dicated that II is synthesized with difficulty, at least by nitration of I. Consequently, biochemists have followed the procedure of Yoshida[3] in which *tris*(*p*-nitrophenyl) phosphate (III) is hydrolyzed in alcoholic NaOH, and II precipitated from the reaction mixture as the calcium salt, the crude product being used as substrate for phosphodiesterase activity.[4] Inasmuch as the calcium salt of *p*-nitrophenyl phosphate (IV) is also water insoluble, the possibility arises that the crude material may be contaminated with IV and that this is responsible, in part, for the observation that II is attacked by certain phosphomonoesterases.[5] This question can be resolved by investigating the behavior of phosphomono- and diesterases against pure II. In this communication we describe a simple method for preparing II in good yield and discuss the preparation and properties of this and related esters of phosphoric acid, about which some erroneous statements appear in the literature.

EXPERIMENTAL

Tris(p-nitrophenyl) phosphate. Garlihe and Laskowski[4] prepared this material by nitrating triphenyl phosphate and obtained a product which crystallized from acetone-water, 1:1, and had a m.p. of 130°. Following their procedure, we obtained a crude product, m.p. 138–145° which, after crystallization from 2-butanone, melted at 154–156°. This is the melting point given by Rapp[2]. An m.p. of 157–159° is reported by Corby, Kenner and Todd.[6]

While the yield of crude III is practically theoretical, the yield of the purified compound by crystallization from 2-butanone was unexpectedly low (15% of theory) in our initial preparation. Upon evaporation of the mother liquor, a large amount of material crystallized out, m.p. 175–176°, whose identity was unknown at the time. This will be referred to later.

Bis(p-nitrophenyl) hydrogen phosphate. Rapp[2] reported the melting point of this compound as 133.5°. Hoeflake[7] has corrected this misinformation and gives the m.p. as 175°. Corby *et al.*[6] record 176–178° for a preparation obtained by the phosphorylation of *p*-nitrophenol. It thus became apparent that the unknown compound, m.p. 175–176°, referred to above, was very likely II. This possibility was confirmed by a mixed melting point determination with II prepared according to Corby *et al.*[6] Investigation of the hydrolytic reaction which yielded II during the preparation of III led to a simple procedure for the preparation of II, illustrated in the following example: 2.4 g. (0.005 mole) *tris* (*p*-nitrophenyl) phosphate, m.p. 154–156, was completely dissolved in about 21 ml. of boiling 2-butanone, 2% (v/v) with respect to water, and refluxed for 3 hr. The solution was evaporated to dryness, first by a stream of air, and finally *in vacuo*. The cake was taken up in 15 ml. boiling chloroform (which did not dissolve all of the solid) and filtered. The crystalline material which separated from the solvent (0.64 g., m.p. 174–175°) was combined with the residue on the filter (0.76 g., m.p. 159–170°) and recrystallized from ethyl *n*-butyrate, yielding 1.1 g. (65% of theory) II, m.p. 175–177°.

(3) S. Yoshida, *J. Biochem.* (*Tokyo*), **34**, 23 (1941).

(4) M. P. de Garlihe and M. Laskowski, *Biochim. et Biophys. Acta*, **18**, 370 (1955).

(5) G. Schmidt, *Methods in Enzymology* Colowick and Kaplan, Ed., Academic Press, New York, N.Y., 1955, II, p. 524.

(6) N. S. Corby, G. W. Kenner, and A. R. Todd, *J. Chem. Soc.*, 1234 (1952).

(7) J. M. A. Hoeflake, *Rec. trav. chim.*, **36**, 26 (1916).

The following comments are of interest. (1) The completeness of the hydrolytic reaction is indicated by the nonappearance of crystalline III which would otherwise separate out from the 2-butanone upon cooling. (2) The addition of mineral acids, *e.g.*, nitric acid, 0.01–0.1*N* with respect to 98% aqueous 2-butanone, did not appear to influence the rate of the reaction. The hydrolysis is, therefore, not strongly catalyzed, if at all, by hydrogen ions. (3) It is not essential to isolate purified III before preparing II. This may be accomplished directly from the crude product resulting from the nitration of triphenyl phosphate. (4) If, however, it is desired to prepare purified III in good yield, (60% or better) it is essential to dry the crude product before crystallizing it from anhydrous solvents. Even the theoretical amount of water may permit appreciable hydrolysis of III, depending upon time and temperature.

