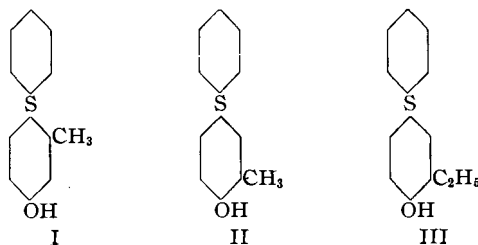


A STUDY OF THE GERMICIDAL ACTIVITY OF AROMATIC SULPHIDES.*

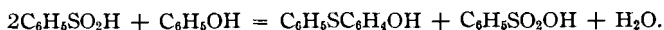
BY E. MONESS, W. BRAKER AND W. G. CHRISTIANSEN.

Hilbert and Johnson (1) found that *p*-hydroxy diphenyl sulphide has a phenol coefficient of 115. They also prepared 4-hydroxy, 4'-methyl diphenyl sulphide, expecting this compound to have a still greater germicidal activity, because it is well known that the introduction of a methyl group into the benzene ring of a phenolic germicide generally increases the activity. However, this compound was found to have a much lower activity, namely, a phenol coefficient of only 50. In view of this unexpected result, we investigated the effect of introducing the alkyl group into the hydroxylated ring of *p*-hydroxy diphenyl sulphide, because we believed that the lower activity of the methylated compound referred to above was due to the presence of the methyl group in the non-hydroxylated ring.

Three compounds were prepared: 4-hydroxy 2-methyl diphenyl sulphide (I); 4-hydroxy 3-methyl diphenyl sulphide (II); and 3-ethyl 4-hydroxy diphenyl sulphide (III).



In the preparation of these compounds, as well as some of the others described below, it was found most convenient to use the Hinsberg (2) reaction, which permits the preparation of our sulphides readily from any derivatives of phenol by reacting with benzene sulphinic acid:



Benzene sulphinic acid was most readily prepared by the method of Knoevanagel (3).

The Hinsberg reaction has the disadvantage of giving a crude product, which according to the author himself, is undistillable. We found that while the crude reaction product obtained in the preparation of *p*-hydroxy diphenyl sulphide could be distilled by exercising great care and using a very high vacuum, such distillation became impossible with the increase in the complexity of the phenol used. We overcame this difficulty by purification of the crude reaction product preliminary to distillation. This consisted essentially in boiling the tarry product with gasoline, which dissolved the sulphide, leaving some of the tar behind. The gasoline extract was then evaporated to dryness, and the resulting residue again treated with gasoline. This treatment yielded a much lighter product, light red instead of almost black, which could be easily distilled *in vacuo*. In this manner the Hinsberg reaction yielded pure products, which were used for the determina-

* Scientific Section, A. P. H. A., Miami meeting, 1931.

tion of their germicidal activity. This study gave very interesting and unexpected results as will appear from the following tabulation.

Dilution in Which Germicide Kills.

Compound.	Typhoid.		Staphylococcus.	
	5 min.	10 min.	5 min.	10 min.
4-hydroxy diphenyl sulphide	15000	20000	5000	5000
2-methyl 4-hydroxy diphenyl sulphide	1000	1500	5000	10000
3-methyl 4-hydroxy diphenyl sulphide	500	500	25000	25000
3-ethyl 4-hydroxy diphenyl sulphide	5000	5000	20000	30000

A definite shift of germicidal activity from typhoid to staphylococcus is noted as a result of the introduction of the methyl group. This shift becomes most pronounced when the methyl group is in the ortho position to the hydroxyl. The introduction of an alkyl group higher than the methyl group, in this case the ethyl group, causes a general rise in germicidal activity as compared with the methyl group. This gives cause to assume that with the alkyl group still higher, there would be a further increase in germicidal activity.

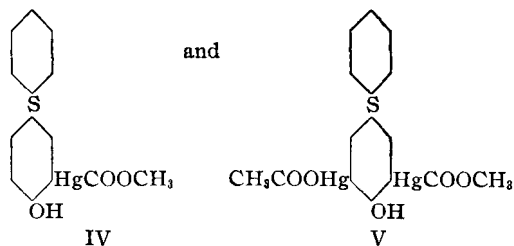
It is also of interest to note that high activity both with respect to typhoid and staphylococcus, can be obtained by using a mixture of equal weights of *p*-hydroxy diphenyl sulphide and 3-methyl 4-hydroxy diphenyl sulphide. Such a mixture gave the following results:

Dilution in Which Germicide Kills.

