organic mercurial such as *PT*–46, wherein the mercury is directly linked to a carbon atom of the cyclic nucleus, is not unlike a diuretic type compound in so far as absorption is concerned. When excretion studies are considered, the difference in behavior of the two types is marked. Nearly all of the injected mercury, in the case of *PT*–46, is retained and stored in the tissues. This difference cannot be ascribed to a variation in the stability of the mercury–carbon linkage but must be due to specific structural design which permits absorption from the circulation with subsequent metabolic destruction of the compound and retention of mercury.

Organic mercurial diuretics appear to exert a direct and pronounced toxic action on the heart when administered in sublethal and lethal doses. The toxic reaction produced by *PT*–44 with theophylline injected intravenously was much more intense than that caused by Salyrgan-Theophylline although both types of compounds possessed the same margin of safety on intramuscular administration.

SUMMARY

1. The relative position of the mercury–carbon linkage in an organic mercurial does not significantly influence the rate of absorption of the compound from muscle.

2. A weakly acid nitrogen heterocycle, theophylline or succinimide, enhances the rate of absorption of nondiuretic as well as diuretic types of compounds.

3. The type and position of mercury–carbon linkage exerts a profound influence on the retention of mercury whether it be administered intravenously or intramuscularly since a nondiuretic type is almost completely retained.

4. Succinimide coupled with a nondiuretic type of derivative does not significantly alter the degree of excretion of mercury.

5. Chemical stability of the mercury–carbon linkage appears to be of secondary importance in so far as absorption and excretion are concerned.

6. An organic mercurial derived from 2-pyridone-5-carboxylic acid was found to be more toxic than Salyrgan.

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Bacteriostatic Properties of Certain Derivatives of Thiophene*

By H. W. Rhodehamel, Jr., and E. F. Degering

The chemical and physical properties of thiophene and its derivatives have been studied extensively by many investigators since its discovery by Victor Meyer in 1883 (1). No systematic study has been made, however, of the bacteriostatic properties of thiophene or its derivatives, although a few thiophene compounds have been used in medicine. Thiophene itself has been used as an antiseptic (2), sodium thiophenesulfonate and diiodothiophene have been used in the treatment of skin diseases (3), and tetrabromothiophene has been recommended as an efficient antiseptic (4). Many investigators believe that the large percentage of thiophene derivatives in ichthyol is responsible for its therapeutic properties (5, 6).

Recently a patent was issued in which it was claimed that aromatic mercury substituted derivatives of thiophene "have extraordinarily high potency as antiseptics and germicides and at the same time are characterized by relatively low toxicity and other desirable properties (7)." Several studies have shown that the replacement of a

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phenyl group by a 2-thienyl group does not alter, qualitatively at least, the pharmacological activity (8, 9, 10, 11).

With these and other facts in mind, it was thought it might be of value to synthesize certain derivatives of thiophene and to test them for bacteriostatic properties. No attempt has been made to produce a series of compounds to obtain comparative results within that series, but to produce instead miscellaneous derivatives which offer a means of comparison with compounds of known antiseptic value and which indicate future trends in research.

EXPERIMENTAL

A number of thiophene derivatives have been made by methods available in the literature. All syntheses started with thiophene obtained either from the Eastman Kodak Company or prepared from the fusion of sodium succinate and phosphorus trisulfide (12).

2-Acetothienone was prepared from thiophene by acetylating with acetyl chloride in the presence of fuming stannic chloride (13). This in turn was oxidized to 2-thiophenecarboxylic acid by hydrogen peroxide and potassium permanganate (14). The 2-thiophenecarboxylic acid was esterified to phenyl 2-thiophenecarboxylate by a modification of a method presented in "Organic Syntheses" (15). 5-Chloromercuri-2-thiophenecarboxylic acid was prepared by the interreaction of 2-thiophenecarboxylic acid and mercuric chloride in the presence of mercuric acetate (16).

The reaction between mercuric acetate and 2-thiophenecarboxylic acid yielded a product whose properties did not correspond to those of the predicted product, 5-acetoxymercuri-2-thiophenecarboxylic acid. The compound obtained is assumed to be the mercuric acetate salt of 2-thiophenecarboxylic acid.

2-Chloromercurithiophene was prepared by the reaction between thiophene and mercuric chloride in the presence of sodium acetate in the cold (17).

2,5-Dichloromercurithiophene was obtained by refluxing thiophene with mercuric chloride in the presence of sodium acetate (18).

2-Chloromercuri-5-chlorothiophene was prepared from 2-chlorothiophene which was made by the method of Töhl and Eberhard (19) and mercuric chloride in the presence of sodium acetate (20).

