It appears remarkable that in a free radical mechanism for thioglycolate oxidation the reaction stops at the disulfide. This might, however, be explained by assuming that in reaction (7) all $\cdot O_2^-$ and $\cdot OH$ radicals are trapped before they can react with the ferric complex. In accordance with this it was found that the addition of 0.1 *M* sodium benzoate[23] did not affect the initial rate of oxygen uptake.

Acknowledgment.—The authors wish to express their very sincere thanks to Professor K. Linderstrøm-Lang for valuable advice and encouragement.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF VIRGINIA]

Some Thionophosphate and Phosphoroamidate Derivatives of Adenosine and Certain Steroids[1]

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RECEIVED DECEMBER 13, 1956

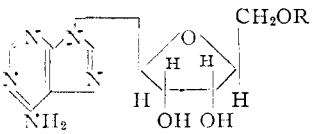
Syntheses of *O,O*-diethyl thionophosphate esters of adenosine, methyl 2,3-isopropylideneribofuranoside, cholesterol, ergosterol and estrone and of two phosphoroamidate derivatives of adenosine are described. These compounds have been prepared as potential antagonists to the corresponding phosphorylated metabolites. Potassium *t*-butoxide smoothly effects the condensation of these complex hydroxy derivatives with diethyl thionophosphorochloridate.

A considerable number of biochemically important substances are phosphorylated prior to being metabolized. The phosphate group in many of the resulting esters seems to bind the substrates to enzyme systems since it frequently remains unchanged in subsequent metabolic conversions. Structural alterations of such phosphate groups may be expected to produce potential inhibitors of enzymes associated with synthetic and catabolic pathways of the corresponding normal metabolites. In previous articles,[2-4] phosphonate derivatives of carbohydrates and of analogs of nucleotides have been described. This paper deals with the synthesis of thionophosphate and phosphoroamidate derivatives.

Thionophosphate esters, $(RO)_3PS$, are known to differ in several respects from the corresponding phosphates. For example, they are much less susceptible to nucleophilic substitution and undergo transformations to thiolophosphates which approximate the isomerization of phosphites to phosphonates.[5] Such differences should affect the biological reactions of thionophosphates even though some of them can be oxidized to the *O*-phosphate esters.[6,7] Tertiary phosphoroamidates could be toxic *per se* and are known to be oxidized enzymatically to toxic *N*-oxides in some instances.[8,9] These products inhibit enzyme systems by phosphorylations similar to those postulated for the attack of organic phosphates on cholinesterases.[10]

The *O,O*-diethyl thionophosphorylation of the alcoholic and phenolic hydroxyl groups of the metabolites used was carried out with diethyl thionophosphorochloridate. According to previous reports, phenolic compounds react more rapidly with this substance in aqueous alkaline solution than in non-polar solvents.[11] A model experiment with a complex primary alcohol, 5-(7-theophyllinyl)-pentanol-1,[2] demonstrated that thionophosphorylation could not be achieved in the presence of such a weak base as pyridine or of sodium carbonate and copper powder in anhydrous benzene. It was concluded that a strong base was required to metalate the hydroxyl groups in order to facilitate the reaction with diethyl thionophosphorochloridate. Potassium *t*-butoxide was chosen because it is soluble in ionizing solvents such as *t*-butyl alcohol and too hindered sterically to react rapidly with the thionophosphorylating agent. The reactions went well under these conditions as described in the Experimental part. The six *O,O*-diethyl thionophosphates obtained are listed in Table I.

By the same general method using potassium *t*-butoxide, 2',3'-isopropylideneadenosine[12,13] was treated with ethyl phosphorodiethylamidochloridate, and the isopropylidene group of the resulting ester was hydrolyzed with dilute sulfuric acid, yielding *O*-ethyl *O*-(5'-adenosyl phosphorodiethylamidate (II). The corresponding *O*-phenyl *O*-(2',3'-isopropylidene-5'-adenosyl phosphorodieth-



I, R = $PS(OC_2H_5)_2$
II, R = $PO(OC_2H_5)N(C_2H_5)_2$

(1) This work was supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

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TABLE I

O,O-DIETHYL THIONOPHOSPHATE DERIVATIVES

ROPS(OC$_2$H$_5$)$_2$ R	Solvent of crystn.	Yield, %	M.p. or b.p., °C. (mm.)	Optical constants	Composition	Analyses, % Calcd. C	Calcd. H	Found C	Found H
Methyl 2,3-isopropylidene-5-D-ribofuranosidyl		66	135 (0.07[a])	n^{30}D 1.466 $[\alpha]^{30}$D −46.5° (*c* 3.81, acetone)	$C_{13}H_{25}O_7PS$	43.81	7.07	43.89	6.96
2′,3′-Isopropylidene-5′-adenosyl picrate	95% EtOH		120–130[b] 175–176		$C_{23}H_{29}N_8O_{13}PS$	40.12	4.25	40.29	4.45
5′-Adenosyl (I)	EtOH	37	178–180	$[\alpha]^{23}$D −15.1° (*c* 2.15, 5% HCl)	$C_{14}H_{22}N_5O_6PS$	40.09	5.29	40.40	5.57
3-Cholesteryl	95% EtOH	66	110–111[c]	$[\alpha]^{23}$D −31.2° (*c* 2.00, $CHCl_3$)	$C_{31}H_{55}O_3PS$	60.10	10.28	68.82	10.65
3-Ergosteryl	EtOH–C_6H_6	58	124–125	$[\alpha]^{23}$D −50.0° (*c* 3.30, $CHCl_3$)	$C_{32}H_{53}O_3PS$	70.03	9.73	70.21	9.80
3-Estronyl[d,e]	Petr. ether, EtOH	46	78–79	$[\alpha]^{23}$D +86.0° (*c* 4.33, $CHCl_3$)	$C_{22}H_{31}O_4PS$	62.53	7.40	62.49	7.61

[a] Yellow oil. [b] Hygroscopic. [c] Colorless plates. [d] The reaction could not be performed in aqueous sodium hydroxide solution. [e] We are grateful to Schering Corporation for supplying the estrone in this study.

ylamidate was obtained in a similar manner, but attempts to hydrogenolyze the phenyl ester group of this compound were unsuccessful.

