Summary

4,4′-Dinitrohydrobenzoin cyclic phosphate was synthesized. Alcoholysis reaction of this compound with various hydroxylic compounds in acid or alkaline medium was found to occur to a greater extent than a similar reaction for non-substituted hydrobenzoin cyclic phosphates. Thus, the new cyclic phosphate was alcoholyzed with polyols such as DL-erythritol and D-mannitol, which were found to be inert to the non-substituted hydrobenzoin cyclic phosphate. The mode of the hydrolysis reaction of methyl 4,4′-dinitrohydrobenzoin phosphate, an intermediate product of methanolysis of 4,4′-dinitrohydrobenzoin cyclic phosphate, in acid and alkaline media was found to be different from that of methyl hydrobenzoin phosphate.

(Received October 21, 1960)

UDC 547.677.5'118.5.07

98. Tyunosin Ukita*1 and Ryuzo Takeshita*2 : Organic Phosphates. XVI.*3 Synthesis and Alcoholysis Reaction of 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate.*4

(*Faculty of Pharmaceutical Sciences, University of Tokyo**1)

The usefulness of hydrobenzoin cyclic phosphate as a phosphorylating agent for hydroxylic compounds has been reported by one of the present authors in a previous paper[1] of this series and in a further study,*3 the enhanced reactivity of a substituted hydrobenzoin cyclic phosphate, 4,4′-dinitrohydrobenzoin cyclic phosphate, in the similar type of alcoholysis reaction and some different mode in hydrolysis reaction of its alcoholysis product, alkyl 4,4′-dinitrohydrobenzoin phosphate, from that of alkyl hydrobenzoin phosphate were also reported.

As a structurally further modified cyclic phosphate, 9,10-dihydro-9,10-phenanthrenediol cyclic phosphate, was synthesized and its behavior in the alcoholysis reaction was investigated, the results of which are described in this paper.

9,10-Dihydro-9,10-phenanthrenediol (trans type) (II), obtained by the reduction of phenanthraquinone (I) with lithium aluminum hydride,[2] was reacted with phosphoryl chloride

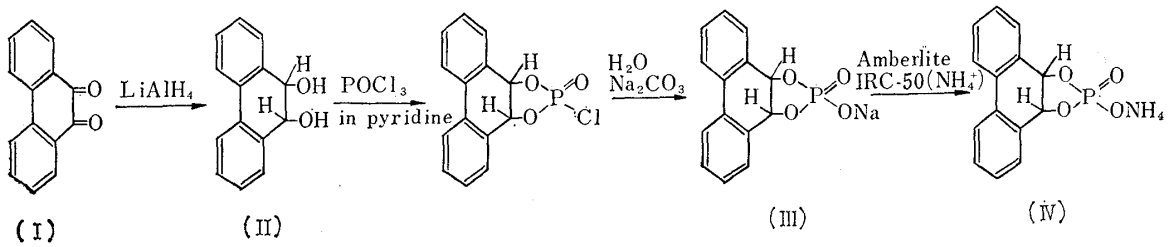


Chart 1.

*1 Hongo, Tokyo (浮田忠之進).
*2 Present Adress : Institute of Public Health, Shirokane-Daimachi, Minato-ku, Tokyo (竹下隆三).
*3 Part XV : This Bulletin, **9**, 600 (1961).
*4 From the thesis of Ryuzo Takeshita for the degree of Doctor of Pharmaceutical Sciences, University of Tokyo, 1959.

1) T. Ukita, K. Nagasawa, M. Irie : J. Am. Chem. Soc., **80**, 1373 (1958).

in pyridine, similarly to synthesis of 4,4′-dinitrohydrobenzoin cyclic phosphate,[1] and 9,10-dihydro-9,10-phenanthrenediol cyclic phosphate (IV) was isolated as its ammonium salt, $C_{14}H_{14}O_4NP$, of colorless needles, m.p. 206° (decomp.). Yield, 91%. Rf_1 0.80 (Chart 1).

