m. p. 121–122°, after recrystallization from acetone, was 47.2%.[10]

5-Nitrofurohydroxamic Acid.—To 21 g. (0.3 mole) of hydroxylamine hydrochloride in 100 ml. of methanol was added 12.0 g. of sodium hydroxide in 60 ml. of methanol, the whole was heated to boiling, cooled, and stirred while 13 g. (0.074 mole) of 5-nitrofuroyl chloride in 50 ml. of ether was added dropwise. Stirring continued for five hours, the reaction mixture was filtered and the filtrate concentrated to dryness. The residue was washed with ether, then extracted in a Soxhlet extractor with ethyl acetate. The ethyl acetate extracts yielded 7.1 g. (56.0%) of product, m. p. 169° (dec.).

Anal. Calcd. for $C_5H_4N_2O_6$: N, 16.28. Found: N, 15.95, 16.00.

5-Nitrofuro-O-methylhydroxamate and Bis-(5-nitrofuro)-O-methylhydroxamate.—A solution of 13.0 g. (0.074 mole) of 5-nitrofuroyl chloride in 100 ml. of absolute ether was added with vigorous stirring to 10 g. (0.212 mole) of O-methylhydroxylamine and the whole allowed to stand overnight. The insoluble material was filtered and extracted with chloroform. The combined chloroform extracts and ether filtrate were concentrated to dryness. The residue, 13 g., was recrystallized successively from 40 ml. and 20 ml. of acetone. The material from the second recrystallization was pure 5-nitrofuro-O-methylhydroxamate, m. p. 151–152°.

(10) Pickard and Neville, *J. Chem. Soc.*, **79**, 847 (1901), prepared this compound, m. p. 124°, without employing sodium ethoxide.

Anal. Calcd. for $C_6H_6N_2O_5$: N, 15.05. Found: N, 15.24.

The acetone filtrates from the above compound were evaporated to dryness and the residue recrystallized from methanol to give bis-(5-nitrofuro)-O-methylhydroxamate, m. p. 105.6–106.0°.

Anal. Calcd. for $C_{11}H_7N_3O_8$: N, 12.92. Found: N, 13.30, 13.30.

2,5-Furodihydroxamic Acid.—This compound was prepared in 60.2% yield by the same procedure used for 5-nitrofurohydroxamic acid. It was recrystallized from hot water and melted above 250°.

Anal. Calcd. for $C_6H_6N_2O_5$: N, 15.05. Found: N, 15.13.

Furylacrylohydroxamic Acid.—This compound was prepared in similar fashion in 46.2% yield, m. p. 137–138° (with dec.) after recrystallization from ethyl acetate.

Anal. Calcd. for $C_7H_7NO_3$: N, 9.15. Found: N, 9.07.

Summary

The preparation of several *exo*-nitro derivatives of 5-nitrofuran has been described.

The preparation of a number of amides and hydroxamic acids of furan has also been described.

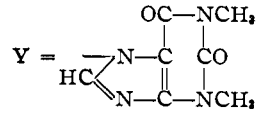
AMES, IOWA RECEIVED NOVEMBER 7, 1949

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION OF LAKESIDE LABORATORIES, INC.]

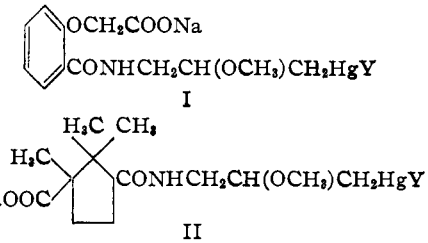
Mercurial Diuretics. I. Addition of Mercuric Acetate to Allyl Urea[1]

BY R. L. ROWLAND, WENDELL L. PERRY, E. LEON FOREMAN[2] AND HARRIS L. FRIEDMAN

Organic mercury compounds have been generally accepted as the most efficient therapeutic agents for stimulation of urine production. This diuretic effect is utilized for the relief of edema, *e. g.*, in treatment of congestive heart failure.[3] The organic mercurial diuretics in accepted clinical use in this country exhibit a similar chemical structure. Salyrgan-theophylline (Mersalyl-Theophylline, U.S.P.) (I),[4] mercuzanthin (Mercurophylline, U.S.P.) (II)[5] and mercuhydrin (Meralluride, N.N.R.) (III)[6] possess the basic structure $RCONHCH_2CH(OCH_3)CH_2HgY$, wherein R includes a carboxyl group. Although the mercurial diuretics originally were utilized in the form of the hydroxymercuri compounds (Y = OH), for the last decade the hydroxy mercurials have been mixed with excess theophylline, presumably with formation of the compound in which



