[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE STATE UNIVERSITY OF IOWA]

Bactericidal Properties of Certain Organomercuric Acetates

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On the basis of certain physical constants of organic compounds Hixon[1] has compiled an electron-sharing ability series of organic radicals. A part of this series is given in the following list in the order of increasing electron-sharing ability: *n*-butyl, ethyl, methyl, benzyl, *p*-tolyl, *p*-methoxyphenyl, phenyl, *o*-chlorophenyl, and *o*-nitrophenyl.

The present investigation was undertaken for the purpose of determining whether there is a relation between the electron-sharing abilities of organic radicals and the toxicities to bacteria of certain organomercuric compounds in which the radicals occur. The compounds used were the organomercuric acetates containing the radicals: *n*-butyl, *n*-propyl, ethyl, methyl, benzyl, *p*-tolyl, *p*-methoxyphenyl, phenyl, *o*-chlorophenyl and *o*-nitrophenyl.

The organomercuric acetates were selected after considerable preliminary study on the preparation and properties of several types of organomercury compounds including the nitrates, the hydroxides and the halides.

For the determination of the bactericidal properties of the organomercuric acetates, eighteen-hour cultures of *Serratia marcescens*, *Eberthella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Aerobacter aerogenes* and *Staphylococcus aureus* were used.

The aromatic organomercuric acetates are more toxic than those in the aliphatic series and were used in more dilute solutions. In the aliphatic series the toxicity of the organomercuric acetates was found to increase as the radical was changed from methyl to *n*-butyl. While the *n*-propyl radical is not shown in Hixon's list, the toxicity of the alkyl mercuric acetates containing the methyl, ethyl and *n*-butyl radicals places them in the same order as given in the electron-sharing ability series. There is no significant difference in the toxicities of the six aromatic organomercuric acetates used.

Experimental

Organomercuric Acetates.—The organomercuric acetates which contain the radicals methyl, ethyl, *n*-propyl, *n*-butyl, phenyl, benzyl, and *p*-tolyl, were prepared by a series of reactions in which the Grignard reagent, the organomercuric halide and organomercuric hydroxide were intermediates. The organomercuric halides were prepared in a manner similar to that given by Whitmore[2] for the preparation of phenyl mercuric bromide. The organomercuric acetates were obtained from the organomercuric halides by the method used by Sneed and Maynard[3] for the preparation of methylmercuric acetate. *o*-Chlorophenyl and *o*-nitrophenylmercuric acetates were prepared by the method used by Hanke.[4] *p*-Methoxyphenylmercuric acetate was prepared by the method of Dimroth,[5] by heating anisole and mercuric acetate.

In Table I are listed the organomercuric acetates, the observed melting points of which differ appreciably from the values recorded in the literature or about which there has been disagreement.

TABLE I

ORGANOMERCURIC ACETATES

Radical	Yield,[a] %	M. p., °C.[b]	Mercury, % Calcd.	Found	
Methyl	70	127–128	73.0	72.8	72.5
Ethyl	60	69–69.8	69.4	69.1	69.0
n-Propyl	60	54.5–55.1	66.2	66.0	66.1
n-Butyl	55	52.5–53.2	63.3	63.5	63.6
Phenyl	80	151.8–152.8	59.71	59.72	59.5
Benzyl	84	128–128.8	57.3	57.26	57.42
p-Tolyl	80	149.6–151	57.3	57.5	57.2

[a] All yields are calculated on the basis of the organomercuric halide. [b] All melting points are corrected.

Method of Determining Bactericidal Properties.—The organisms used were inoculated on to plain agar in sterile bottles and incubated for eighteen hours at a temperature of 37°. Using standard bacteriological procedures suspensions of the washed organisms were made in distilled water. To each of a series of tubes containing 5 cc. of the solutions to be tested was added, at intervals of thirty seconds, 0.5 cc. of the suspension of the eighteen-hour culture of the organism used and the two thoroughly mixed. At definite intervals of time thereafter, subcultures were made of each tube containing the mixture of organism and compound studied, to determine whether the organisms had been killed.

The bactericidal studies with each organism were made using solutions of the acetates of two or more different concentrations. Since the results with all organisms were so similar the following typical examples only are given.

Serratia Marcescens.—Using 0.00008 molar solutions of *n*-butyl, *n*-propyl, ethyl, and methylmercuric acetates

(1) Hixon, THIS JOURNAL, **49**, 1786 (1927); **50**, 168 (1928); **53**, 4367 (1931); **54**, 3971 (1932); **56**, 1329, 1333 (1934).

(2) Whitmore, "Organic Compounds of Mercury," Chemical Catalog Co., N. Y., 1921, p. 173.

(3) Sneed and Maynard, THIS JOURNAL, **44**, 2942 (1922).

(4) Hanke, *ibid.*, **45**, 1321 (1923).

