Dextran Carbanilates.—The rotation of dextran carbanilates was found to be quite insensitive to variations in the cultural conditions under which the dextran was formed[3] or to partial hydrolytic breakdown. However, the presence of increased amounts of non-1,6′-linkages was accompanied by a decrease in the high positive rotation in morpholine found to be characteristic of dextran tricarbanilates. The chemical structures of the NRRL B-742 and NRRL B-1254 dextrans are not yet well enough defined to enable a statement as to whether or not the non-1,6′-linkages occur as points of branching.

Discussion

In the starch series of polysaccharides, the rotational differences between the polysaccharides themselves or their aliphatic triester derivatives[18] is small. This difference was greatly accentuated by conversion of the polysaccharides to the tricarbanilates.[2] A similar advantage is apparent for the polysaccharides discussed here. For example, in water the specific rotations of the dissimilar polysaccharides laminarin ($-14°$[15]) and of the *Phytomonas tumefaciens* polysaccharide ($-10°$[14]) differ only slightly while the specific rotations of their tricarbanilates in pyridine differ by more than 100° (Table I). The differences between the rotations of dextrans and amylaceous polysaccharides are likewise enhanced by use of the tricarbanilate rotations in morpholine.

(18) I. A. Wolff, D. W. Olds and G. E. Hilbert, THIS JOURNAL, **73**, 346 (1951).

Acknowledgment.—The generosity of the following donors of polysaccharides for this study is gratefully acknowledged: J. B. Sumner, Marjorie Austin, R. Hodgson, R. E. Reeves, M. Stacey, W. Z. Hassid, E. Pacsu, K. Meyer, Allene Jeanes and C. Wilham. The nitrogen analyses were performed by Mary Wiele and Joan Heintzman. The percentages of 1,6′-linkages in the dextran samples were determined by J. C. Rankin.

PEORIA, ILLINOIS

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF STANFORD UNIVERSITY]

The Phosphorylation of Chloromycetin[1]

BY HARRY S. MOSHER, JOAN REINHART AND H. C. PROSSER

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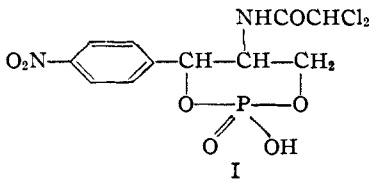
In an attempt to obtain a water-soluble derivative of Chloromycetin, its phosphorylation was studied. With phosphorus oxychloride and tetraphosphoric acid, a cyclic phosphoric acid ester I was formed. This compound was water soluble, very stable to acid hydrolysis and showed no antibiotic activity. Use of crystalline orthophosphoric acid or non-anhydrous phosphorylating conditions was unsuccessful, and often resulted in cleavage of the amide linkage.

The antibiotic Chloromycetin has a low water solubility, which increases its difficulty of administration by the parenteral route. Since many physiologically active compounds exist *in vivo* as the phosphoric acid esters, and since enzymes are present *in vivo* for hydrolyzing such esters, it was desirable to prepare a soluble, phosphoric acid ester of Chloromycetin. Such an ester has been obtained by use of phosphorus oxychloride[2] in pyridine at 0°. Our evidence indicates that this crystalline product has the cyclic structure I.



I

The ester gives an unequivocal monobasic titration curve indicating a single replaceable hydrogen atom. Analytical data as well as neutral equivalent, support the cyclic structure. The ultraviolet absorption spectra for the phosphorylated product is identical to that of Chloromycetin except for a 5 mμ displacement toward the shorter wave lengths. The infrared absorption spectra for the phosphorylated product shows the general absorption around 10 μ, reported to be characteristic of phosphoric acid esters.[3]

Similar types of cyclic phosphate esters have recently been reported, such as the riboflavin cyclic 4′,5′-phosphate,[4] catechol phosphate[5] and the 2′,3′-cyclic phosphates of adenosine, cytidine and uridine.[6] A cyclic phosphate has been postulated as an intermediate in the acid rearrangement of glycerol 2-phosphate to glycerol 1-phosphate.[7]

Since these are all α,β-cyclic phosphates (five-membered ring), it is surprising, in view of the favorable configuration of α,γ-carbon atoms,[8] that the only other isolation of an α,γ-cyclic phosphate (six-membered ring) so far reported, is that of Baddiley and Thain[9] who have prepared a cyclic D-pantothenic acid 2′,4′-phosphate.