Recently a diuretic has been studied in which Y is a carboxymethylmercapto radical (II, Y = SCH_2COOH).[7]



I

II

$NaOOCCH_2CH_2CONHCONHCH_2CH(OCH_3)CH_2HgY$

III

In the course of a study of variation of toxicity and diuretic effect with changes in chemical structure, it was noted that the products obtained from the addition of mercuric acetate to allylurea in methyl alcohol effected in the dog a three- to five-fold greater diuretic response than that resulting

(1) Presented before the Medicinal Division of the American Chemical Society, Atlantic City, September, 1949.

(2) E. S. Miller Laboratories, Los Angeles, Calif.

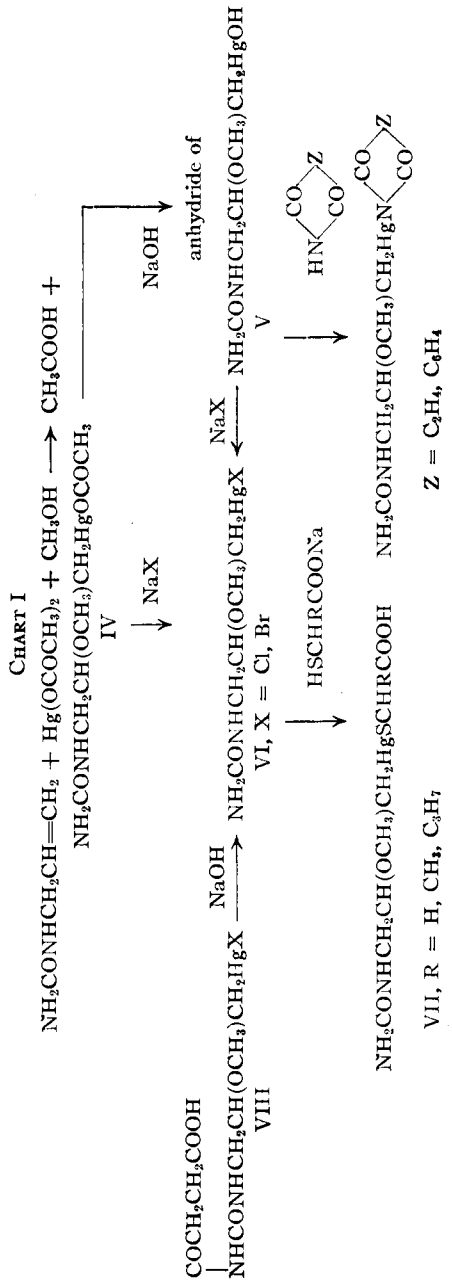
(3) A recent summary of mercurial diuretics is presented by Ray and Burch, *Am. J. Med. Sci.*, **217**, 96 (1949).

(4) Bockmühl and Schwartz, U. S. Patent 1,693,432 (1948); Bockmühl, Middendorf and Fritzsche, U. S. Patent 2,213,457 (1940).

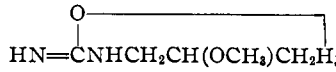
(5) Molnar, U. S. Patent 2,117,901 (1938).

(6) Geiger, Vargha and Richter, U. S. Patent 2,208,941 (1940). In this reference the structure of the product from addition of mercuric acetate to N-(allylcarbamyl)-succinamic acid in methyl alcohol is reported to be N-(3-hydroxy-2-hydroxymercuripropylcarbamyl)-succinamic acid. The formula presented for mercuhydrin in figure III is based upon unpublished studies of D. E. Pearson and Max V. Sigal, Vanderbilt University.