From the structural point of view, the new cyclic phosphate (IV) was assumed to be more unstable to hydrolytic cleavage of one of the phosphate bonds than that involved in hydrobenzoin cyclic phosphate.[2] A comparative test of stability was carried out on 9,10-dihydro-9,10-phenanthrenediol cyclic phosphate (IV), hydrobenzoin cyclic phosphate, and 4,4′-dinitrohydrobenzoin cyclic phosphate*[3] in various pH's at 37°. The test compounds were kept in buffers of various pH, 1*N* hydrochloric acid, or 1*N* sodium hydroxide, at 37° and aliquots were withdrawn at intervals for paper chromatographic detection. Table I shows that at this temperature and between pH 3.5~9.5, three test compounds did not suffer any hydrolysis, but in a more acidic or alkaline condition, i.e. at pH 2.5 and in 1*N* sodium hydroxide, they showed some difference in their stability. In an acidic condition, lability of these compounds were found in the descending order of (IV), 4,4′-dinitrohydrobenzoin cyclic phosphate, and hydrobenzoin cyclic phosphate, while in alkali the order was (IV), hydrobenzoin cyclic phosphate, and 4,4′-dinitrohydrobenzoin cyclic

Table I. Stability Test of Hydrobenzoin Cyclic Phosphate, 4,4′-Dinitrohydrobenzoin Cyclic Phosphate, and 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate in Various pH at 37°

Substrate \ Reaction time (hr.)		1	2	4	8	24	48
Control		−	−	−	−	−	−
0.1*N* HCl	HBCP	++++	++++	++++	++++	++++	++++
	DNHBCP	++++	++++	++++	++++	++++	++++
	PHCP	++++	++++	++++	++++	++++	++++
pH 1.5	HBCP	++++	++++	++++	++++	++++	++++
	DNHBCP	++++	++++	++++	++++	++++	++++
	PHCP	++++	++++	++++	++++	++++	++++
pH 2.5	HBCP	−	−	∓	±	+++	++++
	DNHBCP	−	∓	±	+	+++	+++
	PHCP	∓	±	+	++	++++	++++
pH 3.5~9.5	HBCP	−	−	−	−	−	−
	DNHBCP	−	−	−	−	−	−
	PHCP	−	−	−	−	−	−
0.1*N* NaOH	HBCP	±	±	+	+++	++++	++++
	DNHBCP	−	−	−	+	++	+++
	PHCP	++	+++	+++	++++	++++	++++

Signs indicate the grade of decomposition observed on paper chromatograms, −, ∓, ±, +, ++, +++, ++++, indicating nil, approximately 5%, 10~15%, 25%, 50%, 75%, and 100% decomposition.

Table II. Stability Test of Hydrobenzoin Cyclic Phosphate, 4,4′-Dinitrohydrobenzoin Cyclic Phosphate, and 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate in Pyridine at 80°

Substrate \ Reaction time (hr.)	0.5	1	2	4	6	8
HBCP-NH_4	−	−	∓	+	++	+++
DNHBCP-NH_4	−	±	+	++	+++	++++
PHCP-NH_4	∓	+	++	+++	++++	++++

Signs indicate the grade of decomposition observed on paper chromatograms, −, ∓, ±, +, ++, +++, ++++, indicating nil, approximately 5%, 10~15%, 25%, 50%, 75%, and 100% decomposition.

HBCP : Hydrobenzoin cyclic phospate
DNHBCP : 4,4′-Dinitrohydrobenzoin cyclic phosphate
PHCP : 9,10-Dihydro-9,10-phenanthrenediol cyclic phosphate

2) J. Booth, E. Boyland, E.E. Turner : J. Chem. Soc., **1950**, 1188.

phosphate. They also decomposed in pyridine at 80° in the order of lability similar to that for acid hydrolysis (Table II).

The hydrolysis product of 9,10-dihydro-9,10-phenanthrenediol cyclic phosphate (IV) in acid and alkaline media, and in pyridine was identified on paper chromatogram as 9,10-dihydro-9,10-phenanthrenediol phosphate (V), obtained as its crystalline ammonium salt, $C_{14}H_{16}O_5N_2P \cdot \frac{1}{2}H_2O$, by treatment of (IV) with Amberlite IR-120(H^+) in aqueous solution (Chart 2).