An interesting stability correlation exists between the cyclic pantothenic phosphate and cyclic Chloromycetin phosphate. With both compounds the phosphate group was hydrolyzed very slowly in acid media. The hydrolysis curve of Chloro-

(1) Chloromycetin is the registered trademark which Parke, Davis and Company has adopted for the antibiotic drug, chloramphenicol. See M. C. Rebstock, *et al.*, THIS JOURNAL, **71**, 2460 (1949).

(2) Joan Reinhart, Master's Thesis, 1950, Stanford University.

(3) L. Daasch and D. Smith, *Anal. Chem.*, **23**, 853 (1951).

(4) L. Flexer, W. Farkas Abstract of Papers, XII International Congress of Pure and Applied Chemistry, New York, N. Y., Sept. 10–13 (1951), Biological Chemistry, p. 71.

(5) E. Cherbuliez, *Helv. Chim. Acta*, **34**, 841 (1951).

(6) D. Brown, D. Magrath and A. Todd, *J. Chem. Soc.*, 2708 (1951).

(7) P. Verkade, W. Cohen and J. Stoppelenburg, *Rec. trav. chim.*, **59**, 886 (1940).

(8) It has been shown that six-membered cyclic glycol phosphites are the most stable: A. Arbuzov, V. Zoroastrova, *Bull. acad. sci. U.R.S.S., Classe sci. chim.*, 208 (1948); *C. A.*, **42**, 4932^g (1948).

(9) J. Baddiley and E. Thain, *J. Chem. Soc.*, 3421 (1951).

mycetin cyclic phosphate is shown in Fig. 1. The percentage hydrolysis in 0.5 *N* hydrochloric acid at 100° was determined by a colorimetric method for inorganic phosphorus.[10] The induction lag shown by the hydrolysis curve could have been due to a preliminary partial hydrolysis to a non-cyclic phosphate, but considering the lability of the amide group under the strongly hydrolytic conditions, the induction period was probably due to a change in the kinetics of phosphate hydrolysis after the amide group was removed.

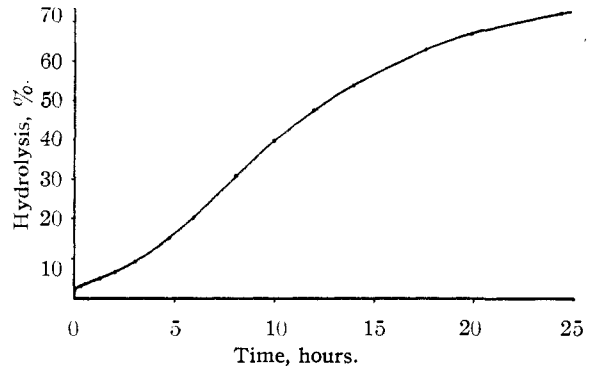


Fig. 1.—Hydrolysis of Chloromycetin cyclic phosphate in 0.5 *N* HCl at 100°.

Baddiley and Thain's cyclic pantothenic acid phosphate was reported by them as not being hydrolyzed by acid or alkali phosphatase in contrast to the non-cyclic pantothenic acid phosphate, and to be inactive in stimulating the growth of *A. suboxydans*. A parallel situation exists with Chloromycetin cyclic phosphate. The lack of *in vivo* antibiotic activity is further evidence for its cyclic nature, which makes it incapable of normal enzymatic hydrolysis.[11] An analogous sulfite ester of Chloromycetin is formed by the action of thionyl chloride, and a carbonate ester by the action of phosgene.[12] The preparation of a cyclic phosphite recently has been reported[13] using phosphorus trichloride at elevated temperature. This very slightly water-soluble product was reported to be active *in vivo* against *Streptococcus pyogene* in mice.