(7) Lehman, *Proc. Soc. Exp. Biol. Med.*, **64**, 428 (1947); Lehman, Taube and King, *ibid.*, **71**, 1 (1949).



from mercuhydrin. The addition of mercuric acetate and methyl alcohol to allylurea is presented in Chart I. The acetoxymercuri compound (IV) obtained from the addition reaction was treated with sodium hydroxide with the intention of isolating 3-hydroxymercuri-2-methoxypropylurea, $NH_2CONHCH_2CH(OCH_3)CH_2HgOH$. Analyses of the product, however, did not agree with this structure; instead, they indicated the structure to be that of an anhydride (V) of the expected compound, presumably of the type



or possibly a polymer of the type $H[NHCONHCH_2CH(OCH_3)CH_2Hg]_xOH$. This anhydride was allowed to react with several compounds containing acidic nitrogen, *e. g.*, succinimide, phthalimide.[8] From the reaction of the anhydride of the hydroxymercuri compound (V) or the acetoxymercuri compound (IV) with sodium chloride or sodium bromide the corresponding halomercuri compounds (VI) were obtained. The chloromercuri compound was converted to mercaptomercuri derivatives (VII) by reaction with α-mercaptoaliphatic acids and sodium hydroxide.[9] The addition of mercuric acetate to allylurea in other alcohols produced the corresponding alkoxy compounds which were isolated as the chloromercuri derivatives.

By reaction with alkali halides Pearson and Sigal[10] have converted the product from the addition of mercuric acetate and methyl alcohol to N-(allylcarbamyl)-succinamic acid into compounds to which they have assigned the structure $HOOCCH_2CH_2CONHCONHCH_2CH(OCH_3)CH_2HgX$ (VIII) where X is halogen. We have been able to hydrolyze VIII, where X is chlorine and bromine, producing compounds which are identical with the chloro and bromo compounds isolated from the addition to allylurea. The relative location of the methoxy and halomercuri radicals in the addition product to allylurea (IV) is therefore the same as that found in VIII.

Pharmacology.—Mr. P. A. Nuhfer of our pharmacology laboratories will report elsewhere upon these compounds. Preliminary findings are as follows: Of this series, only the carboxymethylmercaptomercuri compounds (Table I, compounds 7, 8 and 9) caused little or no irritation upon subcutaneous injection.[11] The fourteen day toxicities (LD_{50} at 14 days, single intravenous dose) were similar and of the same order of magnitude as that of mercuhydrin (III). The immediately fatal dosage (death within one hour following intravenous injection) of compound 7 was *ca.* twenty times that of compound 3 or of mercuhydrin. The compounds prepared from the addition to allylurea produced in dogs a diuretic response three to five times that produced by compounds I, II or III. A response comparable to that obtained from I, II or III could be produced by compound 7 at an appreciably lowered dosage.

(8) The reaction of alkoxyalkylmercury compounds with compounds containing acidic nitrogen is reported by Callsen, U. S. Patent 2,119,701 (1938).

(9) The reaction of organic mercury compounds with thioacids is reported by Kharasch, U. S. Patents 1,589,599 (1926) and 1,672,615 (1928).

(10) D. E. Pearson and Max V. Sigal, Vanderbilt University, private communication.

(11) The determination, based upon the degree of irritation forty-eight hours after subcutaneous injection into the back of the rat, will be described elsewhere by Mr. P. A. Nuhfer.