2,5-Bis(acetoxymercuri)-thiophene was prepared from thiophene and mercuric acetate in the cold (21). 2,3-Bis(acetoxymercuri)-5-nitrothiophene was obtained by refluxing 2-nitrothiophene (22) and mercuric oxide in glacial acetic acid for two hours (23).

Diphenyl-2-thienylacetic acid was prepared by the condensation of benzilic acid with thiophene in glacial acetic acid and concentrated sulfuric acid (24). Triphenylacetic acid was prepared from benzilic acid and benzene in the presence of fuming stannic chloride (25).

Phenyl benzoate was prepared by a modification of a method presented in "Organic Syntheses" (15).

Testing of the Compounds.[1]—Because of the range of solubilities of these compounds and the attending difficulty of obtaining solvents for each, preliminary bacteriostatic testing has been confined to a spot test. In these tests the standard *Staphylococcus aureus* strain No. 209 was used in poured agar plates made in the usual way, both with plain agar and with a 10% serum agar. The undiluted compound was applied like patch tests to both the plain and serum agar. Following incubation at 37° C. for 24 hrs., the zones of clearing about the applied compound were measured. The dimensions of the zone of clearing are indicative of the antiseptic property of the compound being tested, the relationship being

[1] The bacteriostatic testing was carried out by the Lilly Research Laboratories, Indianapolis, Ind.

TABLE I.—BACTERIOSTATIC TESTING

	Compound Tested	Zone of Clearing, Mm., on *Staph. aureus* Poured Agar Plates Using Plain Agar	10% Serum Agar
(1*a*)	Acetophenone	30	10
(1*b*)	2-Acetothienone	20	20
(2*a*)	Benzoic acid	10	Precipitation
(2*b*)	2-Thiophenecarboxylic acid	8	Precipitation
(3*a*)	Phenyl benzoate	1	1
(3*b*)	Phenyl 2-thiophenecarboxylate	5	2
(4*a*)	Triphenylacetic acid	1	1
(4*b*)	Diphenyl-2-thienylacetic acid	9	5
(5*a*)	Phenyl mercuric chloride	10	10
(5*b*)	2-Chloromercurithiophene	10	10
(5*c*)	Pyridyl mercuric chloride	30	12
(6)	2,5-Bis(acetoxymercuri)-thiophene	10	5
(7)	2,3-Bis(acetoxymercuri)-5-nitrothiophene	7	7
(8)	5-Chloromercuri-2-chlorothiophene	7	5
(9)	5-Chloromercuri-2-thiophenecarboxylic acid	10	10
(10)	2,5-Dichloromercurithiophene	7	5
(11)	Product of the reaction between mercuric acetate and 2-thiophenecarboxylic acid	15	16
(12)	Merthiolate (sodium ethyl mercuri thiosalicylate, Lilly)	30	15

directly proportional (*cf.* Circular 198 of the Department of Agriculture (26)).

Interpretations of the Bacteriostatic Testing.—The results of the bacteriostatic testing of certain derivatives of thiophene and certain isosteric compounds are given in Table I. Merthiolate (sodium ethyl mercuri thiosalicylate, Lilly) has also been tested and included as an antiseptic of known properties.

Although it is inadvisable to draw conclusions from too few data, it appears from these tests that the substitution of the thiophene nucleus for a phenyl group either does not change the bacteriostatic properties or renders the derivative somewhat more active. For example, diphenyl-2-thienylacetic acid is nine times more effective against *Staphylococcus aureus* in plain agar than is triphenylacetic acid and five times as effective in serum agar. Similarly, phenyl 2-thiophenecarboxylate exhibits greater activity than phenyl benzoate.

Phenyl mercuric chloride and 2-thienyl mercuric chloride are of about the same activity. Acetophenone and 2-acetothienone are of the same order of activity; one is more active in plain agar, the other in serum agar. These two compounds are both liquids and cannot accurately be compared to the solid derivatives.

2-Thiophenecarboxylic acid demonstrates rather low activity, yet it compares favorably with benzoic acid. Tests could not be carried out for either of these compounds in serum agar since they both cause precipitation of the serum. The other derivatives showed no outstanding activity.

SUMMARY

1. Eleven derivatives of thiophene were prepared and their bacteriostatic properties tested along with closely related compounds of the benzene series and certain other substances against *Staphylococcus aureus* both in plain agar and in 10% serum agar.

2. No compound showed outstanding bacteriostatic activity.

3. The thiophene nucleus substituted in a compound for the phenyl group seems to cause in the derivative either the same or a somewhat enhanced activity against *Staphylococcus aureus* in comparison with the original substance.

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