Typhoid.		Staphylococcus.	
5 min.	10 min.	5 min.	10 min.
15000	15000	10000	20000

The effect of mercuration of these compounds was next studied. The mercury compounds were prepared by dissolving the sulphide in alcohol, and slowly adding to it with heating and stirring, an acidified aqueous solution of mercuric acetate. When the reaction mixture no longer gives a test for mercury ions, the reaction is complete. The solution is filtered and alkalized with dilute alkali. A slight residue is filtered off, and the filtrate carefully neutralized with acetic acid. The compound was isolated as a white powder.

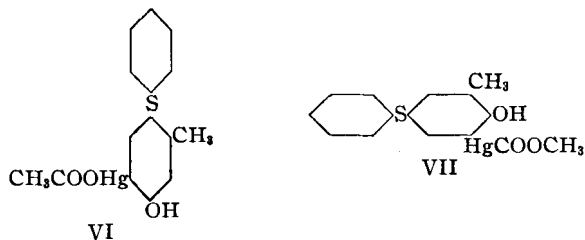
The resultant product is almost always a mixture of the mono- and di-mercury compounds:



In only one instance did we obtain a compound which analyzed for (IV). On the other hand, the preparation of the di-mercury compound (V) proceeds smoothly and is obtained pure without difficulty. (IV) is easily soluble in dilute alkali, but (V) can be kept in alkaline solution only when the solution is protected against absorption of CO₂ from the air by an inert gas such as nitrogen.

Using the method outlined above, 2-methyl 4-hydroxy 5-acetoxy mercuri

diphenyl sulphide (VI) was prepared without difficulty. On the other hand, it was found impossible to prepare in this manner 3-methyl 4-hydroxy 5-acetoxy mercuri diphenyl sulphide:

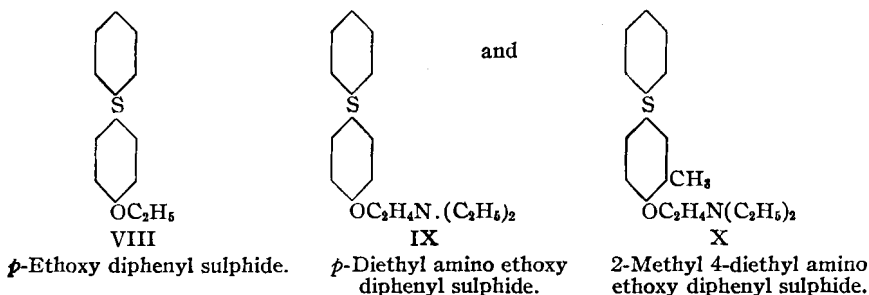


It would seem that the reason for this failure is the position of the methyl group, which being ortho to the hydroxyl, prevents the mercury residue from orienting itself meta to the methyl group.

Compound VI was almost insoluble in dilute alkali; and, therefore, no results are available at present on this compound. The di-mercury compound of *p*-hydroxy diphenyl sulphide (V) is distinctly less germicidal than the mono-mercury compound (IV) and even the latter is not appreciably more active than the *p*-hydroxy diphenyl sulphide (I) from which it was prepared.

In the single instance in which we obtained (IV), the active dilution was 1-15,000 for typhoid and 1-20,000 for staphylococci in 10 minutes. For a mixture of 40% of (IV) and 60% of (V) the germicidal activity was 1-20,000 for typhoid and 1-2500 for staphylococci in 10 minutes. The mercury compounds of *p*-hydroxy diphenyl sulphide have thus no advantage over the sulphide itself, and when the hydroxylated ring contains an alkyl group the mercury derivatives are very insoluble.

The hydroxyl group is as essential to the germicidal activity of the aryl phenolic sulphides as for the ordinary phenols. Any blocking of this group by the formation of ethers has a very great effect in lowering the germicidal activity. Three compounds of this nature were prepared:



In preparing (VIII) *p*-brom phenetol was heated with sodium thiophenate in the presence of Cu, resulting in the splitting off of sodium bromide and the formation of (VIII). (IX) and (X) were prepared by heating an alcoholic solution of the sodium salt of the hydroxy diphenyl sulphide with diethyl amino ethyl bromide.

The germicidal activity for these compounds is as follows:

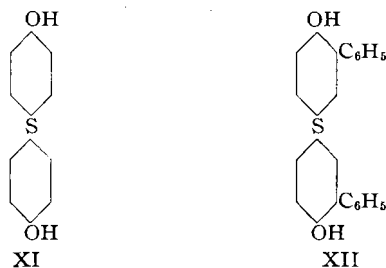
Dilution in Which the Germicide Kills in 10 Mins.