TABLE I

$NH_2CONHCH_2CHORCH_2HgX$

No.	R	X	Formula	M. p., °C.	Recryst. solvent	Analyses, % Mercury[a] Calcd.	Mercury[a] Found	Nitrogen[b] Calcd.	Nitrogen[b] Found
1	CH_3	$OCOCH_3$	$C_7H_{14}O_4N_2Hg$	126–127	*i*-PrOH	51.3	51.4	7.17	7.30
2	CH_3	Cl	$C_5H_{11}O_2N_2HgCl$	152–153	EtOH	54.6	54.7	7.63	7.79
3	CH_3	Br	$C_5H_{11}O_2N_2HgBr$	162–163	EtOH	48.7	48.8	6.81	6.55
4	CH_3	$NC_4H_4O_2$[c]	$C_9H_{15}O_4N_3Hg$	159–160	EtOH	46.7	46.4	9.78	9.59
5	CH_3	$NC_8H_4O_2$[d]	$C_{13}H_{15}O_4N_3Hg$	188–188.5	EtOH	42.0	41.4	8.79	8.83
6	CH_3	$N_4C_7H_7O_2$[e]	$C_{12}H_{18}O_4N_8Hg$	185–187	EtOH	39.3	39.1	16.45	16.07
7	CH_3	SCH_2COOH	$C_7H_{14}O_4N_2HgS$	107–111	...	47.4	47.6	6.63	6.42
8	CH_3	$SCHCH_3COOH$	$C_8H_{16}O_4N_2HgS$	162–163	...	45.9	45.6	6.41	6.37
9	CH_3	$SCHC_3H_7COOH$	$C_{10}H_{20}O_4N_2HgS$	154–155	...	43.2	43.1	6.03	5.96
10	C_2H_5	Cl	$C_6H_{13}O_2N_2HgCl$	150–152	EtOH	52.6	52.6	7.35	7.38
11	C_2H_5	SCH_2COOH	$C_8H_{16}O_4N_2HgS$	115–116	...	45.9	46.2	6.41	6.38
12	*i*-C_3H_7	Cl	$C_7H_{15}O_2N_2HgCl$	149–150	EtOH	50.8	51.0	7.09	7.17
13	*n*-C_4H_9	Cl	$C_8H_{17}O_2N_2HgCl$	119–121	H_2O	49.0	49.0	6.85	6.84
14	*n*-C_4H_9	SCH_2COOH	$C_{10}H_{20}O_4N_2HgS$	86–87	...	43.2	42.6	6.03	6.08

[a] Analyzed on a macro scale by precipitation as mercuric sulfide from hydrochloric acid solution. [b] Kjeldahl nitrogen. [c] Succinimido. [d] Phthalimido. [e] Theophyllinate.

Experimental[12]

3-Acetoxymercuri-2-methoxypropylurea (IV).—To a refluxing solution of 47 g. (0.47 mole) of allylurea in 250 ml. of methyl alcohol was added a hot solution of 140 g. (0.44 mole) of mercuric acetate and 25 ml. of glacial acetic acid in 350 ml. of methyl alcohol. Following the addition which was made in small portions over a one-half-hour period, the reaction mixture was heated under reflux for sixteen hours. The insoluble material was removed by filtration and the filtrate was concentrated at room temperature. The residual mixture of oil and solid was extracted with acetone. The residue weighed 79 g. (46%) and melted at 125–126.5°. After crystallization from isopropyl alcohol, the solid weighed 65 g. (38%).

Anhydride of 3-Hydroxymercuri-2-methoxypropylurea (V).—To a suspension of 7.9 g. (0.020 mole) of 3-acetoxymercuri-2-methoxypropylurea in 6.0 ml. of water was added 7.5 g. (0.019 mole) of 10% sodium hydroxide solution. The insoluble material was removed by filtration and the filtrate was concentrated at room temperature. The residue, after washing with acetone and with hot absolute ethyl alcohol, weighed 4.7 g. (70%) and decomposed at 183°. *Anal.* Calcd. for $C_5H_{10}O_2N_2Hg$: Hg, 60.64; N, 8.47. Calcd. for $C_5H_{12}O_3N_2Hg$: Hg, 57.21; N, 8.03. Found: Hg, 60.11, 60.23; N, 8.36.

In the presentation of this work at the Atlantic City meeting, this compound was presented as of the structure $NH_2CONHCH_2CH(OCH_3)CH_2HgOH$. This was based upon the analytical results obtained from the initial preparation of the product from the reaction of 3-acetoxymercuri-2-methoxypropylurea with sodium hydroxide. Subsequent study of the product of this reaction indicated the anhydride structure.

3-Chloromercuri-2-methoxypropylurea (VI, X = Cl).—The reaction mixture from the addition of mercuric acetate to allylurea in methyl alcohol, after heating six hours under reflux, was clarified by filtration and an aqueous solution containing an equivalent amount of sodium chloride was added to the filtrate. The precipitate after washing with water and ethyl alcohol amounted to 70%; the yield after crystallization from ethyl alcohol was 55%. The same product was obtained by the reaction of the anhydride of 3-hydroxymercuri-2-methoxypropylurea with an equivalent amount of sodium chloride in aqueous solution. The reaction mixture was acidified with acetic acid and the product was obtained after crystallization from ethyl alcohol in 30% yield.