The ammonium salt of 9,10-dihydro-9,10-phenanthrenediol cyclic phosphate (IV) was incubated with various monofunctional hydroxylic compounds in the presence of trifluoroacetic acid or dioxane saturated with dry hydrogen chloride[3] and an aliquot of the reaction mixtures was withdrawn at intervals for paper chromatography. The paper chromatograms obtained from the product of 24-hour incubation in the presence of trifluoroacetic acid gave a common phosphorus spot with Rf_1 0.36, which was identified with 9,10-dihydro-9,10-phenanthrenediol phosphate (V), and, except for *tert*-butanol, other spots with different Rf values were found for different alcohols used. The latter spots were identified with the phosphomonoesters of the alcohols used. The paper chromatograms obtained for reaction mixtures of alcoholysis in dioxane-hydrogen chloride also showed spots for each of the phosphomonoesters of the alcohols used except in the case of *tert*-butanol, besides a common spot corresponding to inorganic orthophosphate. Thus, in this case, no spot of the hydrolysis product of starting cyclic phosphate, i.e. 9,10-dihydro-9,10-phenanthrenediol phosphate (V), was detected. Both of these results indicate that the alcoholysis of (IV) with monofunctional hydroxylic compounds does not produce a phosphodiester-type compound which was the main product in a similar alcoholysis of 4,4′-dinitrohydrobenzoin cyclic phosphate (Chart 2).

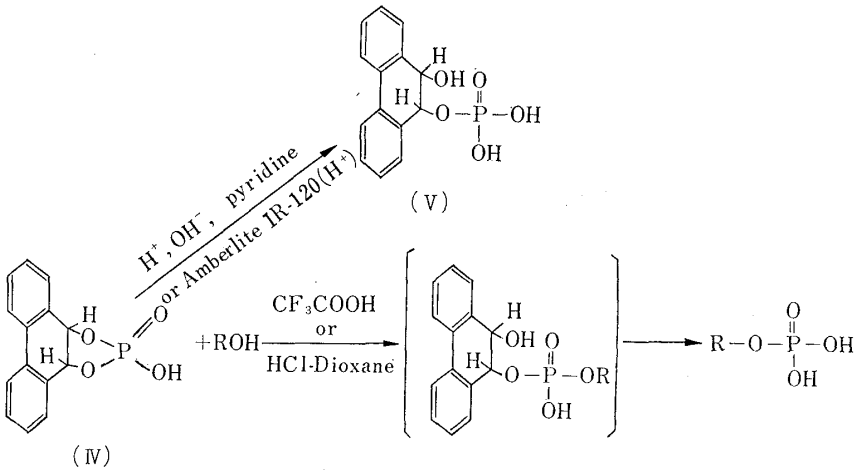


Chart 2.

TABLE III. Hydrogen Chloride-catalyzed Alcoholysis of 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate with Various Alcohols at 37° for 24 hrs.

Alcohol used	Product of alcoholysis Phosphomonoester of the alcohol used Rf_1 (Yield %)	Product of hydrolysis Inorganic phosphate Rf_1 (Yield %)
Methanol	0.13(55)	0.05(45)
Ethanol	0.16(81)	0.05(19)
Isopropanol	0.20(71)	0.05(29)
Butanol	0.36(79)	0.05(21)
tert-Butanol	.....	0.05
Benzyl alcohol	0.32(44)	0.05(56)

3) G.M. Tenner, H.G. Khorana : J. Am. Chem. Soc., **77**, 5349 (1955).

The Rf_1 values and the yield of the phosphorus products in the acid alcoholysis of (IV) are summarized in Table III.

When the ammonium salt of (IV) was warmed with butanol in pyridine at 80° and the products in the reaction mixture were detected by paper chromatography at intervals, three phosphorus spots were detected in the earlier stage of the reaction. The first one, which had the largest Rf value, Rf_1 0.93, corresponded to the phosphodiester-type compound, the second with Rf_1 of 0.80 was that of starting material (IV), and the third (Rf_1 0.36) was that of (V) (Table IV). By prolonged incubation of the reaction mixture, the

Table IV. Alcoholysis of 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate in Pyridine

Alcohol used	Product of alcoholysis Phosphodiester of the alcohol used Rf_1	Product of hydrolysis (V) Rf_1
Butanol	0.93	0.36

size and grade in phosphorus coloration of the first spot gradually increased with simultaneous disappearance of the spot for the starting cyclic phosphate (IV).

