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SOME HIGHLY HALOGENATED PHENOLIC ETHERS AS FUNGISTATIC COMPOUNDS

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The fungistatic activity of the highly halogenated phenols is well known and has been the basis for considerable investigation. For use in the treatment of human infections, solutions of these phenols are too toxic for continued use, being absorbed through the skin. For this reason, these compounds are used mainly as preservatives, as in fungus-resisting paints, mildew-proofing chemicals, and the like (1).

It was thought that certain ethers of these phenols might retain at least some of the fungistatic activity of the parent substances, and at the same time possess lower toxicities. The phenols used were 2,4,6-trichloro-, 2,3,4,6-tetrachloro-, 2,3,4,5,6-pentachloro-, and 2,4,6-tribromo-phenol. Six representative ethers of each phenol were prepared. Since three of the compounds were not obtained sufficiently pure to be tested, results for only twenty-one are given in this report (see Table I). Twelve of the compounds have not been previously described, although the methods of preparation are known.

From the results of the table, it will be seen that in all cases there was a considerable drop in fungistatic ability against *Trichophyton mentagraphtes* #640 of the ether compared to that of the parent phenol. It is interesting to note that the derivatives of tribromophenol possessed only slight activity, although this phenol was apparently the best compound tested. On the other hand, of the ethers derived from the chlorinated phenols, there were several which were appreciably active. The best series was the phenoxyethanols, and even in dilutions of 1:2000, the highest dilution at which the parent phenols showed activity, tetrachlorophenoxyethanol and pentachlorophenoxyethanol were measurably active.

While no definite conclusions may be drawn concerning the relationship between structure and fungistatic strength in this series, it appears that certain generalizations may be made. A halogen atom in the aliphatic portion of the ether produces compounds which have no value as fungistats. Further, the greater the amount of halogen, the lower the activity shown by the compounds (bromoethyl ethers *vs.* dibromopropyl ethers). Alkyl ethers, either saturated or unsaturated (isopropyl and allyl, respectively) appear to be equally active. This activity disappears in the first 10^{-1} dilution. Into this same category fall the phenoxyacetic acids, of current interest as weed killers.

In attempting to evaluate these results, the same test for fungistatic activity was run on propionic acid-sodium propionate, a product which has been used clinically with success. The high degree of activity of this preparation disappears completely on 10^{-1} dilution. Therefore, on the basis of the test employed, several of the ethers prepared in this study appear promising.