3-Bromomercuri-2-methoxypropylurea (VI, X = Br).—The bromomercuri compound was prepared similarly to the chloromercuri compound in 65% yield from the anhydride of 3-hydroxymercuri-2-methoxypropylurea and in 41% yield from addition of aqueous sodium bromide solution to the original reaction mixture of the addition of mercuric acetate to allylurea in methyl alcohol.

3-Succinimidomercuri-2-methoxypropylurea.—To a solution of 2.5 g. (0.025 mole) of succinimide in 50 ml. of water was added 9.0 g. (0.027 mole) of the anhydride of 3-hydroxymercuri-2-methoxypropylurea. The insoluble material was removed by filtration and the filtrate was concentrated at room temperature. The residue, after crystallization from a small volume of ethyl alcohol, amounted to 75%.

Reaction of the Anhydride of 3-Hydroxymercuri-2-methoxypropylurea with Other Acidic Nitrogen Compounds.—The product from the reaction of phthalimide and the anhydride of 3-hydroxymercuri-2-methoxypropylurea precipitated from the aqueous reaction mixture. The yield of crystallized material was 67%. Using theophylline as the acidic nitrogen compound, the yield of crystallized reaction product was 35%.

3-Carboxymethylmercaptomercuri-2-methoxypropylurea (VII, R = H).—To a solution of 8.8 g. (0.095 mole) of thioglycolic acid in 80 ml. of 5% sodium hydroxide solution was added a mixture of 35.5 g. (0.097 mole) of 3-chloromercuri-2-methoxypropylurea and 150 ml. of 2% sodium hydroxide solution. After twenty hours the insoluble material was removed by filtration and 15 ml. of glacial acetic acid were added to the filtrate. The white powder, obtained in 80% yield after washing and drying, resolidified after melting at 107–111° and subsequently decomposed at 138–139°.

Reaction of 3-Chloromercuri-2-methoxypropylurea with Other Thioacids.—If thiolactic acid of α-mercapto-*n*-valeric acid were utilized in the reaction with 3-chloromercuri-2-methoxypropylurea, the yields of product were 70 and 80%, respectively.

Addition of Mercuric Acetate to Allylurea. (a) **In Ethyl Alcohol.**—The addition of mercuric acetate to allylurea in ethyl alcohol was similar to the previously described addition in methyl alcohol. The crude 3-chloromercuri-2-ethoxypropylurea was obtained in 31% yield. It was converted in 80% yield to the thioglycolic acid derivative which, after melting at 115–116°, resolidified and subsequently decomposed at 146°.

(b) **In Isopropyl Alcohol.**—The addition of mercuric acetate to allylurea in isopropyl alcohol was carried out at 50–60°. After the addition of the sodium chloride solution, the reaction mixture was concentrated at room temperature. The yield of product obtained from the concentration amounted to 10%.

(12) All melting points are corrected. We are indebted to Mr. H. C. Krahnke and his staff for analyses.

(c) **In *n*-Butyl Alcohol.**—The addition of mercuric acetate to allylurea in *n*-butyl alcohol was accomplished at a temperature below 70°. After the addition of sodium chloride to the reaction mixture, the solution was concentrated at room temperature. 3-Chloromercuri-2-*n*-butoxypropylurea was obtained in 16% yield. It was converted in 88% yield to the thioglycolic acid derivative, which resolidified after melting at 86–87° and subsequently decomposed at 140–145°.

Hydrolysis of N-(3-Chloromercuri-2-methoxypropylcarbamyl)-succinamic Acid (VIII, X = Cl).—A mixture of 3.0 g. of N-(3-chloromercuri-2-methoxypropylcarbamyl)-succinamic acid[13] and 10 ml. of 10% sodium hydroxide solution was warmed at 80° for two hours. The insoluble material was removed by filtration and the filtrate was acidified with 3 ml. of glacial acetic acid. The crude water-washed product weighed 1.7 g. (75%) and melted at 152–153°. Crystallization from ethyl alcohol did not alter the melting point.

Anal. Calcd. for $C_5H_{11}O_2N_2HgCl$: Hg, 54.63; N, 7.63. Found: Hg, 54.87; N, 7.70.