From the results of this experiment of the butanolysis of (IV) with a basic catalyst, it was assumed that the intermediate phosphodiester-type compounds, alkyl 9,10-dihydro-9,10-phenanthrenediol phosphates, were too unstable in acidic condition to be detected on paper chromatogram that they were immediately converted to the corresponding alkyl phosphomonoesters.

The ammonium salt of (IV) was incubated respectively with 1,2-propanediol in the presence of dioxane-dry hydrogen chloride, and with DL-erythritol and D-mannitol in the presence of N,N-dimethylformamide saturated with dry-hydrogen chloride. Aliquots from these reaction mixtures were applied to paper chromatography at intervals.

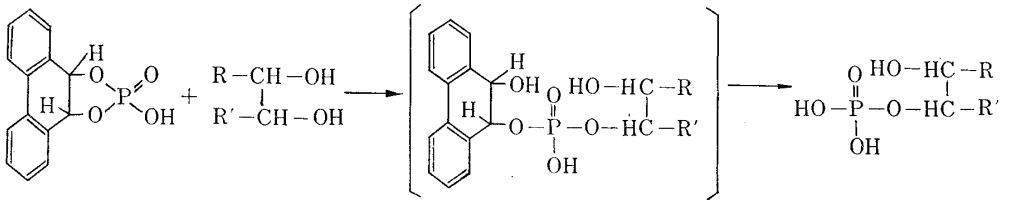
The paper chromatograms of the three reaction mixtures respectively revealed one spot with smaller Rf_1 values, 0.27, 0.20, and 0.18, than that of the starting material (IV) (Rf_1 0.80) but there were no spots with larger Rf_1 values which could be attributed to those of the phosphodiester-type compounds (Chart 3). Of these spots, those obtained for the reaction products of (IV) with DL-erythritol and D-mannitol (Rf_1 0.20 and 0.18) gave positive coloration with the periodate-Schiff reagent. The products corresponding to these three spots were respectively identified with the authentic specimens of 2-hydroxypropyl 1-phosphate (Rf_1 0.27), DL-erythritol 1-phosphate (Rf_1 0.20), and D-mannitol 1-phosphate (Rf_1 0.18). The Rf_1 values and the yields of the phosphorus-containing products from these reactions are summarized in Table V.

Table V. Hydrogen Chloride-catalyzed Alcoholysis of 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate with Polyols

Reaction time (hr.)	Polyol used (Authentic specimen)	Product of alcoholysis Phosphomonoester of polyol used Rf_1 (Yield %)	Product of hydrolysis (V) Rf_1 (Yield %)	Product of hydrolysis inorganic phosphate Rf_1 (Yield %)
24	1,2-Propanediol	0.27 (85)	—	0.05 (15)
—	(2-hydroxypropyl 1-phosphate)	0.27	—	—
3	DL-Erythritol	0.20 (55)	0.36 (31)	0.05 (14)
3	D-Mannitol	0.18 (22)	0.36 (38)	0.05 (20)
—	(DL-erythritol 1-phosphate)	0.20	—	—
—	(D-mannitol 1-phosphate)	0.18	—	—

Throughout the above-mentioned reactions, the alcoholysis reaction of (IV) with monohydroxylic or polyhydroxylic compounds was found to occur more preferentially with the primary than the secondary carbinol groups of the hydroxylic compounds used for alcoholysis to form the phosphomonoester.

In the next series of experiments, alcoholysis of this cyclic phosphate (IV) with some carbohydrates such as D-ribose and D-glucose was attempted. In the presence of dry hydrogen chloride, (IV) was reacted with D-ribose or D-glucose in N,N-dimethylformamide at room temperature, the aliquots were taken from each reaction mixture, and submitted to both paper chromatography and paper electrophoresis (Chart 3). In each case, on paper chromatograms, no phosphorus-positive spot which has larger Rf value than that of (IV) and which could be attributed to that of phosphodiester-type compound, was detected, but each of these reaction mixtures gave one new phosphorus-positive spot with respective Rf_1 value of 0.08 and 0.05. These spots were found positive to reagents for reducing sugar and were identified by paper chromatography with authentic specimens of D-ribose 5-phosphate and D-glucose 6-phosphate, respectively (Table VI).