The compounds listed in Table I are being evaluated for tumor-inhibitory properties by the Cancer Chemotherapy National Service Center.

Experimental[14]

Materials.—Pure, dry *t*-butyl alcohol was obtained by refluxing the Eastman best grade material over calcium hydride and distilling through a 60-cm. packed column. Potassium was dissolved in this liquid under an atmosphere of nitrogen to produce a 3.8% solution of potassium *t*-butoxide.

***O*-Phenyl Phosphorodiethylamidochloridate.**—To a stirred, refluxing solution of 38.0 g. (0.2 mole) of diethyl phosphoramidic dichloride[15] and 18.8 g. (0.2 mole) of phenol in 300 ml. of dry benzene, a solution of 20.2 g. (0.2 mole) of dry triethylamine in 100 ml. of dry benzene was added dropwise over a period of 45 minutes. The mixture was refluxed for an additional 3 hr., cooled, filtered from triethylammonium chloride and the filtrate was concentrated *in vacuo*. The residue was treated with 100 ml. of dry ether, filtered and the solution was fractionated. The yield of colorless oil, b.p. 118° (0.4 mm.), n^{25}D 1.507, was 22.5 g. (45%).

Anal. Calcd. for $C_{10}H_{15}ClNO_2P$: C, 48.49; H, 6.10. Found: C, 48.71; H, 6.34.

General Directions for *O,O*-Diethyl Thionophosphorylations.—The compound to be thionophosphorylated (1 mole equivalent) was added to a stirred 3.8% solution of potassium (1–10 mole equivalents) in dry *t*-butyl alcohol under a dry nitrogen atmosphere, and additional *t*-butyl alcohol was added to produce a clear solution at 25°. A 30–40% *t*-butanolic solution of diethyl thionophosphorochloridate equivalent to the quantity of potassium employed was added dropwise to the mixture at 25°. Stirring under reflux was extended for 1 to 3 hr. and the solvent removed under reduced pressure. The residue was treated with methanol or ethanol to dissolve the product. The filtered solution was concentrated *in vacuo* and the residue recrystallized or distilled. In the case of 2′,3′-isopropylideneadenosine,[12,13] the exactly equivalent amount of potassium was used and the mixture not refluxed but allowed to stand for 30 minutes. It was adjusted to *p*H 7 with 5% hydrochloric acid, the solvent was removed *in vacuo* and the residue extracted with methanol as above. The solid residue from the evaporation of the methanol extract was triturated with dry ether.

***O,O*-Diethyl *O*-(5′-Adenosyl)-thionophosphate.**—The hygroscopic crude sirupy *O,O*-diethyl *O*-(2′,3′-isopropylidene-5′-adenosyl)-thionophosphate was dissolved in 300 ml. of 0.1*N* sulfuric acid and allowed to stand at 27° for 2 days. The solution was neutralized to *p*H 7 with barium hydroxide solution, evaporated under reduced pressure, and the powdered residue was extracted continually with methanol. The extract was concentrated *in vacuo* at 27° to incipient crystallization, the solution was heated to boiling and petroleum ether was added to induce crystallization.

***O*-Phenyl *O*-(2′,3′-Isopropylidene-5′-adenosyl) Phosphorodiethylamidate.**—When 6.15 g. (0.02 mole) of 2′,3′-isopropylideneadenosine, 0.02 mole of potassium *t*-butoxide and 4.95 g. (0.02 mole) of *O*-phenyl phosphorodiethylamidochloridate were allowed to react as described in the general directions and the mixture was worked up, an oily product was obtained which did not crystallize. It was converted to its yellow picrate in ethanolic solution, m.p. 141–143°, after recrystallization from ethanol.

Anal. Calcd. for $C_{29}H_{34}N_9O_{13}P\cdot H_2O$: C, 45.48; H, 4.47. Found: C, 45.45; H, 4.57.

O-Ethyl *O*-(2′,3′-isopropylidene-5′-adenosyl) phosphorodiethylamidate was prepared in the same manner as the *O*-phenyl analog above using ethyl phosphorodiethylamidochloridate.[15] The colorless glassy product was converted to its yellow picrate in ethanol solution. The salt softened at 160° and melted at 169–170°.

Anal. Calcd. for $C_{25}H_{34}N_9O_{13}P\cdot{}^1/_2H_2O$: C, 42.37; H, 4.98. Found: C, 42.05; H, 4.70.

In an experiment using triethylamine in dioxane solution, no reaction was observed.

***O*-Ethyl *O*-(5′-Adenosyl) Phosphorodiethylamidate.**—Hydrolysis of the crude isopropylidene derivative with dilute sulfuric acid as described for the preparation of I furnished a colorless glassy material which was converted to its yellow picrate in boiling water solution. On slow cooling, 13.5 g. (51%) of yellow needles crystallized which, after recrystallization from hot water, melted at 138–140° dec. after sintering at 125°.

Anal. Calcd. for $C_{22}H_{30}N_9O_{13}P$: C, 40.06; H, 4.59. Found: C, 40.01; H, 4.96.

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(14) Melting points are corrected and boiling points are uncorrected. Microanalyses by Miss B. J. Williamson, Mrs. A. Faimann and Mrs. J. Jensen.

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