Compound.	Typhoid.	Staphylococci.
VIII	1000	1000
IX	4000	400
X	5000	400

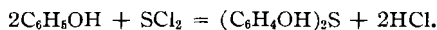
(IX) and (X) are interesting because they yield water-soluble hydrochlorides which are neutral to litmus. Though as germicides they are not very active, there is a possibility of usefulness for these, since they have been found to have trypanicidal activity, and also have distinct activity as local anæsthetics.

This importance of the hydroxyl group for germicidal activity suggested the study of dihydroxy diphenyl sulphides. The greater solubility of the mercury derivatives of such compounds in dilute alkali was the reason for the preparation and testing of some of the latter.

Two compounds were prepared: 4-4' dihydroxy diphenyl sulphide (XI) and 3-3' diphenyl 4-4' dihydroxy diphenyl sulphide (XII):



The method used was that of Tassinari (4) and consisted in reacting a carbon disulphide solution of a phenol with a similar solution of SCl_2 in the cold



Sulphur dichloride was prepared from sulphur chloride- S_2Cl_2 by conducting a stream of dry chlorine into the sulphur chloride in the presence of some activated charcoal as a catalyst, keeping the temperature below $+3^\circ \text{C}$.

The reaction between the phenol and the sulphur dichloride is conducted with cooling, adding the solution of the latter to the solution of the former dropwise, with constant stirring. When the reaction is complete, the solvent is removed by evaporation, and the residue is dissolved in dilute potassium hydroxide (the compound is obtained from this solution by precipitation with CO_2).

The procedure works with modifications also for the reaction between ortho-hydroxy diphenyl and sulphur dichloride.

The presence of the 2 hydroxyl groups in these compounds did not, however, have the expected effect of raising the germicidal activity; on the contrary, the activity was greatly decreased.

Dilution in Which the Germicide Kills in 10 Mins.

Compound.	Typhoid.	Staphylococci.
XI	1500	300
XII	2000	3000

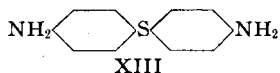
In an attempt to prepare a mono-mercury derivative of XI we found that

again a mixture of mono- and di-mercury compounds resulted. On the other hand, there was no difficulty encountered in the preparation of the di-mercury compound. The expected greater solubility of these compounds in dilute alkali was fully realized. The mixture consisting of 70% mono-mercurated and 30% di-mercurated *p-p'*-dihydroxy diphenyl sulphide dissolved with the use of only one mol. of alkali in excess of the equivalent quantity while the di-mercury compound required 4 mols. in excess; nitrogen protection was not needed for the stability of the latter solution.

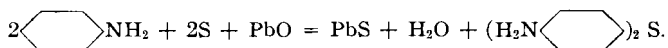
The germicidal activity of these mercury compounds, however, was not as expected: not only was it not higher than the compounds from which they were made, but it was even lower:

Compound.	Dilution in Which Germicide Kills in 10 Mins.	
	Typhoid.	Staphylococci.
70% mono- and 30% di-mercurated <i>p-p'</i> -dihydroxy diphenyl sulphide	750	750
Di-acetoxy mercuri <i>p-p'</i> -dihydroxy diphenyl sulphide	1250	500

In the course of our work on the symmetrical di-aryl sulphides we also prepared 4-4'-diamino diphenyl sulphide:

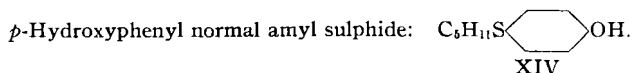


originally with the intention of using it in the preparation of 4-4'-dihydroxy diphenyl sulphide. However, the method of Tassinari was found to be preferable. (XIII) was prepared by the method of Merz and Weith (5). This consists essentially in heating aniline and sulphur together in the presence of lead oxide according to the following reaction:



This compound, too, showed very low germicidal power, and is recorded here, because in conjunction with the results obtained with the dihydroxy diphenyl sulphides, it seems to indicate that the germicidal activity is due neither to the hydroxy group nor to the sulphur linkage alone, but to the grouping, where there is a phenolic ring on one side of the sulphur and a benzene nucleus on the other.

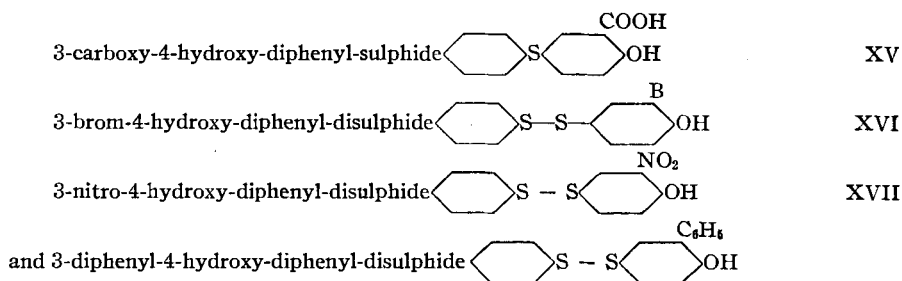
The substitution of an alkyl for the benzene group in the compounds under study was found to produce compounds of comparatively high germicidal potency. This is illustrated in the case of the one compound of this type prepared by us, namely,



