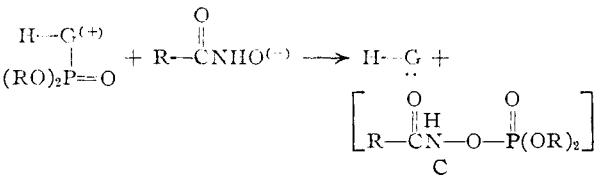


where H-G represents the esteratic site of the enzyme H being an acidic group and the electron pair (..) representing a basic group, X represents an halogen, dialkylphosphate or *p*-nitrophenyl moiety, etc. In the case of TEPP, R = ethyl and X = diethylphosphate, and in the case of DFP, R = isopropyl and X = fluorine. The resulting inhibited enzymes differ, being in the case of TEPP diethylphosphoryl enzyme and diisopropylphosphoryl enzyme in the case of DFP.

The postulated product C has not been described, but diacetylhydroxylamine, an acyl analog, has been prepared. It is easily hydrolyzed in alkali.[14] If C should be unstable in water and have only a transitory existence, these hydroxamic acids might then be described as catalysts for the hydrolysis of the dialkylphosphoryl enzymes.

The failure to obtain complete reactivation was not investigated since it appeared probable that secondary factors which are not of immediate interest were responsible.

Acknowledgments.—The author wishes to thank Dr. Sara Ginsburg for preparing the hydroxamic acids and to acknowledge the assistance of Miss Carole Quan. He also is pleased to acknowledge indebtedness to Dr. David Nachmansohn for fruitful discussions and for his continuing interest.

(14) R. W. J. Taylor and B. Wilson, "Sidgwick's Organic Chemistry," Clarendon Press, Oxford, 1949.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA]

Analogs of Nucleotides. I. Theophyllinylalkylphosphonic Acids and Related Compounds[1]

BY JEKISHAN R. PARIKH AND ALFRED BURGER

RECEIVED DECEMBER 2, 1954

ω-(7-Theophyllinyl)-alkylphosphonic acids (I, n = 3, 4, 5) have been prepared in order to explore synthetic routes to purine glycosidylphosphonic acids. In addition to these compounds, several ω-(7-theophyllinyl)-alkanols and the corresponding alkylthiols have been synthesized.

One of the most distinguishing properties of the tumor cell is its ability to glycolyze rapidly under aerobic or anaerobic conditions. Simple nucleotides such as ATP play an important role in this reaction. The same nucleotides also have been recognized as participating in the synthesis of nucleic acids and this in turn is associated with the multiplication of the neoplastic cell. Numerous attempts have been made to interfere with these important biochemical sequences of the tumor cell by altering the structures of the naturally occurring heterocyclic or carbohydrate moieties or both,[2] with the hope that they may act as antimetabolites or form fraudulent nucleotides.[3]

With this in mind, it was decided to prepare for biological screening against neoplastic growth, compounds in which the normal phosphoric acid ester linkage [ROP(O)(OH)$_2$] would be replaced by an analogous phosphonic acid group [RP(O)(OH)$_2$]. Such compounds might be expected to interfere with some biochemical reactions of the tumor cell involving phosphate ester type nucleotides. This report deals with (7-theophyllinyl)-alkylphosphonic acids (I), and analogous compounds which were needed as models for synthetic routes to purine glycosidylphosphonic acids on which we shall report later. Theophylline was chosen as a purine component because of its ready availability, and the unbranched alkyl chain afforded an opportunity to study the introduction of functional groups in the simplest possible manner.

