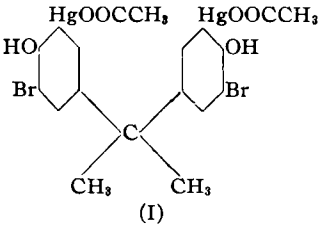
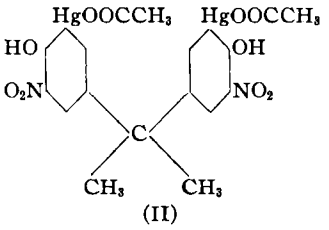
STUDY OF GERMICIDAL AND ANTISEPTIC ACTIVITY OF SOME MERCURY COMPOUNDS.*[1]

BY E. MONESS, S. E. HARRIS AND W. G. CHRISTIANSEN.

Three compounds were selected for this study, 3,3′-dibromo-4-4′-dihydroxy 5-5′-diacetoxymercuri diphenyl dimethyl methane (I); 3,3′-dinitro-4-4′-dihydroxy-5-5′-diacetoxy-mercuri-diphenyl-dimethyl-methane (II); and a mono-acetoxy-mercuri-derivative of 5′,5″-dibromo-resorcinol diphenein (III). The position



(I)



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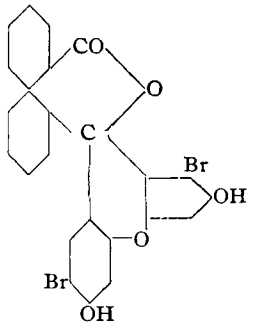

(III)

of the mercury in the product (which will be designated as (IV)) obtained from (III) was not determined. Mercurated dibromo-fluorescein is believed to be mercurated in one of the resorcinol nuclei. However, recent studies in this laboratory on mercurated dibromo-diphenyl-phenol-phthaleins (1) and mercurated derivatives of substituted diphenol-isatin (2) strongly suggest that this type of compound may mercurate in the phthalic acid, diphenic acid or corresponding portions of the molecule.

The three compounds all showed useful germicidal activity:

TABLE I.—TESTED IN DISTILLED WATER.

Compound.	Dilution Killing. Typhoid. 5 Min.	Dilution Killing. Typhoid. 10 Min.	Dilution Killing. Staphylococcus Aureus. 5 Min.	Dilution Killing. Staphylococcus Aureus. 10 Min.
(I)	1–1500	1–1500	1–1500	1–2500
(II)	1–1000	1–1500*	1–5000	1–5000
(IV) *a* 1st batch	1–250	1–2000	1–500	1–1000
b 2nd batch	1–1000	1–4000	1–500	1–1000

* Inhibits growth only—does not kill.

TABLE II.—TESTED IN BLOOD SERUM.

Compound.	Dilution Killing. Typhoid. 5 Min.	Dilution Killing. Typhoid. 10 Min.	Dilution Killing. Staphylococcus Aureus. 5 Min.	Dilution Killing. Staphylococcus Aureus. 10 Min.
(I)	1–500	1–500	1–1000	1–1500
(II)	*Not Tested.*			

* Section on Practical Pharmacy and Dispensing, Madison meeting, 1933.

[1] Research Department of the Chemical and Pharmaceutical Laboratories, E. R. Squibb and Sons, Brooklyn, N. Y.

(IV)	*a*	1st batch	1–100	1–1000	1–250	1–500
	b	2nd batch	1–250	1–1000	1–100	1–500

Compound (IV) was further tested for bacteriostatic activity.

Batch.	Dilution at Which Compound Is Bacteriostatic. Typhoid.		Staphylococcus.	
	24 Hrs.	48 Hrs.	24 Hrs.	48 Hrs.
1	1–20,000	1–10,000	1–30,000	1–20,000
2	1–100,000	1–50,000	1–500,000	1–250,000

The examination also included tests of the action of compounds (I) and (IV) on tissue, with the following results:

Compound I.—1. Non-irritating to shaved unabraded skin and to shaved abraded skin. 2. Produces slight to moderate swelling in subcutaneous tissue on repeated injection persisting for 21–24 days. 3. Autopsies following injection showed no degenerative changes due to the toxicity of the compound in liver or kidney. The autopsies were performed at the end of the test, so that no information was furnished as to possible temporary toxic action.

Compound IV.—1. Non-irritating to shaved and unabraded skin. 2. Only slightly irritating to shaved abraded skin. 3. Produces very slight swelling on subcutaneous injection and a scab at the site of intradermal injection.

