIVER, NEW YORK

(49) The extinction of cytidine at 269 mμ was used for comparison.

COMMUNICATIONS TO THE EDITOR

sym-DIPHENYLPYROPHOSPHORODIAMIDIC ACID: A NEW SUBSTRATE FOR THE COLORIMETRIC ESTIMATION OF ENZYMES

Sir:

N-Phosphorylated nitrogen mustards which have been shown to be non-toxic are potentially cytotoxic agents with a high selectivity for cells with high phosphamidase activity.[1a–g] The distribution of phosphamidase enzymes in malignant tissues is, therefore, a matter of interest in chemotherapy. Studies have shown that some tumors do have high phosphamidase activity.[2a–d] However, confirmation, extension and exploitation of these results have been hampered by the fact that the substrates used are unstable below *p*H 5.0 where the enzyme(s) is active.[3a–c] On the basis of the structural features apparently necessary for enzymatic activity[4] and from theoretical considerations with regard to stability, pyrophosphoroamidic acids, although previously unreported, appeared attractive as substrates for enzymes of the phosphamidase type. In addition, chromogenic pyrophosphoroamidic acids would be satisfactory prototypes for toxagenic analogs potentially useful in the treatment of cancer.

We report here the synthesis of *sym*-diphenylpyrophosphorodiamidic acid (II). O-Benzylphenylphosphoroamidic acid,[5] m.p. 118–119°, on treatment with dicyclohexyl carbodiimide in dimethylformamide gave *sym*-O,O-dibenzyldiphenylpyrophosphorodiamidate (I) (m.p. 142–143°; *Anal.* Calcd. for $C_{26}H_{26}N_2P_2O_5$: C, 61.44; H, 5.16; N, 5.52. Found: C, 61.44; H, 5.53; N, 5.49). Debenzylation of I with hydrogen over palladium afforded the dibasic pyrophosphoroamidic acid II which was isolated both as the dicyclohexylammonium salt (m.p. 223–224°; *Anal.* Calcd. for $C_{24}H_{40}N_4O_5P_2$: C, 54.75; H, 7.62; N, 10.64.

(1) (a) O. M. Friedman and A. M. Seligman, THIS JOURNAL, **70**, 3082 (1948); (b) **76**, 655 (1954); (c) **76**, 658 (1954); (d) A. M. Seligman, M. M. Nachlas, L. H. Manheimer, O. M. Friedman and G. Wolf, *Ann. Surg.*, **130**, 333 (1949); (e) A. M. Seligman, M. Milden and O. M. Friedman, *Cancer*, **2**, 701 (1949); (f) A. M. Rutenburg, L. Persky, O. M. Friedman and A. M. Seligman, *J. Pharm. Exp. Therap.*, III, 483 (1954); (g) O. M. Friedman and E. Boger, THIS JOURNAL, **78**, 4659 (1956).

(2) (a) G. Gomori, *Proc. Soc. Expll. Biol. Med.*, **69**, 407 (1948); (b) J. Meyer and J. P. Weinmann, *J. Dent. Res.*, **32**, 669 (1953); (c) J. Meyer and J. P. Weinmann, *J. Histochem. and Cytochem.*, **1**, 305–314 (1953); (d) **5**, 393 (1957).

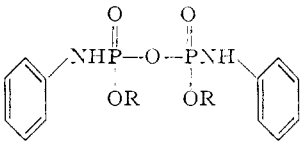
(3) (a) J. D. Chanley and E. Feageson, Abst. of Paper, 130th ACS Meeting, Sept., 1956, 49-O; (b) H. Holter and Si Oh Li, *Compt. Rend. Trav. Lab. Carlsberg*, Ser. Chim., **27**, 393 (1951); (c) K. M. Moller, *Biochem. et Biophys. Acta*, **16**, 162 (1955).

(4) Active substrates have in common structures with at least one ionizable phosphoric acid group, ref. 2a.

(5) V. M. Clark and A. R. Todd, *J. Chem. Soc.*, 2034 (1950); 1499 (1957).

Found: C, 54.85; H, 7.90; N, 10.35) and as the dipotassium salt (*Anal.* Calcd. for $C_{12}H_{12}O_5P_2K_2$: C, 35.64; H, 2.99; N, 6.93. Found: C, 35.40; H, 3.06; N, 7.14) in yields of about 60%.

This substrate II underwent no spontaneous hydrolysis at *p*H 4.0 to *p*H 11.5 after two hours at 37°. It slowly liberated aniline at *p*H 3.5 and did so more rapidly at *p*H 2.5. Aniline was measured colorimetrically.[6] The evidence, moreover, clearly indicates that no hydrolysis of the pyrophosphate, P–O–P, bond had occurred under any of these conditions and that liberation of aniline in acid resulted solely from phosphamide, P–N, bond cleavage. If hydrolysis of the pyrophosphate bond above *p*H 4.0 had occurred phenylphosphoroamidic acid (III) would be formed. This product III[7] is essentially completely hydrolyzed after 30 minutes at *p*H 4.0, conditions at which the substrate II



I, R = $C_6H_5CH_2$
II, R = H

gives no hydrolysis. Thus III can be assayed for. Cleavage of the pyrophosphate bond in II below *p*H 4.0 is measurable simply by assay for inorganic orthophosphate. These assays showed that insignificant amounts of either phenylphosphoroamidic acid III or inorganic phosphate were produced above or below *p*H 4.0, respectively.