Two routes to (7-theophyllinyl)-alkylphosphonic acids were tried. The first one led from dialkyl ω-halogenoalkylphosphonates and silver theophylline to the desired phosphonate esters. The second method, shown in Scheme 1 (Th = 7-theophyllinyl), consisted of the conversion of ω-(7-theophyllinyl)-alkyl iodides to the corresponding phosphonate esters by the Michaelis–Arbuzov reaction.[4] The latter procedure proved to be more fruitful. The esters were hydrolyzed to the free phosphonic acids I; the derivatives with n = 3, 4 and 5 have been prepared.

$$\text{Th-Ag} + \text{I(CH}_2)_n\text{Cl} \longrightarrow \text{Th(CH}_2)_n\text{Cl} \longrightarrow \text{Th(CH}_2)_n\text{I} \longrightarrow \text{Th(CH}_2)_n\text{P(O)(OC}_2\text{H}_5)_2 \longrightarrow \underset{\text{I}}{\text{Th(CH}_2)_n\text{P(O)(OH)}_2}$$

Scheme 1

A threefold excess of the chloroalkyl iodides was necessary to obtain optimum yields of the ω-(7-theophyllinyl)-alkyl chlorides and suppress the simultaneous formation of ω-bis-(7-theophyllinyl)-alkanes.

Since 4-(7-theophyllinyl)-butyl iodide and its next higher alkyl homolog had become readily available, these compounds also were converted to the corresponding ω-(7-theophyllinyl)-alkanols and alkylthiols. The alkanols were obtained by

(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.

(2) G. B. Brown, *Texas Rep. Biol. Med.*, **10**, 961 (1952).

(3) H. E. Skipper, *Cancer Research*, **13**, 545 (1953).

(4) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 121.

way of the acetates, and the thiols through the corresponding isothiouronium salts. The two alkanol derivatives have been tested for diuretic activity by Prof. C. L. Gemmill of the Department of Pharmacology, University of Virginia, because of the reported diuretic action of hydroxyethyl- and dihydroxypropyltheophylline,[5] but proved inactive in rats on oral administration.

Compounds III, V, VI, VII and VIII (see Table I), injected intraperitoneally into mice, have been tested by Dr. C. Chester Stock of the Sloan–Kettering Institute for inhibition of the development of Sarcoma 180 but have been found inactive.

Experimental[6]

3-Iodopropylphosphonic Acid.—A solution of 10.6 g. (0.052 mole) of 3-bromopropylphosphonic acid[7] and 15 g. (0.1 mole) of sodium iodide in anhydrous acetone was refluxed for 24 hours. Acetone was removed and the residue was treated with methanol and with acetone. The yield was 6.3 g. (48%). Recrystallization from dilute acetone gave shiny plates which melted at 182–183°.

Anal. Calcd. for $C_3H_8IO_3P$: C, 14.41; H, 3.22. Found: C, 14.02; H, 3.03.

3-(7-Theophyllinyl)-propylphosphonic Acid Dihydrate (II).—Dimethyl 3-iodopropylphosphonate was prepared from 3-iodopropylphosphonic acid and excess diazomethane in ether solution. The ether solution was washed with 5% sodium carbonate solution and with water, dried over anhydrous sodium sulfate and the solvent was removed. The residual oil (2.78 g., 0.01 mole) was added to a suspension of 2.87 g. (0.01 mole) of silver theophylline[8] in 100 ml. of N,N-dimethylformamide and the mixture was heated and stirred at 140° for 6 hours. It was filtered, the dimethylformamide was removed under reduced pressure and the residue was taken up in 20 ml. of ethanol. The solution deposited 0.6 g. of theophylline. The alcoholic filtrate was evaporated under vacuum, the semi-oily residue was dissolved in acetonitrile, filtered and the solution was evaporated on a steam-bath. The crude oily phosphonate ester was hydrolyzed with 10 ml. of hot concentrated hydrochloric acid for 3 hours, the solution was filtered and evaporated under vacuum. The dark oil was washed with acetone and crystallized from absolute ethanol–acetone and finally from absolute ethanol.

1-Chloro-4-(7-theophyllinyl)-butane (III).—To a suspension of 27.55 g. (0.096 mole) of carefully dried silver theophylline in 500 ml. of anhydrous sulfur-free xylene was added 61.0 g. (0.28 mole) of 1-chloro-4-iodobutane, and the mixture was refluxed and stirred for 24 hours. It was filtered while hot and allowed to cool. Colorless crystals (2.5 g.) separated out. The crystalline material was washed with dilute ammonium hydroxide and water in order to remove some theophylline, and recrystallized from cyclohexanone. It melted at 294–295° and was identical with 1,4-bis-(7-theophyllinyl)-butane (VII).