Solutions Used in Tests.—The compound (IV) was prepared for test by dissolving in water containing 2 molecular equivalents of sodium hydroxide and diluting to a final concentration of 2%. This solution was diluted with distilled water immediately prior to the germicidal and bacteriostatic tests. The same solution was used in the animal experiments.

Solution of Compound (I) was effected in an exactly similar manner, except that the original concentration was 1–500. Compound (II), however, required a large excess (about 15 mols.) NaOH to produce a clear 1–250 or 1–500 solution. The latter concentration was used for testing.

The animal experiments were conducted on albino rats.

EXPERIMENTAL.

The preparation of these compounds was carried out along familiar lines, mercuration of the suitable intermediates being effected in boiling alcohol solution.

Resorcinol Diphenein.—The diphenein required for compound (IV) was prepared by the procedure of Dutt (3). The starting material was technical 70% phenanthrene which was oxidized to phenanthraquinone by the method of Oyster and Adkins (4), and subsequently to diphenic acid according to German Patent 516,282, using sodium peroxide as the oxidizing agent. Diphenic anhydride was then prepared by the method of Oyster and Adkins (5) and condensed with resorcinol (3). The product, however, did not melt at 172° C. as stated by Dutt, but softened at 70° C. and is completely melted at 100° C. The bromine derivative, however, was prepared without difficulty.

Dibromo-Resorcinol-Diphenein.—Thirty-five cc. of a 20% solution of bromine in glacial acetic acid was added to a well-stirred solution of 8.6 Gm. of resorcinol diphenein in 170 cc. glacial acetic acid. The mixture was heated on the steam-bath for fifteen minutes and poured into 2000 cc. of cold water. The yellow flocculent precipitate was filtered off, washed with water and dried, giving a yellow powder.

0.2277 Gm. of substance required 7.92 cc. of $N/10$ $AgNO_3$ solution.

Br found, 27.83%.
Calculated for $C_{26}H_{14}O_5Br_2$, 28.26%.

Mercuration of Dibromo-Resorcinol-Diphenein.—Fifteen Gm. of dibromo-resorcinol-diphenein was dissolved in 250 cc. of alcohol and the solution refluxed with good stirring. A solution of 8.41 Gm. of mercuric acetate in 42 cc. of water slightly acidified with acetic acid was then added dropwise during 45 minutes to the well-stirred, boiling diphenein solution. After boiling and stirring for a further period of 15 minutes a brown precipitate had formed and the clear superna-

tant liquid gave a negative test for inorganic mercury with ammonium sulphide and sodium hydroxide. The precipitate was filtered off, washed with alcohol and dried *in vacuo*. Yield—16 Gm. The substance was a brownish purple powder insoluble in the common organic solvents but readily soluble in alkali hydroxide solutions. Such solutions were dark red by transmitted light, opaque with a greenish fluorescence by reflected light, and did not stain the skin.

0.2523 Gm. of sample gave 0.0728 Gm. of mercury.
Hg found, 28.80%.
Calculated for $C_{26}H_{16}O_6Br_2Hg$, 24.22%.

The high mercury content indicates contamination with the diacetoxy mercuri compound or hydrolysis to the hydroxymercuri derivative. This point was not investigated since the use of the compound in aqueous alkaline solutions inevitably transforms any acetoxy derivative to the hydroxy form.

4,4'-Dihydroxy-Diphenyl-Dimethyl-Methane.—One hundred and eighty-eight Gm. phenol, 30 Gm. acetone and 15 Gm. phosphorus oxychloride were mixed at 20° C. The temperature was then raised to 40–45° C. and a further 15 Gm. phosphorus oxychloride added. The mixture was allowed to stand for 72 hours during which time it set to a solid mass. This was broken up, treated with water and steam distilled to remove excess phenol. The residue was cooled with vigorous stirring. The granular product was filtered off, washed with water, dried and purified by distillation *in vacuo*.

B. p., 225–230° C./4 mm. M. p., 144° C.
Yield—119 Gm.
0.2297 Gm. gave CO_2 0.6627 Gm.; H_2O 0.1458 Gm.

	Carbon.	Hydrogen.
Found Found	78.7%	7.11%
Calculated for $C_{15}H_{16}O_2$	78.9%	7.06%

3,3'-Dinitro-4,4'-Dihydroxy-Diphenyl-Dimethyl-Methane.—Thirty-one Gm. of 4,4'-dihydroxy-diphenyl-dimethyl-methane was dissolved in 300 cc. glacial acetic acid and to the well-stirred solution 14 Gm. concentrated nitric acid was added dropwise. After standing twenty minutes the reaction mixture was poured into 1500 cc. of cold water. The gummy precipitate was washed with cold water and recrystallized from 250-cc. absolute alcohol. It formed a yellow micro crystalline powder.