Further experiments were tried in an attempt to prepare a non-cyclic phosphate. Use of commercial tetraphosphoric acid[14] resulted in a simpler preparation of the cyclic phosphate I. No other phosphorus containing derivatives of Chloromycetin could be isolated. Poggi and Serchi[15] claim to have prepared a normal phosphate ester with the primary hydroxyl group of Chloromycetin, although no analytical or pharmaceutical data were given. They employed phosphoric acid and mixtures of the latter with phosphorus pentoxide at 50° for 30 minutes to two hours. We have been unable to duplicate their original experiments using concentrations of orthophosphoric acid from 85% to the crystalline 100% acid. The only reported characterization of their product was a melting point of 132–134° and solubility data. These data correspond roughly with the properties we have found for the cyclic ester I.

It has been hypothesized[16] that in the hydrolysis of phosphorus pentoxide, tetraphosphoric acid may be an intermediate. We have found that commercial "tetraphosphoric acid" can give rise to the cyclic phosphate ester.

Acknowledgment.—We wish to thank Parke, Davis and Company for the Chloromycetin used in this investigation, for the biological tests, and for fellowship support.

Experimental

Cyclic Phosphate Formation Using Phosphorus Oxychloride.—D-(−)-*threo*-1-*p*-Nitrophenyl-2-amino-1,3-propanediol (Chloromycetin), 3.8 g., was dissolved in 9 ml. of dry pyridine. A solution of 1.6 ml. (1.5 equivalents) of freshly distilled phosphorus oxychloride in 10 ml. of pyridine was added slowly with stirring at 0°. After a few minutes a precipitate of pyridine hydrochloride formed, and the mixture was hydrolyzed in ice-water. Barium hydroxide was added to reach *p*H 7; after filtering, the solution was lyophilized. Extraction of the powdery product with acetone gave a solution which yielded an amorphous precipitate on the addition of ether. The solid was purified by two such precipitations giving 2.6 g. of a barium salt.

The barium salt was titrated with dilute sulfuric acid. After removing barium sulfate the solution yielded on lyophilization 1.42 g. (31%) of the phosphorylated product. The ester was crystallized by slow evaporation of an aqueous solution giving a dihydrate melting at 95°. The ester was also obtained as needle clusters from 50% ether–alcohol. The melting point of carefully dried material in a sealed capillary was 134–137°. Water solubility was some 20 times greater than for Chloromycetin. The fact that a monobasic titration curve was obtained eliminates the possibility of a normal phosphoric acid ester; 196.45 mg. of I required 4.20 ml. of 0.1199 *N* sodium hydroxide solution.

Anal. Calcd. for $C_{11}H_{11}NO_7Cl_2P$: C, 34.31; H, 2.88; N, 7.28; P, 8.05; neut. equiv., 385. Found: C, 33.4; H, 3.13; N, 6.35; P, 8.0; neut. equiv., 389 ± 3.

The combustion analysis[17] proved difficult and required the addition of chromium trioxide. Phosphorus was determined by a modified Fiske–SubbaRow colorimetric method.[18] Phosphorus determination prior to hydrolysis showed the absence of inorganic phosphate. The optical rotation of a product from another run (7.9% phosphorus) was $\alpha^{27}D$ −16.2° (*c* 2.3 in water).