As regards stability to spontaneous hydrolysis this substrate II is, therefore, eminently satisfactory for enzymatic work. Extracts of intestine with high alkaline phosphatase activity[8] active against various phosphamides[9] were significantly active against this substrate II.

Preliminary results, however, suggest that the active enzyme is not alkaline phosphatase since maximum hydrolysis occurred at a new *p*H between 7.6–8.0, and of the two intestinal extracts studied the partially purified one was significantly more active against β-glycerol phosphate and β-napthyl phosphate than it was against the substrate II. The relationship of this "pyrophosphamidase" to phosphamidase and its distribution in tissues are under study that will be reported later.

Acknowledgments.—This work was supported by a research grant (No. C-2130) from the National Institutes of Health, U.S. Public Health Service. The authors also gratefully acknowledge assistance from Mrs. Phyllis R. Kaplan and Mr. Harold Sommer.

DEPARTMENT OF CHEMISTRY
BRANDEIS UNIVERSITY
WALTHAM 54, MASSACHUSETTS
ELIAHU BOGER
ORRIE M. FRIEDMAN

RECEIVED JANUARY 22, 1958

(6) F. Michael and J. Schierholt, *Ber.*, **85**, 1089 (1952).
(7) Prepared by a procedure kindly provided by Dr. J. D. Chanley.
(8) Kindly supplied by Dr. Gerhardt Schmidt.
(9) G. Schmidt and S. J. Thannhauser, *J. Biol. Chem.*, **149**, 369 (1943); G. E. Perlmann, in "A Symposium on Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. 2, Johns Hopkins Press, Baltimore, Md., 1952, p. 167; T. Winnick, *Arch. Biochem.*, **12**, 209 (1947).

METHYLATION OF ALCOHOLS WITH DIAZOMETHANE

Sir:

Methylation of alcohols by the Williamson synthesis requires strongly basic conditions and there is no satisfactory general methylation procedure effective under neutral or mildly acidic conditions. In principle, diazomethane with an acidic catalyst should methylate alcohols, but the usual protonic acid, such as hydrochloric acid, is unsatisfactory because it is itself methylated with diazomethane. Fluoroboric acid, however, promised to serve as a useful catalyst since it would be consumed in reaction with diazomethane only by some process involving rupture of a B–F bond.[1] This expectation was indeed realized and a novel method has been developed for methylation of alcohols in high yields under mild conditions.

Methylations of alcohols were carried out in diethyl ether or methylene chloride at 0–25° in the presence of 0.6–8 mole per cent. of fluoroboric acid. Methyl ethers of simple primary or unhindered secondary alcohols thus were formed rapidly in 84–98% yields. Moderately hindered secondary alcohols and tertiary alcohols reacted more slowly, yields were lower and methylation was accompanied by some polymethylene formation, minimized at lower temperatures. Typical cases were *n*-octanol (87%), cyclohexanol (92%), cholesterol (95%), α-cholestanol (98%), β-cholestanol (98%), dimethylphenylcarbinol (30%) and *t*-amyl alcohol (66%).

Competition experiments showed the ratios of the rates of primary, secondary and tertiary butyl alcohols = 2.2:1.3:1.0 and β (equatorial) and α (axial) cholestanol = 1.3:1. Clearly, these acid-catalyzed methylations lack high steric selectivity. Triphenylcarbinol and isoborneol could not be methylated by this method.

The new reagent provides a unique tool for the methylation of certain alcohols containing other sensitive groups. For example, testosterone and desoxycorticosterone have been converted directly to their methyl ethers—a difficult, if not impossible, transformation by any previously available direct methods. Testosterone methyl ether, which does not appear to have been described before, melts at 127–127.5°, $[\alpha]_D^{CHCl_3}$ +106.3° (found: C, 79.21; H, 9.71; OCH_3, 10.5). Ascorbic acid gave a hitherto unknown trimethyl ether, m.p. 99.5–101°, $(\alpha)^{25}D$ + 33° (H_2O); C, 49.48; H, 6.49; OCH_3, 42.3; which we consider to be 2,3,6-trimethylascorbic acid on the basis of its oxidation with weakly alkaline periodate, and by its infrared and ultraviolet spectra and the changes in the latter in the presence of dilute alkali.

Fluoroboric acid also has been shown to catalyze the otherwise sluggish reaction of weakly acidic phenols with diazomethane. Estradiol was thus converted to the dimethyl ether in 81% yield under conditions which, although forcing, gave no reaction at all in the absence of the catalyst. Other weakly acidic phenols of pK_a 9.36 to 10.17 gave poor to fair yields of methyl ethers.

Formation of α-alkoxyketones from diazoketones

(1) Cf. the reaction of ethyl diazoacetate and ethanol with various mineral acids, particularly fluoroboric acid, as catalysts, J. D. Roberts, C. M. Regan and I. Allen, THIS JOURNAL, **74**, 3679 (1952).