Effect of the Trifluoromethyl Group on Biological Activity of Certain Arylmercurials

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The effect of the trifluoromethyl group on the biological properties of four new isomeric (trifluoromethyl) phenylmercuric chlorides was evaluated with a representative strain of *Micrococcus pyogenes* (var. *aureus*) using the Vincent paper disk modification of the Oxford cup method. This extension of the chemical investigations was motivated by numerous reports in the literature which indicate that there are certain relationships between the physico-chemical properties of organo-metallic compounds and their respective biological activities. The data given reveal some interesting relationships between the chemical structure and antibacterial activity among some of these new organo-mercury compounds. Results with the above method of evaluation and concentrations of 1:1,000 indicate that *ortho* and *para* (trifluoromethyl) phenylmercuric chlorides are more effective than the corresponding *meta* compound. The bis (trifluoromethyl) mercury compound and the *meta* (trifluoromethyl) phenylmercuric chloride showed activities comparable to that of phenylmercuric chloride.

THIS PRELIMINARY EVALUATION of the antibacterial activity of some new isomeric (trifluoromethyl) phenylmercuric chlorides was conducted as a part of the general investigation of the electronic effect of the trifluoromethyl group on the properties of certain organo-mercury compounds (1). This extension of the chemical study was motivated by numerous reports in the literature which indicate that certain relationships between the physico-chemical properties of organo-metallic compounds and their respective biological activities (2) exist.

Inorganic mercury compounds such as mercuric chloride, mercury oxycyanide, and potassium tetraiodomercurate have long been known as antiseptic agents. The chief objections to their therapeutic use are their toxicity and irritating action upon tissue (3).

Various theories for the mechanism of action of these mercurials have been proposed (3). Fildes (4) suggested that the antibacterial activity of mercurials is due to the interference with sulfhydryl (S-H) containing compounds which are essential cellular metabolites. This suggestion led to the use of phenylmercuric salts—notably the acetates, borate, and nitrate. The acid radical influences the solubility and other physical properties of the cation but does not alter its antibacterial activity (3). Since organo-mercuric compounds do not readily liberate mercuric ions, they are reported to be less toxic, less irritating, less corrosive to surgical instruments, and retain a higher degree of antibacterial activity in the presence of organic matter than inorganic mercuric compounds (3).

The antibacterial activity of various substituted phenylmercurials has been investigated and compared (5); the antibacterial activity of some fluorine-substituted aromatic mercurials also has been reported (6).

A survey of the literature reveals that in many areas of medicinal and pharmaceutical chemistry, one of the approaches often employed in searching for new drugs is the synthesis of structural modifications of prototype compounds which are known to possess interesting biological properties. During the past decade many efforts have been devoted to the incorporation of the trifluoromethyl group into such prototype molecules—in some instances in place of a methyl group, and in some cases instead of a chlorine atom. It is generally anticipated that such trifluoromethyl analogs might be acceptable as medicinal agents in view of the known unique chemical and physiological stability of the trifluoromethyl group (7).

Since it has been shown that the trifluoromethyl group affects the physico-chemical properties of certain organo-mercurials of this type, it was of interest to determine the effect of the trifluoromethyl group on the biological properties of these agents.

EXPERIMENTAL

Many methods have been developed and used to conduct antibacterial tests (3). The Oxford cup method (8), with several modifications (9), has been a widely accepted procedure because of its commensurate accuracy, speed, and minimum of labor. The filter paper disk modification of the Oxford method also has been successfully used. For expediency, this method is considered very practical in preliminary evaluations; consequently, the Oxford method as modified by Vincent (10) was employed in this study.

MATERIAL

The representative strain, *Micrococcus pyogenes* (var. *aureus*), used in the investigation was provided by the Microbiology Department, University of Texas, Austin. The nutrient agar and nutrient broth employed were from Difco Laboratories, Detroit, Mich.

Phenylmercuric chloride was prepared according to the method of Gilman, *et al.* (11), and trifluoromethyl phenyl mercuric chlorides were prepared by the method described by Maung and Lagowski (12). The bis (trifluoromethyl) mercury was prepared by the method described in the literature (13, 14). All the compounds were purified by sublimation *in vacuo* prior to use.

PROCEDURE AND RESULTS

Since the mercurials used were practically insoluble in water (except the bis (trifluoromethyl) mercury), test samples of 1:1,000, 1:10,000, and 1:100,000 were prepared in the following manner. The accurately-weighed quantity of required mercurial

Received June 3, 1963, from the College of Pharmacy, University of Texas, Austin.
Accepted for publication July 17, 1963.
The authors gratefully acknowledge the financial support from the R. A. Welch Foundation and a scholarship from the Burmese Government.