TABLE I

HALOGENATED PHENOLS AND THEIR ETHERS

NAME	EMPIRICAL FORMULA	M.P., °C	HALOGEN ANALYSIS, %	FUNGISTATIC ACTIVITY IN MM. OF INHIBITION AGAINST *Trichophyton mentagraphytes* # 640			
				5%	0.5%	0.05%	0.005%
2,4,6-Trichlorophenol	$C_6H_3Cl_3O$	67	—	Comp.[a]	Comp.	9	0
2,3,4,6-Tetrachlorophenol	$C_6H_2Cl_4O$	69	—	Comp.	Comp.	10	
2,3,4,5,6-Pentachlorophenol	C_6HCl_5O	190	—	15	13	5	0
2,4,6-Tribromophenol	$C_6H_3Br_3O$	94	—	Comp.	Comp.	5	0
2-(Trichlorophenoxy)-ethanol (2)	$C_8H_7Cl_3O_2$	77	—	Comp.	5	0	0
2-(Tetrachlorophenoxy)-ethanol	$C_8H_6Cl_4O_2$	51–53	Calc'd 51.41 Found 51.84	25	17	±	0
2-(Pentachlorophenoxy)-ethanol	$C_8H_5Cl_5O_2$	92–94	Calc'd 57.14 Found 57.45	11	10	9	0
2-(Tribromophenoxy)-ethanol (2)	$C_8H_7Br_3O_2$	116	—	5	±	0	
Trichlorophenoxyacetic acid (3)	$C_8H_5Cl_3O_3$	179–181	—	Comp.	0	0	
Tetrachlorophenoxy-acetic acid	$C_8H_4Cl_4O_3$	170–172	Calc'd 49.28 Found 49.47	8	±	0	
Pentachlorophenoxy-acetic acid	$C_8H_3Cl_5O_3$	187–189	Calc'd 54.66 Found 54.32	10	2	0	
Tribromophenoxyacetic acid (3)	$C_8H_5Br_3O_3$	200	—	3	0	0	
Trichlorophenoxyethyl bromide (4)	$C_8H_6BrCl_3O$	47–48	—	2	±	0	
Tetrachlorophenoxy-ethyl bromide	$C_8H_5BrCl_4O$	43–45	Calc'd 65.55 Found 65.19	2	±	0	
Pentachlorophenoxy-ethyl bromide	$C_8H_4BrCl_5O$	77–79	Calc'd 69.00 Found 68.28	5[b]	0	0	
Tribromophenoxyethyl bromide	$C_8H_6Br_4O$	84–86	Calc'd 73.03 Found 73.5	0	0	0	
Allyl trichlorophenyl ether (5)	$C_9H_7Cl_3O$	43–45	Calc'd 46.8 Found 46.61	Comp.	±	0	
Allyl tetrachlorophenyl ether	$C_9H_6Cl_4O$	B.P. 150/ 6 mm.	Calc'd 52.3 Found 52.5	Comp.	0	0	
Allyl pentachlorophenyl ether	$C_9H_5Cl_5O$	101–103	Calc'd 57.84 Found 58.02	3	±	0	

[a] "Comp." indicates complete inhibition on a 90-mm. petri dish.

[b] Initial solution strength was 2.5%.

[c] Initial solution strength was 20%, and dilutions were 10^{-1}, to 2% and 0.2%.

The analyses reported in this table were run by Mr. A. E. Stickels.

TABLE I—*Continued*

NAME	EMPIRICAL FORMULA	M.P., °C	HALOGEN ANALYSIS, %	FUNGISTATIC ACTIVITY IN MM. OF INHIBITION AGAINST *Trichophyton mentagraphytes* # 640			
				5%	0.5%	0.05%	0.005%
Allyl tribromophenyl ether	$C_9H_7Br_3O$	77	—	5	0	0	
β,γ-Dibromopropyl trichlorophenyl ether	$C_9H_5Br_2Cl_3O$	54–56	Calc'd 67.34 Found 67.19	0	0	0	
β,γ-Dibromopropyl pentachlorophenyl ether	$C_9H_6Br_2Cl_5O$	122–124	Calc'd 72.23 Found 73.1	0[b]	0	0	
β,γ-Dibromopropyl tribromophenyl ether	$C_9H_8Br_5O$	42.5–43.5	—	0	0	0	
Isopropyl pentachlorophenyl ether	$C_9H_7Cl_5O$	44.46	Calc'd 57.49 Found 57.85	6	0	0	
Isopropyl tribromophenyl ether (6)	$C_9H_9Br_3O$	40	—	6	±	0	
Propionic acid-sodium propionate	—	—	—	Comp.[c]	0	0	

TABLE II

COMPARATIVE TOXICITY* OF HALOGENATED PHENOLS AND THEIR ETHERS

COMPOUND	M.L.D. PER KILOGRAM
Pentachlorophenol	75 mgm.
Pentachlorophenoxyacetic acid	125 "
Pentachlorophenoxyethanol	250 "
Isopropylpentachlorophenyl ether	300 "
Tetrachlorophenol	250 "
Tetrachlorophenoxyethanol	250 "
Allyl tetrachlorophenyl ether	375 "
Trichlorophenol	250 "
Trichlorophenoxyethanol	250 "
Allyl trichlorophenyl ether	300 "
Tribromophenol	375 "
Sodium propionate-propionic acid	1000 "

* All toxicities are based on intraperitoneal injection in 20 gm. mice.