Diphenyl hydrogen phosphate. This was prepared according to Asakawa[8] as well as Brigl and Muller,[9] neither of whom report its melting point. It is given by Rapp[2] as 56°. Hoeflake[7] has corrected this to 70°. This is the melting point we also found for the anhydrous compound crystallized from water and dehydrated *in vacuo*.

It has been mentioned that Rapp[2] was unsuccessful in preparing II by the nitration of I. The following example illustrates that such a preparation is feasible: 500 mg. of diphenyl hydrogen phosphate (m.p. 69–70°) was treated with 1.6 ml. HNO_3 (sp. gr. 1.5) by adding increments of the former to the latter while maintaining the temperature at about 10°. The solution was then diluted with 5 ml. cold water and filtered. The precipitate was dried *in vacuo* over KOH and crystallized from *n*-butyl acetate, yielding 274 mg. of product, m.p. 175–176°. This method offers no advantage over the hydrolysis of III, especially as triphenyl phosphate (Eastman-Kodak P 1149) is commercially available whereas diphenyl hydrogen phosphate is not.

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(8) K. Asakawa, *J. Biochem.* (*Tokyo*), **11**, 143 (1929).

(9) P. Brigl and H. Muller, *Ber.*, **72**, 2121 (1939).

Some New Derivatives of Pentachlorophenol and Their Fungistatic Activities

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Pentachlorophenol is a fungistatic agent of well-known activity. It is used as a chemical preservative,[1] but is too toxic for use on plants, usually causing burns.

In view of the reported fungistatic activity of pentachlorophenoxyethanol[2] and related compounds, it seemed of interest to prepare and test other derivatives of pentachlorophenol. It was hoped that certain derivatives might be less phytotoxic than the parent substance and at the same time retain the fungistatic activity. In the present investigation, eight esters, two ethers, and six salts of pentachlorophenol have been synthesized, most of them novel compounds. These have

(1) T. S. Carswell and H. K. Nason, *Ind. Eng. Chem.*, **30**, 622 (1938).

(2) C. W. MacMullen, U. S. Patent **2,416,263** (Feb. 18, 1947); L. C. Felton and C. B. McLaughlin, *J. Org. Chem.*, **12**, 298 (1947).

TABLE I

PROPERTIES AND FUNGISTATIC ACTIVITY OF VARIOUS NEW DERIVATIVES OF PENTACHLOROPHENOL

Compound	M.P., °C.	Formula	Analyses, % Calculated C	H	—	Found C	H	—	Fungistatic Activity[a] M.f.	A.o.
Pentachlorophenyl-*beta*-chloropropionate	108–109	$C_9H_4Cl_6O$	30.20	1.13	59.6Cl	30.32	1.15	59.6Cl	D	D
benzoate	161–2[b]		—	—	—	—	—	—	D[b]	D
p-chlorobenzoate	104	$C_{13}H_4Cl_6O_2 \cdot H_2O$	36.90	1.43	50.4Cl	36.20	1.44	50.5Cl	A	AA
p-nitrobenzoate	187–189	$C_{13}H_4Cl_5NO_4$	37.59	0.97	3.37N	37.12	1.14	3.59N	C	D
dimethylcarbamate	149–151	$C_9H_6Cl_5NO_2$	32.10	1.79	4.15N	32.26	1.72	4.11N	C	C
benzenesulfonate	158–158.5	$C_{12}H_5Cl_5O_3S$	35.50	1.24	7.89S	35.40	1.59	7.95S	D	D
p-toluenesulfonate	157–158	$C_{13}H_7Cl_5O_3S$	37.13	1.68	7.62S	37.50	1.83	7.41S	D	D
p-chlorobenzenesulfonate	148–149[c]	$C_{12}H_4Cl_6O_3S$	—	—	—	—	—	—	D	D
propargyl ether	140.5–141.5	$C_9H_3Cl_5O$	35.50	0.99	58.2Cl	35.28	1.06	58.6Cl	D	D
Diethyl pentachlorophenoxy malonate	75–76	$C_{13}H_{11}Cl_5O_5$	36.80	2.61	41.8Cl	36.71	2.51	40.2Cl	D	D
Pentachlorophenol, salt with dimethyl isopropanolamine	133–134	$C_{11}H_{14}Cl_5NO_2$	35.75	3.82	3.80N	35.60	3.91	3.74N	A	A
isopropylaminoethanol	151.5–153.5	$C_{11}H_{14}Cl_5NO_2$	35.75	3.82	48.0Cl	35.72	3.89	48.5Cl	A	A
N-ethylethanolamine	138.5–140	$C_{10}H_{12}Cl_5NO_2$	33.80	3.38	49.9Cl	33.80	3.54	49.7Cl	A	B
8-hydroxyquinoline	112.5–113.5	$C_{15}H_8Cl_5NO_2$	43.75	1.96	3.41N	43.95	1.84	3.87N	AA	B
quinoline	113	$C_{15}H_8Cl_5NO$	45.55	2.04	3.54N	45.73	1.88	3.56N	A	A
dibutylaminoethanol	–[d]	$C_{16}H_{24}Cl_5NO_2$	43.70	5.50	3.19N	44.03	5.49	3.42N	B	C