Infrared spectrum, principal bands, μ (nujol mull, Perkin-Elmer model 21)

W	3.05, 6.00–6.04, 6.35, 6.44, 7.56, 8.02, 13.56
M	6.18, 7.89, 8.22–8.34, 9.00, 12.90–13.07–13.22, 14.94
M–S	11.00, 11.36, 12.30, 14.18–14.26, 14.54
S	5.83, 6.56, 7.37, 9.40, 9.99, 10.33

The infrared spectrum of Chloromycetin has recently been published.[19]

Cyclic Phosphate Formation Using Tetraphosphoric Acid.—A solution of 7.58 g. of Chloromycetin in 75 g. of tetraphosphoric acid stood for one week in a vacuum desiccator and was then hydrolyzed in 300 ml. of ice-water. Barium carbonate was added to reach *p*H 5.2. The solution was filtered, and the precipitated barium phosphate thoroughly washed with cold water. A separate methanol wash yielded 1.5 g. of recovered Chloromycetin. The barium

(10) I. Berenblum and E. Chain, *Biochem. J.*, **32**, 295 (1938).

(11) We are indebted to Dr. Glazco of Parke, Davis and Co. for these tests. The cyclic ester I was inactive against cultures of *S. sonnei* and several other organisms, at a concentration of 25 μg./0.1 cc. This cyclic ester is poorly absorbed from the intestinal tract of the rat. When administered parenterally, it is rapidly excreted through the kidneys. No antibiotic activity was found in the urine or blood of these rats indicating that it was not hydrolyzed to Chloromycetin.

(12) G. W. Moersch and A. C. Moore, U. S. Patent 2,587,641, March 4, 1952.

(13) E. P. Taylor, *J. Pharm. and Pharmacol.*, **5**, 254 (1953).

(14) Monsanto Chemical Company.

(15) A. R. Poggi and G. Serchi, *Rev. asoc. bioquim. Argentina*, **15** 273 (1950); *Sperimentale, Sez. chim. biol.*, **3**, 100 (1952).

(16) R. N. Bell, L. F. Audrieth and O. F. Hill, *Ind. Eng. Chem.*, **44**, 568 (1952).

(17) Microchemical Specialties, Berkeley, Calif.

(18) C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).

(19) P. Sensi and O. Fagioli, *Gazz. chim. ital.*, **83**, 76 (1953).

salt could be obtained as an amorphous white solid. The solution was treated with 1 *M* sulfuric acid to give the free acid ester without isolating the barium salt. Lyophilization yielded an oil which crystallized on the addition of a small amount of water, to yield 3.7 g. (41%) (51% based on recovered Chloromycetin) of the phosphate ester. On recrystallization it had the same melting point, mixed melting point and monobasic neutralization equivalent as the product from phosphorus oxychloride. A residual phosphorus containing oil could not be crystallized and contained a small amount of Chloromycetin free base sulfate of m.p. 218–221° (uncor.).

Use of Orthophosphoric Acid.—Crystalline orthophosphoric acid[20] was used in attempts to phosphorylate L(+)-Chloromycetin under the following conditions: 30 min. at 50°; 60 min. at 50°; three weeks at room temperature. A large excess of the acid was used to obtain homogeneous solutions. The isolation procedure was essentially the same as that employed using tetraphosphoric acid.

The short run; 30 min. at 50°, duplicated Poggi's[15] conditions and gave an almost complete recovery of unchanged Chloromycetin. Other experiments using 85%, "97%" and "100%" phosphoric acid prepared by mixing phosphorus pentoxide with sirupy phosphoric acid and varying the time and temperature, were unsuccessful. Longer reaction times gave considerable Chloromycetin free base (hydrolyzed amide group) which sometimes crystallized from the final lyophilized oils as the free base sulfate.

(20) W. H. Ross and R. M. Jones, THIS JOURNAL, **47**, 2165 (1925).

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[CONTRIBUTION FROM THE ROSS CHEMICAL LABORATORY, ALABAMA POLYTECHNIC INSTITUTE]

Synthesis of Aromatic Phosphonic Acids and their Derivatives. III. Some Amino and Hydroxy Substituted Acids[1]

BY VERNON L. BELL, JR., AND GENNADY M. KOSOLAPOFF

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The diazonium fluoborate method for synthesis of aromatic phosphonic acids with functional substituents was examined for possible improvements in the operating techniques. As a result, improved yields of several compounds were obtained. Typical organic substitutions of functional groups in such compounds were examined.