The xylene filtrate from this compound was evaporated under vacuum and the residual 1-chloro-4-(7-theophyllinyl)-butane was recrystallized.

1-Iodo-4-(7-theophyllinyl)-butane (IV).—A solution of 13.5 g. (0.05 mole) of 1-chloro-4-(7-theophyllinyl)-butane and 15.0 g. (0.1 mole) of sodium iodide in 250 ml. of dry acetone was refluxed for 24 hours in the presence of 1 g. of cuprous iodide. The reaction mixture was filtered from inorganic salts, evaporated, and the residue dissolved in warm toluene. This solution was washed with 5% sodium thiosulfate solution and water, dried and the solvent was removed under reduced pressure. The oily residue crystallized slowly.

Diethyl 4-(7-Theophyllinyl)-butylphosphonate (V).—A solution of 12.67 g. (0.035 mole) of 1-iodo-4-(7-theophyllinyl)-butane in 16.6 g. (0.1 mole) of triethyl phosphite was heated in an oil-bath at 160–170° for 5 hours. On standing overnight at room temperature the crystalline ester precipitated. An additional quantity was obtained by diluting the mother liquor with petroleum ether. Recrystallization from isooctane furnished fine small colorless needles, soluble in water, ethanol and acetone, and sparingly soluble in ether, dioxane and benzene.

4-(7-Theophyllinyl)-butylphosphonic Acid (VI).—A solution of 1 g. (0.0026 mole) of diethyl 4-(7-theophyllinyl)-butylphosphonate in 10 ml. of concentrated hydrochloric acid was refluxed for 3 hours and evaporated under reduced pressure. The resinous residue was washed with acetone and ether and recrystallized from ethanol–acetone.

1,4-Bis-(7-theophyllinyl)-butane (VII).—To a solution of 18.0 g. (0.1 mole) of anhydrous theophylline in 300 ml. of dry butanol containing 2.3 g. (0.1 mole) of sodium was added 21.85 g. (0.1 mole) of 1-chloro-4-iodobutane and the mixture was refluxed for 19 hours. On cooling it deposited colorless crystals which were washed with petroleum ether, dilute ammonium hydroxide and water.

1-Iodo-5-(7-theophyllinyl)-pentane (IX).—1-Chloro-5-(7-theophyllinyl)-pentane (VIII) was prepared in 61% yield from silver theophylline and pentamethylene chloroiodide as described above for the lower homolog, but using dimethylformamide as a reaction medium. The compound appeared as long, shiny, colorless needles.

The conversion to the iodo compound IX was carried out as described above for the butane derivative.

A small amount (0.18 g.) of **1,5-bis-(7-theophyllinyl)-pentane (XI)** separated from the benzene solution of 1-chloro-5-(7-theophyllinyl)-pentane while this was washed with water. It is described in Table I.

5-(7-Theophyllinyl)-amylphosphonic Acid (X).—A solution of 21 g. (0.056 mole) of 1-iodo-5-(7-theophyllinyl)-pentane in 28 g. (0.17 mole) of triethyl phosphite was heated at 160–170° for 6 hours. The excess triethyl phosphite was distilled off under reduced pressure, the oily residue was taken up in benzene and the phosphonate ester was extracted with water. The aqueous solution was evaporated under vacuum; the oily residue (13.3 g., 61%) did not solidify. It was refluxed with 100 ml. of concentrated hydrochloric acid for 3 hours, the solvent was removed at the aspirator and the oily residue washed with dioxane, ether and acetone. It crystallized from a 1:5 ethanol–acetone mixture.

ω-(7-Theophyllinyl)-alkyl Acetates.—4-(7-Theophyllinyl)-butyl acetate (XII) and 5-(7-theophyllinyl)-amyl acetate (XIII) were prepared in an analogous manner. A mixture of 8 millimoles of the respective ω-(7-theophyllinyl)-alkyl iodide and 0.01 mole of silver acetate in 30 ml. of glacial acetic acid was shaken at 25° for 24 hours. Silver iodide was filtered, the acetic acid was evaporated under reduced pressure, the oily residue was taken up in 5 ml. of water and the aqueous solution made ammoniacal. The precipitated oils solidified soon.