TABLE I.—ZONES OF INHIBITION OBSERVED WITH AQUEOUS SUSPENSIONS OF SOME FLUOROARYLMERCURIALS

Compd.	Concn.	Diam. of Inhibition, Zones in mm.	Av. Diam. Inhibition Zones in mm. ± A. D.
C_6H_5HgCl	1:1,000	21, 22, 20, 20, 20	20.6 ± 0.72
	1:10,000	19, 18, 17, 17, 18	17.8 ± 0.64
	1:100,000	16, 15, 15, 15, 17	15.6 ± 0.72
o-$CF_3C_6H_4HgCl$	1:1,000	30, 30, 30	30.
	1:10,000	19, 20, 20, 20	19.8 ± 0.35
	1:100,000	16, 16, 17, 16	16.2 ± 0.35
m-$CF_3C_6H_4HgCl$	1:1,000	22, 22, 22	22
	1:10,000	19, 19, 19	19
	1:100,000	18, 17, 17	17.3 ± 0.43
p-$CF_3C_6H_4HgCl$	1:1,000	24, 24, 23	23.7 ± 0.43
	1:10,000	19, 19, 19	19
	1:100,000	16, 17, 17	16.7 ± 0.43
$(CF_3)_2Hg$	1:100	22 (37)[a], 23 (39)[a], 24 (38)[a]	23.0 ± 0.67 (38.0 ± 0.67)[a]
	1:1,000	20 (27)[a], 20 (27)[a], 20 (26)[a]	20.0 (26.7 ± 0.43)[a]
	1:10,000	15, 15 (d)[b], (d)[b]	15

[a] Values in parentheses indicate practical inhibition. [b] d = Diffuse secondary zone.

for each sample was dissolved in a minimum amount of acetone, then poured into 75 ml. of 0.5% tragacanth mucilage. This mixture was stirred for approximately 30 minutes, warmed in a water bath to remove the acetone, and then made up to the required volume (100 ml.) with distilled water. Each test sample of the bis (trifluoromethyl) mercury was prepared by dissolving the drug, accurately weighed, in the required amount of distilled water.

Each plate was prepared by distributing 0.1 ml. of the inoculated broth (broth incubated for 24 hours with test organism) in 15 ml. of warm Difco agar, then pouring the mixture into the Petri dish.

Paper disks, 12 mm. in diameter, were saturated with the test samples, superimposed on the media, and the plates incubated at 37° for 24 hours. All tests were performed three or more times. Distilled water and a tragacanth mucilage prepared in the manner described above without the drugs were used as controls; results expressed as zones of inhibition appear in Table I.

DISCUSSION AND SUMMARY

All the test mercurials gave zones of inhibition with the dilutions employed. The tragacanth control showed some diffusion of material around the paper disks; however, no distinct zones of inhibition could be observed with any of the controls.

It is apparent from Table I that with 1:1,000 dilution, the *ortho* and *para* (trifluoromethyl) phenylmercuric chlorides were the most active of the compounds studied; the *meta* compound was the least active among the isomeric (trifluoromethyl) phenylmercuric compounds; the bis trifluoromethyl mercury and the *meta* trifluoromethyl compound exhibited activities comparable to that of phenylmercuric chloride.

Considering these concentrations, it is of interest to note the relationship between the position of the trifluoromethyl group on the ring and the relative antibacterial activity. A similar relationship has been reported among the halogenomercury compounds (XC_6H_4HgCl, X = F, Cl, Br, and I), where the *ortho* and *para* compounds are more active than the corresponding *meta* compound (5, 6).

It might be expected from the consideration of ionization constants and solubility products shown in Table II that the trifluoromethyl analogs would be less effective than phenylmercuric chloride. However, extensive hydrolysis of the (trifluoromethyl) phenylmercuric compound and the phenylmercuric compound will tend to minimize differences in the tendency of the chloride to ionize; no justifiable conclusion can be made regarding a relationship between ionization constants or solubility products of the compounds and their antibacterial activity. But if the antibacterial activity of mercury compounds is postulated to be due to the complexing ability of the RHgX, *i.e.*, to its ability to combine with the sulfhydryl (S-H) groups on certain enzyme systems (3), it is not surprising that $CF_3C_6H_4HgCl$ and XC_6H_4HgCl (X = F, Cl, Br and I) are more active than C_6H_5HgCl.