Toxicities for mice on a number of the compounds in the series were determined and it can be seen that as far as a single dose is concerned, the phenolic ethers are generally less toxic than the parent phenols (see Table II). Because of its considerable fungistatic activity, the lower toxicity of pentachlorophenoxyethanol compared to pentachlorophenol is of particular interest.

On the basis of the preliminary work reported here, it may be seen that several of the new compounds have favorable therapeutic indices. The phenoxyethanols in particular constitute a series of compounds which show definite promise as fungistatic substances.

EXPERIMENTAL

Technique of fungistatic test. A slight modification of the cup plate test of Emmons (7) was employed. Six Sabouraud Dextrose Agar (BBL) plates were inoculated with *Trichophyton mentagraphytes* #640 (supplied by Dr. E. L. Keeney of the Department of Medicine, Johns Hopkins University) and allowed to grow for fourteen days at 27°. The fungus mats were then scraped from the agar surface and placed in a flask containing glass beads and a small volume of saline. After shaking vigorously for ten to fifteen minutes, the beads and mycelium filaments were strained from the spore suspension, using several thicknesses of cheese-cloth.

The spores were counted with a hemocytometer and the suspension so diluted as to contain 10,000,000 spores of the trichophyton per millimeter. Physiological saline (0.85%) was used as the diluent and aseptic technique was observed throughout the preparation of the spore suspension. This suspension can be stored successfully in the refrigerator at 4–10° in screw-cap bottles for several months.

Pour-plates for the test proper were prepared by adding 0.5 cc. of the spore suspension to 22–24 cc. of Sabouraud Dextrose Agar, previously cooled to 42°. After the plates were hardened in a refrigerator for fifteen minutes, holes were cut in the center of the agar with a sterile brass cork borer, 15 mm. in diameter. It is unnecessary to seal the bottom of the hole after the plug has been removed.

One-tenth cc. of the solution or suspension to be tested was then pipetted into the hole. The plates were incubated for four days at 27°. Inhibition was measured in millimeters from the edge of the cup to the nearest place that the fungus growth appears.

Polyethylene glycol-400 was used as a vehicle and diluent for the compounds in this investigation. Dilutions of the sodium propionate-propionic acid were also made with this solvent.

Preparation of phenoxyethanols. The general procedure involved refluxing for three hours 0.1 mole of phenol in 80 cc. of water containing 4.3 g. NaOH with 0.2 mole of ethylene chlorohydrin. The unchanged phenol was washed out with 10% NaOH and the product crystallized from ethanol or dilute ethanol.

Preparation of phenoxyacetic acids. One-tenth mole of phenol was refluxed for three hours with 0.1 mole of chloracetic acid in 80 cc. of H_2O containing 0.2 mole of NaOH. On cooling, the product crystallized and was recrystallized from ethanol or benzene.

Preparation of phenoxyethyl bromides. The procedure used was that of Jacobs and Heidelberger (4).

Preparation of the allyl phenyl ethers. One-tenth mole of phenol in 40 cc. of ethanol containing 0.1 mole of NaOH was refluxed and 0.15 mole of allyl chloride added dropwise over a period of ten minutes. Refluxing was continued for one hour and the reaction mixture added to two volumes of water. The product was water-insoluble. With the exception of allyl tetrachlorophenyl ether, which was distilled, the products were recrystallized from ethanol.

Preparation of the dibromopropyl phenyl ethers. The calculated quantity of bromine in $CHCl_3$ was added to a solution of the allyl ether in $CHCl_3$. The solvent was distilled off and the product recrystallized from ethanol.

Preparation of the isopropyl ethers. One-tenth mole of phenol in 60 cc. of ethanol containing 0.1 mole of KOH was heated in a sealed tube with 0.2 mole of isopropyl chloride for six hours at 140–150°. The reaction mixture was poured into several volumes of water and the product recrystallized from ethanol.

SUMMARY

Six classes of ethers of highly halogenated phenols were prepared and tested for fungistatic activity.

Twelve of the compounds prepared are new.

On the basis of the test employed, the phenoxyethanols reported in this work show definite promise as fungistatic or fungicidal compounds.

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