This was prepared by the reduction of *p*-methoxy benzene sulphonyl chloride to the corresponding *p*-methoxy thiophenol, and reacting the sodium salt of the latter with normal amyl bromide in alcoholic solution. The methoxy compound was subsequently demethylated yielding (XIV). This compound was active against typhoid in a dilution of 1 in 2500 in 10 minutes, and against staphylococci

in a dilution of 1 in 10,000 in 5 minutes, and compares favorably with the activity of 2-methyl-4-hydroxy-diphenyl sulphide.

We had also intended to study the effect on the germicidal power of aryl phenolic sulphides of introducing carboxyl, halogen, nitro and phenyl groups into the ring containing the hydroxyl group. It was found, however, that under the conditions of the Hinsberg reaction only the compound containing the carboxyl group gave the desired sulphide; in the other instances disulphides were obtained. While it is true that the Hinsberg reaction tends to give a small percentage of disulphide even with phenol, a tendency which is increasingly apparent with the introduction of the higher alkyl groups, it is interesting to note that in the cases mentioned above, the product is almost exclusively the disulphide. Four compounds were thus prepared:



In each case the corresponding phenol was reacted with benzene sulphinic acid according to the Hinsberg reaction, and purified as already described.

The germicidal power of all of these compounds was found to be low:

Compound	Dilution in Which Germicide Kills.		
	Typhoid.		Staphylococci.
XV	500	in 10 mins.	500
XVI and XVII	1000		1000
	in 1 min. but inactive in greater dilutions.		

EXPERIMENTAL PART.

Preparation of p-Hydroxy Diphenyl Sulphide by Modified Hinsberg Reaction.

Seventy-three grams of benzene sulphinic acid (2 mols.) were treated with 26.3 Gm. (1 mol.) of phenol. Eight grams of concd. H_2SO_4 were employed as the condensation agent. The mixture was heated on the steam-bath for 8 hours. It was then shaken with Na_2CO_3 solution and excess phenol driven over by steam. Concentrated HCl acid was then added and a dark red oil separated out. The latter was extracted with ether, then the ether solution was washed successively with Na_2CO_3 solution and water. The extract was dried over Na_2SO_4 and the ether then distilled off. Thirty-six grams of crude product were thus obtained corresponding to a 65% yield.

The crude *p*-hydroxy-diphenyl-sulphide was purified by gasoline, cf. previous discussion, and the oil thus obtained was distilled under diminished pressure. Twenty-five grams of a light yellow oil were thus obtained. By redistillation we obtained 20.0 Gm. of a clear viscous oil, the boiling point of which at 3-4 mm. was 162-164° C.

Yield—36.0% of pure compound.

Analysis: Calculated for $C_{12}H_{10}OS$: S, 15.86%. Found: S, 15.65%.

This compound was also prepared by the Grignard and Ziegler reactions as described by Hilbert and Johnson (1) but we obtained better yields by the modified Hinsberg reaction.

Preparation of 3-Methyl-4-Hydroxy Diphenyl Sulphide.

The method used in this experiment was that employed in the preparation of *p*-hydroxy diphenyl sulphide, but using ortho cresol instead of phenol. A 42% yield of a crystalline compound was obtained. The boiling point at 4 mm. was 185–190° C., melting point 72° C.

Analysis: Calculated for $C_{13}H_{12}OS$: S, 14.81%. Found: S, 14.85%.

Preparation of 2-Methyl-4-Hydroxy Diphenyl Sulphide.

This compound was prepared by the modified Hinsberg reaction using meta cresol. The boiling point of the substance was 192–197° C. at 5 mm.

Analysis: Calculated for $C_{13}H_{12}OS$: S, 14.81%. Found: S, 14.48%.

Preparation of 3-Ethyl-4-Hydroxy Diphenyl Sulphide.

The *o*-ethyl phenol necessary for the preparation of this sulphide was prepared by a modification of the method of Resenmund and Schnurr (6), rearranging the acetic ester of phenol to the corresponding ketone with subsequent reduction of the latter.

Phenyl acetate, prepared in the usual manner, was rearranged as follows: To 65 Gm. of phenyl acetate, 28 Gm. of $AlCl_3$ was added slowly, the reaction proceeding with evolution of HCl and rise of temperature. After all the $AlCl_3$ had been added, the mixture was heated on the oil-bath for 1½ hours at a temperature of 170–180° C. The melt which is a reddish brown glass was powdered and added in small portions to one liter of approximately normal HCl. A dark, almost black, oil separates out, which was steam distilled, yielding 27.5 Gm. of ortho-acetyl phenol, since the para compound does not come over with steam. This was distilled *in vacuo* and all came over at 103–105° C. at 13-mm. pressure.