With the trifluoromethyl and the halogen analogs, the electron attracting trifluoromethyl group and the halogen might alter the electronic environ-

TABLE II.—IONIZATION CONSTANTS AND SOLUBILITY PRODUCTS OF TRIFLUOROMETHYL PHENYL MERCURIC COMPOUNDS (12)

Cation	Constant	OH^-	Cl^-	Br^-	I^-
p-$CF_3C_6H_4Hg$	K_i	8.3×10^{-11}	...	...	...
	K_{sp}	...	1.2×10^{-10}	1.0×10^{-12}	7.7×10^{-16}
o-$CF_3C_6H_4Hg$	K_i	9.1×10^{-11}	4.6×10^{-6}	...	...
	K_{sp}	...	2.6×10^{-10}	6.3×10^{-12}	3.5×10^{-15}
m-$CF_3C_6H_4Hg$	K_i	1.2×10^{-10}	6.0×10^{-6}	...	...
	K_{sp}	...	4.8×10^{-10}	8.3×10^{-12}	3.9×10^{-5}
C_6H_4Hg	K_i	1.3×10^{-10a} 1.0×10^{-10b}	...	...	...
	K_{sp}	...	5.0×10^{-10}	1.8×10^{-12}	1.0×10^{-15}

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ment of the mercury atom and make it a more effective electron-pair acceptor (15).

If the antibacterial activity changes with the electronic effect of the halogen and the trifluoromethyl groups, substitution in the *ortho* and *para* positions would affect the electronic environment of the mercury atom more than substitution in the *meta* position, and the activity of former compounds would be greater than the latter chemical. This was observed with the high concentrations.

It might be postulated that with very dilute samples certain factors such as the dispersion of the drug, the media, or the dilution factor might overshadow or interfere in some manner with the electronic effect of the trifluoromethyl group. Perhaps other testing techniques which are more sensitive should be employed to test the antibacterial activity of the very dilute samples.

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Colorimetric Determination of Chlorpheniramine Maleate

By JOSEPH HUDANICK

A sensitive colorimetric method has been developed for determining chlorpheniramine maleate in various pharmaceutical preparations. The method is especially suitable as a rapid control method.

CHLORPHENIRAMINE MALEATE, a pyridine derived antihistamine, is presently marketed in a variety of pharmaceutical preparations. Its determination is especially difficult when present in small amounts and in combination with certain other antihistamines and related compounds. Ordinary methods (1–3) such as ultraviolet, chromatographic, and gravimetric techniques, are time consuming and relatively nonselective. Jones and Brady (4) describe a general colorimetric method for determining pyridine derived antihistamines which is a modification of the Koenig reaction (5, 6). This method in its present form is of low sensitivity and unsuitable for mixtures of antihistamines. By changing the reaction conditions and using sulfanilic acid instead of aniline and an acetate buffer instead of phthalate, chlorpheniramine formed a very intense transient yellow suitable for quantitative analysis. The intensity of the color (absorption peak at 480 mμ) is approximately 40 times that obtained by the Jones and Brady method and about three times as sensitive as the ultraviolet method. In addition, the color is very reproducible (standard deviation 0.4%) and unaffected by the presence of methapyrilene HCl and pyrilamine maleate, two pyridine derived antihistamines which are commonly associated with chlorpheniramine in many preparations. The very closely related compounds, pheniramine maleate and brompheniramine maleate, react the same as chlorpheniramine maleate. The method has been found suitable for determining chlorpheniramine in many varied tablet combinations and timed release pellets.

Received June 5, 1963, from the Rexall Drug Co., St. Louis, Mo.
Accepted for publication July 20, 1963.

EXPERIMENTAL

Buffered Sulfanilic Acid Solution.—Dissolve 2.5 Gm. of sulfanilic acid and 4.00 Gm. of anhydrous

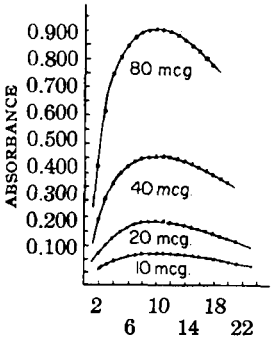

Fig. 1.—Plot of absorbance *vs.* time for specific concentrations of chlorpheniramine maleate.

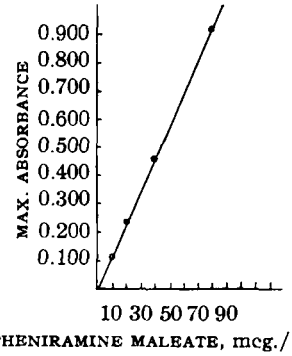

Fig. 2.—Plot of maximum absorbance of developed color *vs.* concentration.