ω-(7-Theophyllinyl)-alkanols (XIV), (XV).—An ethanolic solution of 0.07 mole of the ω-(7-theophyllinyl)-alkyl acetates just described, containing a slight excess of potassium hydroxide was refluxed for 2 hours, the alcohol was removed under vacuum and the residue was dissolved in 100 ml. of water. The aqueous solution was extracted repeatedly with chloroform, the combined extracts were dried and the solvent removed. The oily residue crystallized on standing.

ω-(7-Theophyllinyl)-alkylisothiouronium Salts (XVI), (XVII).—These compounds were prepared from the corresponding ω-(7-theophyllinyl)-alkyl iodides and thiourea by the general directions of Johnson and Sprague.[9] The reflux time was 4 hours.

ω-(7-Theophyllinyl)-alkanethiols (XVIII), (XIX).—These compounds were prepared by decomposition of the corresponding isothiouronium salts with ammonium hydroxide according to the procedure of Phillips and Shapiro.[10] The reaction time was one day.

Pentamethylene-1,5-bis-isothiouronium Picrate.—This compound was prepared from pentamethylene dibromide by the customary procedure.[11] It crystallized from ethanol, m.p. 239.5–240.5°.

(5) H. Hensel, *München. Med. Wochschr.*, **96**, 381 (1954).

(6) All melting points are corrected. Microanalyses by Miss P. L. Paynter. The yields, physical constants, solvents of recrystallization and analytical data of all compounds containing theophyllinyl groups are listed in Table I.

(7) Reference 4, p. 287.

(8) A. Kossel, *Ber.*, **21**, 2166 (1888).

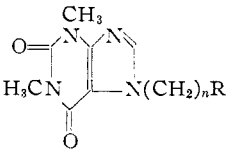
(9) T. B. Johnson and J. M. Sprague, THIS JOURNAL, **58**, 1348 (1936); J. M. Sprague and T. B. Johnson, *ibid.*, **59**, 1837 (1937).

(10) M. A. Phillips and H. Shapiro, *J. Chem. Soc.*, 584 (1942).

(11) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 193.

TABLE I

ω-SUBSTITUTED ω'-(7-THEOPHYLLINYL)-ALKANES



No.	R	n	Yield, %[a]	M.p., °C. (cor.)[b]	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
II	$PO(OH)_2 \cdot 2H_2O$	3		187–188[c]	$C_{10}H_{15}N_4O_5P \cdot 2H_2O$	35.50	35.75	5.66	5.24
III	Cl	4	77	92–92.5[d]	$C_{11}H_{15}ClN_4O_2$	48.79	48.72	5.58	5.57
IV	I	4	90	105–105.5[e]	$C_{11}H_{15}IN_4O_2$	36.47	36.99	4.17	4.10
V	$PO(OC_2H_5)_2$	4	88	118.5–119.5[f]	$C_{15}H_{25}N_4O_5P$	48.38	48.00	6.76	6.67
VI	$PO(OH)_2$	4	75	210–211[g]	$C_{11}H_{17}N_4O_5P$	41.77	42.04	5.41	5.37
VII	7-Theophyllinyl	4	52	294–295[h]	$C_{18}H_{22}N_8O_4$	52.16	52.27	5.35	5.42
VIII	Cl	5	61	79.5–80[d]	$C_{12}H_{17}ClN_4O_2$	50.61	49.69	6.01	5.77
IX	I	5	77	81.5–82[i]	$C_{12}H_{17}IN_4O_2$	38.30	38.05	4.55	4.82
X	$PO(OH)_2$	5	51	222.5–224.5[c]	$C_{12}H_{19}N_4O_5P$	43.63	43.91	5.79	5.93
XI	7-Theophyllinyl	5		232.5–233[g]	$C_{19}H_{24}N_8O_4$	53.25	53.31	5.64	5.66
XII	$OCOCH_3$	4	91	87.5–88.5[j]	$C_{13}H_{18}N_4O_4$	53.05	53.34	6.16	6.26
XIII	$OCOCH_3$	5	87	66–67[i]	$C_{14}H_{20}N_4O_4$	54.53	54.67	6.53	6.50
XIV	OH	4	85	117–118[i]	$C_{11}H_{16}N_4O_3$	52.36	52.49	6.39	6.08
XV	OH	5	94	121–122[i]	$C_{12}H_{18}N_4O_3$	54.12	54.01	6.81	6.90
XVI	$SC(=NH)NH_2 \cdot HI$	4	81	218–219[g]	$C_{12}H_{19}IN_6O_2S$	32.88	33.20	4.36	4.58
XVII	$SC(=NH)NH_2 \cdot HI$	5	98	216–217[k]	$C_{13}H_{21}IN_6O_2S$	34.51	34.64	4.67	5.03
XVIII	SH	4	97	167–168[l]	$C_{11}H_{16}N_4O_2S$	49.23	49.54	6.01	5.48
							49.66		5.89
XIX	SH	5	96	129.5–130.5[l]	$C_{12}H_{18}N_4O_2S$	51.05	51.32	6.42	6.17