1,2-propanediol	$R=CH_3$,	$R'=H$
DL-erythritol	$R=CH_2OH-(CHOH)_2$,	$R'=H$
D-mannitol	$R=CH_2OH-(CHOH)_3$,	$R'=H$
D-ribose	$R=CHO-(CHOH)_2$,	$R'=H$
D-glucose	$R=CHO-(CHOH)_3$,	$R'=H$

Chart 3.

TABLE VI. Hydrogen Chloride-catalyzed Alcoholysis of 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate with Aldoses

Aldose used (Authentic specimen)	Product of alcoholysis: Phosphomonoester of aldose used					Product of hydrolysis: (V)	Product of hydrolysis: inorganic phosphate
	Rf_1	Rf_2	Rf_3	Rf_4	Mo.	Rf_1	Rf_1
D-Ribose	0.08	0.50	0·46	0.47	0.70	0.36	0.05
D-Glucose	0.05	0.38	0.37	0.26	0.68	0.36	0.05
(D-ribose 5-phosphate)	0.08	0.50	0.46	0.47	0.70	—	—
(D-glucose 6-phosphate)	0.05	0.38	0.37	0.26	0.68	—	—

Mo. indicate the movement of phosphate relative to orthophosphate after electrophoresis.

Furthermore, from the reaction mixture of (IV) and D-ribose in a preparative scale, after fractionation of the reaction mixture by column chromatography by use of cellulose powder and final purification by paper electrophoresis, D-ribose 5-phosphate was isolated as its barium salt, $C_5H_9O_8BaP$, in 16% yield. On periodate oxidation, this phosphate consumed 2.97 moles of the reagent to afford glycolaldehyde 3-phosphate which was identified with the authentic specimen by paper chromatography (Fig. 1).

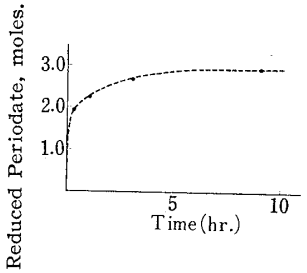


Fig. 1. Periodate Oxidation of the Product produced by Alcoholysis Reaction of 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate with D-Ribose

Experimental

Paper Chromatography——The following solvent systems were used for paper chromatography: (1) iso-PrOH-conc. NH_4OH-H_2O (7:1:2); (2) PrOH-conc. NH_4OH-H_2O (6:3:1)[4]; (3) iso-PrOH-conc. NH_4OH-CCl_3COOH-H_2O (7:5:0.3:25)[5]; (4) *tert*-BuOH-picric acid-H_2O (80:4:20)[6]. The Rf values obtained by these solvent systems are designated respectively as Rf_1, Rf_2, Rf_3, and Rf_4. Chromatography was performed as follows: A sample containing 10~40 γ as P was applied to Toyo Roshi No. 53 filter paper and run ascendingly for 15 hr., using the solvent system (1), (2), or (3), and descendingly for 24 hr. with the solvent system (4). P was detected by the method of Bandurski and Axelrod.[7] 1,2-Glycol group and reducing sugar were detected by spraying the periodate-Schiff reagent[8,9] and aniline hydrogenphthalate,[10] respectively.

Paper Electrophoresis——The buffer employed as the solvent system was prepared as follows: A mixture of 20 cc. of BuOH, 2 cc. of AcOH, and 10 cc. of pyridine was made up to 1 L. with distilled H_2O. The material was applied on a strip (28×19 cm.) of Toyo Roshi No. 27 filter paper (starting line was placed at 8 cm. from one edge of the paper set on cathode side) and after being moistened with the buffer solution (pH 5.6), the strip was subjected to electrophoresis at a potential of 18 V/cm. for 1 hr. The detection of the spots on paper was made by the same techniques as those used for paper chromatography.