Twenty-four grams of ortho-acetyl phenol was added to 500 cc. of a 50–50 mixture of concentrated hydrochloric acid and water, 50 cc. of alcohol and 25 Gm. of zinc amalgam. This was heated on the steam-bath with vigorous stirring for 8 hours, or until the oil no longer gave a purple color with ferric chloride. The oil was steam distilled and extracted with ether. After evaporating off the ether, the oil was redistilled, yielding 12 Gm. of ortho-ethyl phenol:

Analyses: $\left. \begin{array}{l} \text{Carbon: Found, 78.5\%; calculated, 78.6\%} \\ \text{Hydrogen: Found, 8.1\%; calculated, 8.2\%} \end{array} \right\} \text{ for } C_8H_{10}O.$

The *o*-ethyl phenol was then reacted with benzene sulphinic acid, as described for 4-hydroxy diphenyl sulphide. After the gasoline purification, 14 Gm. of a red oil were obtained from the 12 Gm. of ortho-ethyl phenol used, which is a yield of 61.9%. This was distilled giving 9 Gm. of light yellow oil, which came over

between 190–205° C. at 7-mm. pressure. This analyzed 16.53% for sulphur as against 13.91% for the calculated value, giving rise to the supposition that some disulphide was present. This was therefore reduced with zinc and hydrochloric acid and was steam distilled. Thiophenol was found in the distillate. The contents of the flask were then extracted with ether (having previously dissolved all of the zinc with hydrochloric acid) and after evaporation of the solvent, 3.4 Gm. of a clear oil were obtained, which was the desired 3-ethyl-4-hydroxy diphenyl sulphide.

Analysis: Sulphur: Found, 13.89%; calculated for $C_{14}H_{14}SO$ 13.91%.

Preparation of p-Ethoxy Diphenyl Sulphide.

Two and nine-tenths grams of sodium (7) were dissolved in 40 cc. of absolute alcohol and 14.3 Gm. of thiophenol added. The alcohol was then distilled off. Twenty-eight grams of *p*-brom phenetol and 0.4 Gm. of copper powder were added. The reaction mixture was then heated on the oil-bath at 280° C. for 8 hours. The semi-solid mass was then extracted with alcohol, acidified and after adding zinc, was steam distilled. The zinc salt solution was extracted with ether; the latter was dried over anhydrous sodium sulphate and evaporated. The yield was very small.

Analysis: Sulphur: Found, 13.45%; calculated for $C_{14}H_{14}SO$ 13.91%.

Preparation of the Hydrochloride of Diethyl Amino Ethyl Ester of p-Hydroxy Diphenyl Sulphide.

Six and six one-hundredth grams of *p*-hydroxy diphenyl sulphide were added to a solution of 1.38 Gm. of sodium in 58 cc. of absolute alcohol. To the resulting solution 7.83 Gm. of diethyl amino ethyl bromide hydrobromide, dissolved in 58 cc. of alcohol, were added. The mixture was refluxed on the steam-bath for 15 hours. The salt resulting from the reaction was filtered off, and the alcohol evaporated off. The residue was taken up in dilute HCl. Any unreacted hydroxy diphenyl sulphide was removed by extracting with ether. The solution was then made alkaline and steam distilled to remove any excess diethyl amino ethyl bromide. The alkaline solution was now extracted with ether, and the dried ether extract was treated with dry HCl gas. This precipitated the hydrochloride of diethyl amino ethyl ether of *p*-hydroxy diphenyl sulphide.

Analysis: Sulphur: Found, 9.05%; calculated for $C_{18}H_{24}SONCl$ 9.48%.

The compound dissolves easily in water and is neutral to litmus and congo red.

The diethyl amino ethyl ether of 3-methyl 4-hydroxy diphenyl sulphide was prepared in the manner outlined above. The hydrochloride, after pouring off the ether, and drying retains free HCl tenaciously.

Analyses: Sulphur: Found, 8.70%; calculated, 9.10% }
 Chlorine: Found, 12.04%; calculated, 10.07% } for $C_{19}H_{26}SONCl$.

This indicates the presence of about 4% of free HCl still present. This can be eliminated by protracted heating at 100° C.

Preparation of 4-4'-Dihydroxy Diphenyl Sulphide (4).