M. p., 132° C.
0.3115 Gm. gave 23.5 cc. moist N_2 at 24° C. and 767 mm.
N found, 8.5%.
Calculated for $C_{15}H_{10}N_2O_6$, 8.9%.

3,3'-Dinitro-4,4'-Dihydroxy-5,5'-Diacetoxy-Mercuri-Diphenyl-Dimethyl-Methane.—10.5 Gm. of 3,3'-dinitro-4,4'-dihydroxy-diphenyl-dimethyl-methane was dissolved in 250 cc. of alcohol and the solution stirred under a reflux condenser. A solution of 19 Gm. of mercuric acetate in 100 cc. water and 5 cc. glacial acetic acid was then added and the mixture refluxed and stirred for 18 hours, until a side test showed absence of ionic mercury. The precipitate was then filtered off, washed with alcohol and ether and dried. The product formed a bright yellow powder insoluble in alcohol and the common organic solvents but soluble in alkali hydroxide solutions to yield fluorescent brown solutions.

Yield, 17.0 Gm.
0.1995 Gm. gave 0.0948 Gm. Hg.
Hg found, 47.5%.
Calculated for $C_{19}H_{18}N_2O_{10}Hg_2$, 48.1%.

3,3'-Dibromo-4,4'-Dihydroxy-5,5'-Diacetoxy-Mercuri-Diphenyl-Dimethyl-Methane.—28 Gm. 4,4'-dihydroxy-diphenyl-methane was dissolved in 250 cc. glacial acetic acid and 40 Gm. bromine added dropwise with good stirring. The solution was then allowed to stand fifteen minutes and

poured into 1500 cc. of water. The sticky precipitate was washed free of acid with water and mercurated without further purification, because attempts to crystallize this substance led to decomposition with the formation of red coloring matters.

The hot solution of 23 Gm. mercuric acetate in 100 cc. of water and 5 cc. glacial acetic acid was added to a refluxing solution of 11 Gm. crude 3,3′-dibromo-4,4′-dihydroxydiphenyl-dimethylmethane with continuous stirring. About fifteen minutes were required for the addition of the mercuric acetate solution. A white precipitate commenced to form during the addition of the mercuric acetate and continued for about 45 minutes. At the end of this time inorganic mercury could no longer be detected in the solution. The latter was cooled and the heavy white precipitate collected by filtration, washed with ether and dried.

Yield, 18 Gm.
M. p.—decomposes 250° C.
0.1704 Gm. gave 0.0775 Gm. Hg.
Hg found, 44.5%.
Calculated for $C_{19}H_{18}O_6Br_2Hg_2$, 44.5%.

The product formed a white amorphous powder, insoluble in the common organic solvents but soluble in a considerable excess of alkali hydroxide solution.

The mercury analyses reported here were carried out by the Whitmore gold crucible method.

The biological tests on compounds reported herein were made in the Biological Research Laboratories of E. R. Squibb and Sons and we gratefully acknowledge their assistance.

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(2) Harris and Christiansen, *Ibid.*, 23, 109 (1934).
(3) Dutt, *J. Chem. Soc.*, 123, 225 (1922).
(4) Oyster and Adkins, *J. A. C. S.*, 43, 208 (1921).
(5) Oyster and Adkins, *Ibid.*, 43, 205 (1921).

VEGETABLE EXTRACTS AND BLOOD-SUGAR.*

BY PAUL S. JORGENSEN AND E. V. LYNN.

Just after the discovery of insulin Collip (1) prepared from various vegetables, as well as from animal tissue, extracts which were capable of producing hypoglycemia in animals. The effects differed from those by insulin in requiring a comparatively long period before their appearance. A further very interesting observation he made was that the serum or defibrinated blood of an animal, made hypoglycemic by insulin, by the plant extracts, by chemicals, by starvation or by pancreatectomy, had similar marked lowering effect on the sugar in another animal and could even cause death. The decrease in sugar and toxicity could thus be transmitted from one individual to another, apparently without limit.

Thalkimer and Perry (2), in a very limited study, reported an insulin-like action by injection of raw potato juice. Winter and Smith (3) noted similar results from extracts of yeast. Others (4) have partly confirmed or directly contradicted this testimony.

In 1927 Allen (5) described the action of myrtomel (earlier called myrtillin), an extract made from leaves of the genus *Vaccinium* by a process similar to that for

* Seattle, Washington, June 20, 1934.