Synthesis of Ammonium 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate (IV)——To an ice-cold solution of 2.8 g. of $POCl_3$ in 10 cc. of dehyd. pyridine, 2.0 g. of 9,10-dihydro-9,10-phenanthrenediol[3] (II) dissolved in 50 cc. of dehyd. pyridine was added. By the same procedure as in the synthesis of 4,4′-dinitrohydrobenzoin cyclic phosphate,*3 sodium salt (III) of 9,10-dihydro-9,10-phenanthrenediol cyclic phosphate was isolated and its aqueous solution was passed through a column of Amberlite IRC-50 (NH_4^+). The effluent and washings were combined, lyophilized, and the slightly yellow powder thus obtained was dissolved in a minimum volume of EtOH. Recrystallization was effected by addition of Et_2O to the EtOH solution to give colorless long needles, m.p. 206° (decomp.). The product was dried over P_2O_5 to a constant weight. Yield, 91%. *Anal.* Calcd. for $C_{14}H_{14}O_4NP$ (ammonium salt): C, 57.73; H, 4.80; N, 4.80; P, 10.65. Found: C, 57.47; H, 4.97; N, 5.13; P, 10.79.

Stability Test of Hydrobenzoin Cyclic Phosphate, 4,4′-Dinitrohydrobenzoin Cyclic Phosphate, and 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate in Acid and Alkaline Media——A solution of 2 mg. each of the ammonium salts of hydrobenzoin cyclic phosphate, 4,4′-dinitrohydrobenzoin cyclic phosphate, and 9,10-dihydro-9,10-phenanthrenediol cyclic phosphate respectively dissolved in 0.2 cc. each of 0.1*N* HCl, 0.1*N* NaOH, the Clark buffer[11] having four kinds of different pH of 1.5, 2.5, 3.5 and 6.5, or Michaelis buffer[12] of pH 8.0 and 9.5, was incubated at 37°. Each 0.01 cc. of the reaction mixtures was withdrawn at intervals and chromatographed on paper. The results obtained are given in Table I.

Stability Test of Hydrobenzoin Cyclic Phosphate, 4,4′-Dinitrohydrobenzoin Cyclic Phosphate, and 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate (IV) in Pyridine——To each of three tubes containing 0.2 cc. of dehyd. pyridine, 2 mg. each of the ammonium salt of hydrobenzoin cyclic phosphate, 4,4′-dinitrohydrobenzoin cyclic phosphate, or 9,10-dihydro-9,10-phenanthrenediol cyclic phosphate was added and the solutions were warmed at 80~85°. Each 0.01 cc. of the solutions was withdrawn at intervals and submitted to paper chromatography. The results are given in Table II.

Hydrolysis Product (V) of 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate (IV)——To a solution of 200 mg. of the ammonium salt of (IV) in 10 cc. of distilled H_2O, 2 cc. of freshly prepared Amberlite IR-120 (H^+) was added. The mixture was warmed at 80~85° for 10 min. and filtered. The filtrate was passed through a column of Amberlite IRC-50 (NH_4^+) and the effluent and the washings were lyophilized. The white powder thus obtained was recrystallized by dissolving it in a minimum volume of MeOH and adding Et_2O to it to produce colorless crystals, m.p. 204~205° (decomp.). Rf_1 0.36. The product was dried over P_2O_5 in vacuum to a constant weight. *Anal.* Calcd.

4) C.S. Hanes, F.A. Isherwood: Nature, **164**, 1107 (1949).
5) J.P. Ebel: Bull. soc. chem. biol., **34**, 330 (1952).
6) H.S. Loring, L.W. Levy, L.K. Moss: Anal. Chem., **28**, 539 (1956).
7) R.S. Bandurski, B. Axelrod: J. Biol. Chem., **193**, 405 (1951).
8) J.G. Buchanan, C.A. Dekker, A.G. Long: J. Chem. Soc., **1950**, 3162.
9) J. Baddiley, J.G. Buchanan, R.E. Handschumacher, J.F. Prescott: *Ibid.*, **1956**, 2818.
10) S.M. Partridge: Nature, **164**, 443 (1949).
11) W.M. Clark: "The Determination of Hydrogen Ions," (1920). Williams and Wilkins Co., Baltimore, U.S.A.
12) L. Michaelis: J. Biol. Chem., **87**, 33 (1930).

for $C_{14}H_{21}O_5N_2P \cdot \frac{1}{2}H_2O$ (Ammonium salt): C, 52.83; H, 4.75; N, 4.40; P, 9.01. Found: C, 53.54; H, 5.00; N, 4.46; P, 9.08.