No depression of melting point was noted of a mixture of this solid with the 3-chloromercuri-2-methoxypropylurea obtained *via* the addition of mercuric acetate to allylurea. The products obtained by these two different routes were identical when compared in respect to crystalline appearance, diuretic response and toxicity.

Hydrolysis of N-(3-Bromomercuri-2-methoxypropylcarbamyl)-succinamic Acid (VIII, X = Br).—A mixture of 6.0 g. of N-(3-bromomercuri-2-methoxypropylcarbamyl)-succinamic acid[13] and 20 ml. of 10% sodium hydroxide solution was heated at 80° for one and one-half hours. After another one and one-half hours at room temperature the solid material was removed by filtration and the filtrate was acidified with acetic acid. The precipitate after crystallization from absolute ethyl alcohol melted at 162°; yield 2.9 g. (60%). The melting point was not depressed by mixture with the bromomercuri compound obtained *via* the addition of mercuric acetate to allylurea.

Anal. Calcd. for $C_5H_{11}O_2N_2HgBr$: Hg, 48.72; N, 6.81. Found: Hg, 48.66; N, 6.83.

(13) Prepared by the method of Pearson and Sigal which is similar to the method used above in the preparation of 3-chloromercuri-2-methoxypropylurea from the anhydride of 3-hydroxymercuri-2-methoxypropylurea.

Summary

A series of compounds of the structure $NH_2CONHCH_2CHORCH_2HgX$ has been prepared utilizing the addition of mercuric acetate and alcohols to allylurea. These compounds effect greater diuresis in dogs than do those mercurial diuretics in medical use.

MILWAUKEE 1, WIS. RECEIVED JANUARY 25, 1950

[CONTRIBUTION FROM THE RESEARCH DIVISION OF THE UPJOHN COMPANY]

A Paper Chromatographic Technique and its Application to the Study of New Antibiotics

BY D. H. PETERSON AND L. M. REINEKE

The necessity for early identification of antibiotics produced by a given culture has been well recognized by those working in the field. Past experience has shown that considerable time can be spent in purification studies only to find eventually by spectrum and toxicity tests that one has emerged with a known antibiotic such as, for example, streptothricin. It is increasingly apparent that the majority of cultures produce more than one antibiotic and hence antibacterial spectra and toxicity studies are generally of limited value in early isolation studies. This paper is therefore primarily concerned with a paper chromatographic method, its application to the early identification of antibiotics and factors affecting the procedure.

The classical rediscovery of paper partition chromatography by Consden, Gordon and Martin[1] has already contributed essential information to many biochemical and chemical problems. This unique and powerful tool has been widely adopted but its application to various problems has not yet been fully exploited.

(1) (a) In 1861 Schoenbein [*Verhandl. naturforsch Gesel Basel*, **III**, 249 (1861) and **IV**, 1 (1864)] in studying the formation of ozone with electrical discharges observed the different heights to which the components of a mixture rose when a strip of filter paper was dipped into the solution. He termed this use of the paper strip as "capillary analysis." (b) R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).

The use of paper chromatography involves generally two basic principles. First it is necessary to have a chemical compound or its derivative of such nature as to distribute appropriately between one solvent as the stationary phase on the paper (water in this case) and another solvent as the mobile phase. Secondly, it is important to have some method of locating the position of the substance chromatographed by means of a chemical, physical or biological test. In addition adsorption on the paper plays a greater or lesser role depending upon the total system.

One of the most desirable features of the paper chromatographic technique is the resolution and identification using as little as 0.1–2.0 microgram of starting material.

Goodall and Levi[2] first applied a paper strip method successfully to the study of various penicillins in a mixture. Winsten and Eigen[3] modified the technique of the British workers[2] and applied it to studies on the streptomycin complex. Winsten and Spark[4] modified the original technique[2] and applied the method to the various penicillins contained in culture filtrates. Kleuner[5] has recently published a method and

(2) R. R. Goodall and A. A. Levi, *Nature*, **158**, 675 (1946).
(3) W. A. Winsten and E. Eigen, THIS JOURNAL, **70**, 3333 (1948).
(4) W. A. Winsten and A. H. Spark, *Science*, **106**, 192 (1947).
(5) R. G. Kleuner, *J. Bact.*, **57**, 101 (1949).