[a] ED50 values in p.p.m. are summarized as follows: D = >1000; C = 100–1000; B = 10–100; A = 1–10; AA = 0.1–1, against *Monilinia fructicola* (M.f.) and *Alternaria oleracea* (A.o.).

[b] Previously prepared by H. Biltz and W. Giese, *Ber.* **37,** 4010 (1904) and reported to m. 164–165°. This material is claimed as a mildew-proofing agent for textiles by A. L. Houk, U.S. Patent **2,430,017** (Nov. 4, 1947).

[c] Previously prepared by H. R. Slagh and E. C. Britton, *J. Am. Chem. Soc.*, **72,** 2808 (1950) and reported to m. 146.5–147.5°.

[d] This material was an oil which decomposed upon attempted distillation.

undergone preliminary evaluation for ability to prevent germination of spores of *Alternaria oleracea* Milb. and *Monilinia fructicola* (Wint.) Honey on glass slides.

The chemical and physical properties and fungistatic activities of the new compounds are summarized in Table I. The series exhibiting the highest fungistatic activity was the amine salts. This was the only group of compounds with activity of the same order as copper sulfate. Among the amine salts, the 8-hydroxyquinoline salt showed outstanding activity. This probably is in some measure due to the high fungistatic activity of the 8-hydroxyquinoline itself. All of the compounds tested were phytotoxic.

Although the tests made and number of compounds evaluated were not extensive, one broad generality may be made concerning the relationship between the fungistatic activities and the structures of the materials. The fungistatic activities are highest in those materials in which the pentachlorophenoxy portion of the molecule is ionic, as in the salts.

EXPERIMENTAL

The carboxylic and sulfonic acid esters, with the exception of the β-chloropropionate, were prepared by the reaction of pentachlorophenol with the appropriate acid chloride in pyridine. The β-chloropropionate was prepared by the reaction of β-chloropropionyl chloride with sodium pentachlorophenate in methyl ethyl ketone at 5–10°.

The ethers were prepared by the reaction of sodium pentachlorophenate with the appropriate bromine compound in alcohol.

The salts were prepared by mixing pentachlorophenol with the appropriate nitrogen compound in isopropyl alcohol, benzene, or *n*-hexane. The salts were solids, with the exception of the dibutylaminoethanol salt, which was purified by washing with *n*-hexane.

Biological evaluation. The preliminary data on the fungitoxicity of the compounds described were obtained with the test tube dilution technique[3] used for determining inhibition of spore germination. Spores from 10-day-old cultures of *Alternaria oleracea* and *Monilinia fructicola* served as test objects. Chemicals were prepared for testing by suspending or dissolving in distilled water at an initial concentration of 1000 p.p.m. by first adding a solvent, usually acetone, to make a final volume of 5 per cent and an emulsifier (Triton X-155) to equal 0.01 per cent. Test compounds were given alphabetical ratings which corresponded to the concentration that inhibited germination of half of the spores (ED_{50}) in the test drops: AA = 0.1 to 1.0 p.p.m.; A = 1.0 to 10 p.p.m.; B = 10 to 100 p.p.m.; C = 100 to 1000 p.p.m.; and D = >1000 p.p.m. Fungicides with known performance for a given use were included in all evaluations for comparative purposes. In the spore germination on slides, copper sulfate was used as a standard where an A rating for *Alternaria oleracea* and an AA rating for *Monilinia fructicola* were consistently reproducible. Concentrations were based on copper present. These tests were performed at the Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, N. Y.

(3) American Phytopathological Society. Committee on Standardization of Fungicidal Tests. *Phytopathology,* **37,** 354 (1947).

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