(5) Dimroth, *Ber.*, **35**, 2867 (1902).

the organisms did not grow after exposures of six, ten, sixteen and twenty minutes, respectively.

Klebsiella Pneumoniae.—With 0.00008 molar solutions of *n*-butyl, *n*-propyl, ethyl, and methylmercuric acetates the organisms did not grow after exposures of four, eight, fourteen, and eighteen minutes, respectively.

E. Coli.—Using solutions (concentration 1:100,000) of benzyl, *p*-tolyl, *p*-methoxyphenyl, phenyl, *o*-chlorophenyl, and *o*-nitrophenylmercuric acetates the organisms did not grow after exposures of fifteen, fifteen, eighteen, eighteen, eighteen, and eighteen minutes, respectively.

Summary

The organomercuric acetates containing the radicals methyl, ethyl, *n*-propyl, *n*-butyl, benzyl, *p*-tolyl, *p*-methoxyphenyl, phenyl, *o*-chlorophenyl and *o*-nitrophenyl have been prepared and the bactericidal action determined with several organisms.

In the aliphatic series the toxicity of the compounds increases uniformly from methylmercuric acetate to *n*-butylmercuric acetate.

The toxicity of the aromatic mercuric acetates is greater than that of the aliphatic compounds. There is no significant difference in the toxicities of the six aromatic organomercuric acetates used.

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Sterols. XXIV. Sitostenone and Stigmastenone

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Tallol-sitosterol is one of the most readily available sterols since, in contrast to the difficulty of isolation of pure phytosterols from wheat germ oil, it may be obtained easily in a pure state from pine oil.[1] Although their identity has not been established with certainty, β-sitosterol from wheat germ oil,[2] cinchol from cinchona bark,[3] and tallol-sitosterol are probably the same substance. Therefore, we shall, for convenience, designate tallol-sitosterol simply as sitosterol.

Bengtsson[4] found no depression in melting point on mixing comparable derivatives of stigmastanol and sitostanol; since these derivatives included the ketones and hydrocarbons as well as a number of esters, it is highly probable, if not certain, that sitosterol is 22-dihydrostigmasterol.

We have compared the melting points of a number of new substances related to stigmasterol and sitosterol and find no depression in melting point on mixing corresponding derivatives. The accompanying chart (Fig. 1) shows the relationship of these derivatives to the parent sterols. It may now be said with certainty that sitosterol from pine oil is identical with 22-dihydrostigmasterol; since the complex interconversions reported here exclude any chance coincidences.

Stigmasterol (I) and sitosterol (X), upon dehydrogenation with copper powder at 200° under reduced pressure, yield stigmastenone (II) and sitostenone (IX), respectively. The latter has been prepared previously by Heiduschka and Gloth[5] by the oxidation and subsequent debromination of sitosterol dibromide. α-Fucostenone, prepared in a somewhat similar manner from α-dihydrofucosterol,[6] is probably identical with sitostenone. Both stigmastenone (II) and sitostenone (IX), on catalytic hydrogenation, and subsequent treatment with sodium in boiling xylene to render small amounts of allo compounds precipitable with digitonin, yield 24-ethyl-*epi*-coprostanol (V).[7] This, upon oxidation with chromium trioxide in acetic acid, gives 24-ethylcoprostanone (VII). The latter, when reduced with aluminum isopropylate, gives a mixture of 24-ethyl-*epi*-coprostanol (V) and 24-ethylcoprostanol (β) (VI) which may be separated by the use of digitonin. 24-Ethylcoprostanol (β) (VI) on treatment with sodium in boiling xylene, is epimerized to give as the chief product, 24-ethyl-*epi*-coprostanol (V). The bromination of 24-ethylcoprostanone (VII) in acetic acid gives 4-bromo-24-ethylcoprostanone (VIII), which, on treatment with pyridine, gives sitostenone (IX) identical with that obtained by the dehydrogenation of sitosterol (X).

(1) Sandqvist and Bengtsson, *Ber.*, **64**, 2167 (1931).

(2) Anderson, Burr and Shriner, THIS JOURNAL, **48**, 2987 (1926).

(3) Strain, in "Organic Chemistry," ed. by H. Gilman, John Wiley and Sons, Inc., New York, 1937, Vol. II, Chap. 15.

(4) Bengtsson, *Z. physiol. Chem.*, **237**, 46 (1935).

(5) Heiduschka and Gloth, *Arch. Pharm.*, **257**, 415 (1915).

(6) Coffey, Heilbron and Spring, *J. Chem. Soc.*, 738 (1936).

(7) The name coprostanol seems preferable to coprosterol since the former, but not the latter name, corresponds to the epimer, cholestanol. The suffix sterol should, perhaps, be applied only to carbinols unsaturated in the first or second ring. It should be noted that the only known naturally occurring substance affected by this suggestion is coprostanol.