[a] Yield before recrystallization. [b] Superscripts in this column refer to solvents of crystallization. [c] Absolute ethanol–acetone and absolute ethanol. [d] Ligroin. [e] Benzene–petroleum ether and 95% ethanol. [f] Isoöctane. [g] 95% ethanol. [h] Cyclohexanone. [i] Benzene. [j] Benzene–isoöctane. [k] Absolute ethanol. [l] 50% ethanol.

Anal. Calcd. for $C_{19}H_{22}N_{10}O_{14}S_2$: C, 33.62; H, 3.26. Found: C, 34.01; H, 3.32.

5-Iodopentanol.—A solution of 25.0 g. (0.2 mole) of 5-chloropentanol[12] and 60.0 g. (0.4 mole) of sodium iodide in 500 ml. of anhydrous acetone was refluxed in the presence of 1 g. of cuprous iodide for 24 hours and worked up as usual. The oily product weighed 34.5 g.

A sample was converted to the **phenylurethan** derivative, fine colorless needles from petroleum ether, m.p. 61.5–62.5°.

Anal. Calcd. for $C_{12}H_{16}INO_2$: C, 43.25; H, 4.84. Found: C, 43.37; H, 4.78.

The 1-naphthylurethan derivative crystallized from petroleum ether, m.p. 84.5–85°.

Anal. Calcd. for $C_{16}H_{18}INO_2$: C, 50.14; H, 4.73. Found: C, 50.10; H, 4.73.

Reaction of Silver Theophylline with 5-Iodopentanol.—When a mixture of 5.74 g. (0.02 mole) of silver theophylline and 4.7 g. (0.022 mole) of crude 5-iodopentanol was heated in 200 ml. of dimethylformamide at 130–135° for 4 hours and worked up as described for analogous cases above, 1 g. of fine long colorless needles melting at 232–233° was obtained. A mixture melting point of this material with 1,5-bis-(7-theophyllinyl)-pentane (XI) showed no depression. Chloroform extractions of the concentrated aqueous mother liquors, treatment of the residue with methanol, evaporation of the filtered methanol solution and crystallization from benzene yielded 1.3 g. of colorless material, m.p. 120–121°. A mixture melting point with a sample of 5-(7-theophyllinyl)-pentanol (XV) showed no depression.

1,5-Bis-(9-adenyl)-pentane.—To a thin paste of 2 g. (8.2 millimoles) of finely ground silver adenine in 100 ml. of anhydrous xylene was added 1.9 g. (8.2 millimoles) of 1-chloro-5-iodopentane with vigorous stirring. The mixture was refluxed and stirred for 24 hours, filtered and the filtrate was evaporated under reduced pressure. The resulting oily residue (1.5 g.) did not crystallize. **The picrate** crystallized from 95% ethanol, m.p. 189.5–190.5°.

Anal. Calcd. for $C_{21}H_{21}N_{13}O_7$: C, 44.44; H, 3.73. Found: C, 44.73; H, 3.76.

CHARLOTTESVILLE, VA.

(12) Purchased from Columbia Organic Chemicals Co., Inc., Columbia, S. C.