One finds little information in the literature on the possibilities of functional group replacements in organic compounds of phosphorus in general and in phosphonic acids, specifically. The purpose of the present investigation was to examine some of these replacements as a method of synthesis of aromatic phosphonic acids with hydroxy and amino substitution. The recent development of the diazonium fluoborate method for phosphonation reactions was selected as the starting point inasmuch as this method affords definite isomers of the desired starting materials.[2]

There are three rather obvious methods for preparation of hydroxy-substituted aromatic phosphonic acids. The first of these, diazo-replacement of an amino group, has been used with moderate success by Doak and Freedman.[3] A second method is that involving an alkaline hydrolysis of halogen-substituted aromatic phosphonic acids. Replacement of chlorine by the hydroxy group in compounds bearing a nitro group in *o*-position in respect to the chlorine was reported some time ago by Arnold and Hamilton,[4] but one is unable to find in the literature any data on reactions of this type involving otherwise unsubstituted halophosphonic acids. The third possible method involves the ether cleavage in ether-substituted phosphonic acids, such as anisolephosphonic acids. No data on such reactions can be found in the literature.

All three methods were examined as to possible utility with the several specific examples described in the Experimental section. It was readily found that any method which involves drastic high temperature treatment results in formation of phenol as the consequence of the cleavage of the phosphorus-to-carbon link. In contrast to the ready ammonolysis of halobenzenephosphonic acids, the hydrolytic reaction appears to be applicable only to "activated" halogen derivatives of the type studied by Arnold and Hamilton. In fact, the thermal stability of the hydroxybenzene- and methoxybenzenephosphonic acids was found to be rather low. It appears that the first method of their synthesis, mentioned above, appears to be the only practicable one at this time.

The stability of the carbon-to-phosphorus bond in *o*-aminobenzenephosphonic acid is known to be of low order, as shown by rapid dephosphonation in bromine water.[5] We have found that *p*-aminobenzenephosphonic acid also shows this low order of stability of the carbon-to-phosphorus link. Thus, *o*- or *p*-substitution with strongly *o*–*p*-directing groups renders the carbon-to-phosphorus bond very susceptible to cleavage, a situation which is rather unusual in the family of substances noted for the high order of stability of the phosphonate group. The ease of dephosphonation shown by *p*-hydroxybenzenephosphonic acid is illustrated by formation of tribromophenol at room temperature with bromine water and by formation of phenol on heating the acid not only with dilute hydrochloric acid but even with water alone.

Experimental Part

The Phosphonation by the Diazonium Fluoborate Method.—The fluoborates used as the starting materials in the various syntheses were obtained through the sodium fluoborate procedure.[6]

Preparation of *p*-bromobenzenephosphonic acid by the procedure outlined by Doak and Freedman gave a 61% yield of the product,[7] which agreed with the previous work. In the preparation of the *o*-isomer, we obtained a consistent

(1) For part II see THIS JOURNAL, **70**, 3465 (1948).
(2) G. O. Doak and L. D. Freedman, *ibid.*, **73**, 5658 (1951).
(3) G. O. Doak and L. D. Freedman, *ibid.*, **74**, 753 (1952).
(4) G. B. Arnold and C. S. Hamilton, *ibid.*, **63**, 2637 (1941).
(5) G. M. Kosolapoff, *ibid.*, **71**, 4021 (1949); G. O. Doak and L. D. Freedman, ref. 3.
(6) A. Roe, "Organic Reactions," Vol. 5, John Wiley and Sons, Inc., New York, N. Y., 1949, p. 217.
(7) G. O. Doak and L. D. Freedman, THIS JOURNAL, **75**, 683 (1953).