Ninety-four grams of phenol were dissolved in 150 cc. of CS₂. The solution was cooled and held at 0-5° C. SCl₂ in 100 cc. of CS₂ was then added dropwise with constant stirring; HCl came off steadily and a solid mass separated from the solution. The CS₂ was then distilled off, leaving a grayish solid mass. This was dissolved in KOH, diluted with about 5 volumes of water, and filtered from the insoluble matter. CO₂ was passed into the solution, and silvery crystals deposited. This was isolated giving 27 Gm. of a melting point of 152° C. as compared with 150-151.5° observed by Tassinari.

The filtrate was further treated with CO₂ and yielded a tarry substance which was not further investigated.

The SCl₂ was prepared from S₂Cl₂ as follows:

One hundred and thirty-five grams of S₂Cl₂, together with 2 Gm. of "Nuchar," were placed in a flask, and a steady stream of dry chlorine was conducted into the mixture with mechanical stirring and cooling in an ice-salt mixture. The temperature was kept below 3° C., since SCl₂ decomposes at room temperature. When the increase in weight was equal to 71 Gm., the reaction was completed.

Preparation of 3-3'-Diphenyl, 4-4'-Dihydroxy Diphenyl Sulphide.

The method used was as above, using *o*-hydroxy diphenyl instead of phenol.

On distilling off the CS₂ a viscous oil is obtained. Unreacted *o*-hydroxy diphenyl was distilled off *in vacuo* and the residue was dissolved in KOH and treated with CO₂. This yielded a grayish curdy precipitate, which on heating at 110° C., solidifies into a dark, glassy substance, which can be crushed to violet, sticky powder, analyzing too high for sulphur.

The procedure was therefore modified, removing the unreacted *o*-hydroxy diphenyl by steam distillation. The solution remaining in the distillation flask was then extracted with ether, and the solvent evaporated off. The residue was heated for 2 hours at 110° C., and the resultant product was a brown, transparent viscous liquid.

Analysis: Sulphur: Found, 8.20%; calculated for C₂₄H₁₈SO₂ 8.62%.

Attempts to further purify this substance by crystallization from solvents were unsuccessful.

Preparation of p-Hydroxy Phenyl Amyl Sulphide.

Potassium methoxy benzene sulphonate which had been prepared from anisol (8) was converted into *p*-methoxy benzene sulphon chloride by reacting with POCl₃. The sulphon chloride was reduced to *p*-methoxy thiophenol with tin and hydrochloride acid.

Eight and nine-tenths grams of *p*-methoxy thiophenol were dissolved in 50 cc. of alcohol containing 0.970 Gm. of Na. An alcoholic solution of 6.21 Gm. of *n*-amyl bromide was added. An immediate precipitate of NaBr was formed. The reaction mixture was refluxed on the steam-bath for 3 hours. The alcoholic solution was then filtered from NaBr and upon evaporation of the alcohol, 6.4 Gm. of a light yellow oil were obtained. The yield was 67.8%.

Analysis: Calculated for $C_{12}H_{18}OS$, S, 15.24%; found, S, 15.23%.

Five grams of the above methoxy compound were added to a mixture of 22.6 Gm. of acetic anhydride and 9.9 Gm. of 48% HBr acid. The solution was heated on the steam-bath for 8 hours. Excess HBr acid and acetic anhydride were distilled off *in vacuo*. The oil which remained was dissolved in alkali and the latter solution was extracted with ether to remove undemethylated compound. Acidification of the alkaline solution resulted in precipitation of a black oil. The latter was dissolved in ether and the ethereal solution shaken with charcoal. After filtration the ether was evaporated and the red oil which remained was distilled, the boiling point at 2-3 mm. being 123-130° C.

Analysis: Calcd. for $C_{11}H_{16}OS$, S, 16.23%; found, S, 16.27%.

Preparation of Mono-Acetoxy Mercuri-p-Hydroxy Diphenyl Sulphide.

One gram of *p*-hydroxy diphenyl sulphide prepared by the Grignard reaction was dissolved in 20 cc. of alcohol. The solution was heated on the steam-bath and 1.5 Gm. of mercuric acetate dissolved in 7 cc. of water was added slowly with stirring. The solution was refluxed for one hour when a test with $(NH_4)_2S$ indicated the absence of inorganic mercury. The precipitate formed during the reaction was filtered and purified by dissolving in aqueous alkali and precipitating with acetic acid; 0.1 Gm. of substance was obtained.

The filtrate of the reaction mixture was diluted with water and after saturating with NaCl a gummy mass was obtained. This was treated with alkali and the latter solution acidified. A white precipitate was obtained. The yield was 0.5 Gm.

Analysis: Calc. for $C_{14}H_{12}O_3HgS$, Hg, 43.46%; found, 42.60%.