Alcoholysis Reaction of 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate (IV) with Various Mono- and Poly-functional Hydroxylic Compounds——To a series of tubes containing 2 mg. of the ammonium salt of (IV) dissolved in 0.2 cc. each of MeOH, EtOH, iso-PrOH, BuOH, *tert*-BuOH, benzyl alcohol, or 1,2-propanediol, 0.05 cc. of CF_3COOH or 0.2 cc. of dioxane saturated with dry HCl was added. On the other hand, in the case of DL-erythritol and D-mannitol, 10 mg. each of the compound was dissolved in 0.2 cc. of N,N-dimethylformamide and, after adding 2 mg. of ammonium salt of (IV) to each, the mixture was saturated with dry HCl. All the mixtures were incubated at 37° and the aliquots of each reaction mixture were withdrawn at intervals to be submitted to paper chromatography. In order to observe the Rf_1 value of phosphorus in the case of mono-functional hydroxylic compounds and 1,2-propanediol, one of the chromatograms was sprayed with phosphorus reagent. From another chromatogram, the corresponding parts containing the phosphorus compounds were cut out. On the other hand in the case of polyfunctional hydroxylic compounds, each chromatogram obtained in two runs was respectively sprayed with phosphorus reagent and the periodate-Schiff reagent for detecting the products which contained both P and 1,2-glycol moiety. From another chromatogram obtained in the third run, the parts corresponding to the spots positive to both reagents were cut out. The cuttings were boiled with $HClO_4$ and submitted to the determination of P by the Allen method.[13] Untreated pieces of the filter paper of the same sizes as the cuttings served as blanks. The results are given in Tables III and IV.

Alcoholysis Reaction of 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate (IV) with Carbohydrates——To a solution containing 10 mg. of D-ribose or D-glucose dissolved in 0.2 cc. of N,N-dimethylformamide, 2 mg. of the ammonium salt of (IV) was added and the mixture was saturated with dry HCl. After allowing to stand for 1 hr., 0.02 cc. of the reaction mixture was submitted to paper chromatography with four kinds of solvent systems and to electrophoresis. The results are given in Table V.

Isolation of Alcoholysis Product of 9,10-Dihydro-9,10-phenanthrenediol Cyclic Phosphate (IV) with D-Ribose: D-Ribose 5-Phosphate——To a solution containing 1.5 g. of D-ribose dissolved in 20 cc. of N,N-dimethylformamide 1.0 g. of the ammonium salt of (IV) was added and the mixture was saturated with dry HCl. As soon as the crystals of NH_4Cl began to appear in the solution, the bubbling of dry HCl was stopped and the reaction mixture was kept at room temperature for 1 hr. The mixture was diluted with 50 cc. of distilled H_2O and Ag_2CO_3 was added to remove the Cl^- ion. After standing overnight, the precipitate that appeared was collected by centrifugation and washed with distilled H_2O. The filtrate and washing were combined and H_2S was passed through the mixture to remove Ag^+ ion. After removal of Ag_2S by centrifugation, the solution was concentrated to 15 cc. at below 40° in vacuum.

This solution was chromatographed through a column of cellulose powder (Toyo Roshi, 100~200 mesh) by the ascending run with the solvent system (1). The fractions containing phosphorus compounds having Rf_1 0.03~0.13 were combined and dried at room temperature. The residue obtained was extracted with 50 cc. of distilled H_2O and, after decationization of the solution with Amberlite IR-120 (H^+), the solution was adjusted to pH 8.0 with a small volume of $Ba(OH)_2$ solution. The excess of Ba^{2+} ion was removed by passing CO_2 through the solution and precipitated $BaCO_3$ was removed by centrifugation. The supernatant was evaporated to dryness in vacuum to furnish a slightly yellow powder in 16% yield.

The solution of the barium salt of the product in a minimum volume of H_2O was decationized with Amberlite IR-120 (H^+). The acidic solution was streaked on a filter paper, and the strip subjected to electrophoresis. Along the traveling direction, both sides of the strip were cut off and the cuttings were sprayed respectively with reagents for P and for reducing sugar to detect the location to which the desired compound traveled. From the major untreated part of the strip, the corresponding parts positive to above detection were cut off and the cuttings were extracted with 3 cc. of distilled H_2O. The solution was passed through a column of Amberlite IRC-50 (Ba^{2+}), and the effluent and washings were combined. The mixture was concentrated *in vacuo* or lyophilized.