Mercuration of p-Hydroxy Diphenyl Sulphide as Obtained through Modified Hinsberg Reaction.

Four and eight-tenth grams of *p*-hydroxy diphenyl sulphide were dissolved in 100 cc. of absolute alcohol and heated under reflux with stirring. A solution of 4.9 Gm. of mercuric acetate in 50.0 cc. of 70% alcohol was added dropwise. The solution was refluxed for one hour at the end of which time the test for inorganic mercury was negative. The alcoholic solution was decanted from the precipitate formed during the mercuration and the filtrate was made just acid; the resulting precipitate was filtered, washed with alcohol and dried *in vacuo*.

Yield, 1.2 Gm. Analysis: Calcd. for $C_{14}H_{12}O_3HgS$, S, 6.93%; found, S 5.50%.

The precipitate from which the original reaction mixture was decanted was dissolved in glacial acetic acid and alcohol and treated with NaOH until the solution was just acid. The white precipitate was filtered, washed with alcohol and dried *in vacuo*.

Yield, 0.85 Gm. Analysis: Calcd. for $C_{14}H_{12}O_3HgS$, S, 6.93%; found, 5.40%.

Evidently the products were alike and were a mixture of mono- and di-acetoxy mercuri *p*-hydroxy diphenyl sulphide.

A mercury analysis was made on the compound obtained.

Calcd. for $C_{14}H_{12}O_3HgS$, Hg, 43.55%; found, Hg, 51.2%.

The high mercury assay checks the low sulphur determination in that both are indicative of the presence of 40% of the mono and 60% of the di-mercury compound.

Preparation of Diacetoxy Mercury Para Hydroxy Diphenyl Sulphide.

The method employed above was followed. Six grams of *p*-hydroxy diphenyl sulphide were reacted with 8.6 grams of mercuric acetate in 5% alcohol. Four and three-tenths grams of a white power were isolated.

Analyses: Calcd. for $C_{16}H_{14}O_6Hg_2S$, Hg, 4.40%; found, Hg, 4.70%, 4.64%.

Preparation of 3-3'-Diacetoxy Mercuri, 4-4'-Dihydroxy Diphenyl Sulphide.

Five grams of 4-4'-dihydroxy diphenyl sulphide were dissolved in 70 cc. of alcohol and a solution of 14.6 Gm. of mercury acetate in 80 cc. of water acidified with acetic acid was added all at once. The reaction mixture was heated on the steam-bath with stirring for $\frac{1}{2}$ hour, and a yellow heavy precipitate appeared. This was filtered off, washed and dried. The dry compound turned black in color. It was purified by redissolving in alkali and reprecipitating with acetic acid several times. A light colored substance was finally obtained weighing 2.0 Gm.

Analyses: Sulphur: Found, 4.16%; calculated 4.35% }
Mercury: Found, 55.00%; calculated 54.60% } for $C_{18}H_{14}O_6SHg_2$.

Preparation of Mono-Mercurated 4-Hydroxy-2-Methyl-Diphenyl Sulphide.

In this experiment the procedure employed in the previous mercurations was followed, using 3.2 Gm. of sulphide and 4.79 Gm. of mercuric acetate.

Analysis: Calculated for $C_{18}H_{14}O_3HgS$, S, 7.01%; found, S, 6.74%.

Preparation of 4-Hydroxy-3-Carboxy Diphenyl Sulphide.

The Hinsberg reaction was used in the synthesis of this compound. Thirty-two and five-tenths grams of benzene sulphinic acid were reacted with 13.0 Gm. of salicylic acid. The mixture was heated for $4\frac{1}{2}$ hours. It was then extracted with hot water which removed all constituents except the sulphide. Six and six-tenths grams of crude compound were obtained; yield 28.4%. By distillation of the substance we obtained 2.3 Gm. of crystalline material boiling between $162-167^\circ$ at 3-4 mm.

Analysis: Calculated for $C_{18}H_{10}O_3S$, S, 13.0%. Calculated for $C_{18}H_{10}O_3S_2$, S, 23.0%; found, S, 15.3%.

Evidently a mixture of 83.0% of monosulphide and 17.0% of disulphide had been formed.

Preparation of 4-Hydroxy-3-Nitro-Diphenyl Disulphide by Means of the Hinsberg Reaction.

From 27.0 Gm. of *o*-nitro phenol 14.0 Gm. of product were obtained, corresponding to a 30% yield. It was distilled *in vacuo*; the fraction boiling between $175-181^\circ$ at 5 mm. was collected. The compound was a greenish yellow oil.

Analysis: Calcd. for $C_{12}H_8O_3NS_2$, S, 22.9%; found, S, 22.0%.