The compound thus obtained was dissolved in a minimum volume of H_2O and after removal of insoluble material by centrifugation, Me_2CO was added to the supernatant. The precipitate that appeared was again dissolved in a minimum volume of distilled H_2O, 4 to 5 volumes of EtOH were added to the solution, and the white flocculent precipitate that appeared was separated by centrifugation. It was washed successively with EtOH and Et_2O by centrifugation and dried over P_2O_5 at 55° for 3 hr. *Anal.* Calcd. for $C_5H_9O_8BaP$: C, 16.43; H, 2.48; P, 8.49. Found: C, 16.41; H, 3.10; P, 8.77. Rf_4 0.47.

On oxidation of this product with periodate at pH 5.0, it consumed 2.97 moles of the reagent after 9 hr. at 10°. The consumption curve of the periodate is given in Fig. 1.

13) R. J. Allen: Biochem. J., **34**, 858 (1940).

A part of the expenses of this work was supported by the Grant-in-Aid for Scientific Research provided by the Ministry of Education to which the authors' thanks are due. Thanks are also due to Mr. D. Ohata, Iatrochemical Institute of Pharmacological Research Foundation, and to Misses M. Iwanaga and S. Ohno, Institute for Infectious Diseases, University of Tokyo, for the microanalyses.

Summary

9,10-Dihydro-9,10-phenanthrenediol cyclic phosphate (IV) was synthesized and its alcoholysis reaction with various hydroxylic compounds was investigated. The new cyclic phosphate was found to be alcoholyzed in acidic media by mono- and polyfunctional hydroxylic compounds, as well as by some aldoses, while the latter was found to be inert to the similar alcoholysis of hydrobenzoin or 4,4′-dinitrohydrobenzoin cyclic phosphates. D-Ribose 5-phosphate was produced by this type of reaction. The intermediate phosphodiester-type compound in the above alcoholysis reaction, alkyl 9,10-dihydro-9,10-phenanthrenediol phosphate, was found to be not stable enough in acid media to be detected by paper chromatography.

(Received October 21, 1960)

UDC 616-006-085 : 547.233.4

99. Masahiro Torigoe : Studies on Carcinostatic Substances. XXXV.*1 Chemical and Antitumor Properties of Quaternary Derivatives of N-Alkoxy-2,2′-dichlorodiethylamine.

(*Iatrochemical Institute of Pharmacological Research Foundation**2)

The previous investigation (Part XXXIV) revealed the strong antitumor activity of 2,2-bis(2-chloroethyl)isoxazolidinium chloride (I). One thing to be noted about this compound is that the compound (I) is regarded as a quaternary derivative of N,N,O-trisubstituted hydroxylamine. On the other hand, as reported in earlier stage of the investigation of this work,[1] N-(2-chloroethoxy)-N-methyl-2-chloroethylamine was neither effective on experimental tumor nor chemically active as an alkylating agent.

From these observations, attempt was made to prepare two new linear derivatives of (2-chloroethoxy)-bis(2-chloroethyl)methylammonium halide and some related compounds and discussions are made here on their chemical and biological properties. The compounds and their summarized properties to be discussed are shown in Table I.

The compounds (II), (III), (IV), and (V) were synthesized according to the processes shown in Chart 1.

As anticipated, (II) and (III) showed very potent antitumor activity against Yoshida sarcoma, while (IV) was completely inactive and (V) was slightly effective. Although even the latter two compounds yielded an active secondary amine, viz. 2,2′-dichlorodiethylamine, by catalytic reduction on more drastic condition than in case of reduction of (I), this reductive activation seemed not to occur *in vivo*.

Of course, (II) and (III) were proved to be reduced by milder reduction yielding N-

---

*1 This paper constitutes a part of a series entitled "Studies on Carcinostatic Substances" by M. Ishidate and Y. Sakurai. Part XXXIV : This Bulletin, **9**, 485 (1961).

*2 Designation now changed to Cancer Chemotherapy Section, Sasaki Institute, 26 Nishigahara 1-chome, Kita-ku, Tokyo (鳥越政宏).

1) M. Ishidate, *et al.* : Gann, **47**, 375 (1956).