Preparation of 4-Hydroxy-3-Brom Diphenyl Disulphide by the Hinsberg Reaction.

In this experiment we expected to prepare 4-hydroxy-3-brom diphenyl sulphide from *o*-brom phenol and benzene sulphinic acid. However, analysis of the reaction product indicated it to be the disulphide.

The yield was 26.5% and the boiling point of the disulphide was 173–179° at 3–4 mm.
Analysis: Calcd. for $C_{12}H_9OBrS_2$, S, 20.9%; found, S, 21.7%.

Preparation of 4-Hydroxy-3-Phenyl Diphenyl Disulphide by the Hinsberg Reaction.

We expected to obtain 4-hydroxy-3-phenyl diphenyl sulphide from *o*-hydroxy diphenyl and benzene sulphinic acid. However, analysis showed the product to be a disulphide.

Analysis: Calcd. for $C_{18}H_{14}OS_2$, S, 20.66%; found, S, 21.1%.

CONCLUSIONS.

1. A number of aryl phenolic sulphides have been prepared, as well as some mercury derivatives of some of them, and their germicidal activity has been studied.

2. The introduction of a methyl group into the ring containing the hydroxyl group in *p*-hydroxy diphenyl sulphide decreases the typhoid activity and increases the staphylococcus activity; this is most pronounced when the methyl group is ortho to the hydroxyl.

3. The introduction of an ethyl instead of a methyl group in that ring causes a general rise in germicidal activity for both types of bacilli, giving rise to the assumption that germicidal activity can be still further increased with an increase in the size of the alkyl group.

4. The symmetrical dihydroxy diphenyl sulphides have only low germicidal activity.

5. The mercury derivatives of the aryl phenolic sulphides have no higher germicidal activity than the compounds from which they are derived. The di-mercury compounds are less active than the mono-mercury compounds.

6. The ether derivatives of aryl phenolic sulphides have very low germicidal activity showing that the sulphur linkage alone is not the cause of the high activity of these compounds.

7. Alkyl phenolic sulphides have about the same order of activity as the corresponding aryl compounds.

8. The introduction of the carboxyl group instead of the alkyl group in the ring containing the hydroxyl group in *p*-hydroxy diphenyl sulphide, causes a lowering of germicidal activity.

9. Using the Hinsberg reaction on ortho-nitro phenol, ortho-brom phenol and ortho-hydroxy diphenyl produces the disulphide instead of the sulphide. These disulphides are of a low order of activity.

10. A method of purification of the crude product of the Hinsberg reaction prior to distillation has been developed, which makes it possible to obtain pure products by distillation when phenols of increasing complexity are used.

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EXAMINATION OF THE JUICES OF THE LEAVES OF MONARDA PUNCTATA AND MONARDA FISTULOSA.*

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In order to supplement our knowledge concerning these two plants, it was thought advisable to investigate several physical and chemical characteristics of the freshly expressed juice from the leaves. The leaves were picked and immediately placed in bottles of approximately one-liter capacity with 25 cc. of ether and stoppered securely. The bottles were then placed in a refrigerator over night. The leaves were ground in a food chopper and the sap expressed in a mechanical press. The juice was clarified in a centrifuge and the refractive index determined at 20° C. with an Abbe refractometer.

DETERMINATION OF THE BOUND WATER CONTENT.

The method devised by Newton and Gortner¹ was used for this purpose. The percentage of total solids was obtained by use of the refractive index by consulting the table of Schönrock.² The freezing point of the sap was then determined using the ordinary cryoscopic method for this purpose. A sample of the juice was then taken which contained exactly 10 Gm. of water and 0.01 mol. of sugar was added and the freezing point of the sugar solution determined as above. From the data obtained in this manner, the bound water content was calculated using the Newton-Gortner formula for this purpose. The following is a table of the results:

Juice.	Ref. Index.	P. c. Water.	F. P. Sap.	F. P. Sap and Sugar.	Depr.	P. c. Bound Water.
<i>M. fistulosa</i>	1.3437	92.7	-1.03° C.	-3.37° C.	2.34° C.	9.7
<i>M. punctata</i>	1.3437	92.7	-0.91° C.	-3.15° C.	2.24° C.	6.2

A check on the accuracy of the refractive index as a method for the determination of total solids was obtained by evaporating a sample of the sap to constant weight at 60° C. The average results of three samples each of the juices of the leaves of the two plants are as follows: 92.2 p. c. water in the case of *M. punctata*

* From the Laboratory of Edward Kremers.

¹ R. Newton and A. G. Gortner, *Bot. Gaz.*, 74 (1922), 442.

² "Off. & Tent. Methods of Analysis," *A. O. A. C.*, 3rd Edition, page 510.