Summary

1. Condensation reactions with 3-nitro-4-halogenophenylarsonic acids and a series of aliphatic amino compounds, namely, iso- and *n*-amyl, iso- and *n*-butyl, *n*-propyl and ethanol amines, and glycine were successfully completed.

2. The corresponding amino derivatives of the above condensation products were prepared.

3. 3-Nitro-4-bromophenylarsonic acid was condensed with phenol and a series of substituted phenols, namely, *p*-chlorophenol, *o*- and *p*-cresols and *p*-nitrophenol, leading to a series of phenyl ether derivatives. The carboxyl derivatives were also prepared through the oxidation of the methyl derivatives.

4. The corresponding amino derivatives of the substituted phenol condensation products were synthesized. The lactam of 2-amino-4-arsono-2′-carboxyphenyl ether was obtained through the elimination of water from 2-amino-4-arsono-2′-carboxyphenyl ether. This resulted in the formation of a seven-membered ring compound.

5. A study was made of the effects of experimental conditions on the reaction of *p*-bromophenylarsonic acid with aniline.

LINCOLN, NEBRASKA

[CONTRIBUTION FROM THE PLAUT RESEARCH LABORATORY OF LEHN & FINK, INC.]

HALOGEN DERIVATIVES OF MONOHYDROXYDIPHENYLMETHANE AND THEIR ANTIBACTERIAL ACTION

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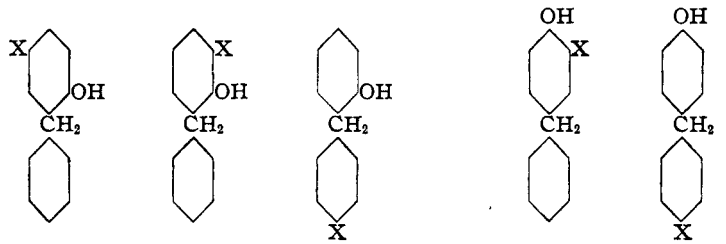
It was shown in a recent communication that monosubstituted halogen derivatives of 2,4-dihydroxydiphenylmethane are potent bactericides for *Eberthella typhi* and *Staphylococcus pyogenes aureus*.[1] Distinct differences in antibacterial action were observed depending upon the position of the substituting halogen. Thus with both the chloro and the bromo derivatives, it was found that a greater germicidal efficacy toward both test microörganisms was obtained in the case of substitution in the nucleus bearing the two hydroxyl groups than in that of substitution in the other nucleus. While *E. typhi* appeared to be more susceptible to the action of the compounds of this group than *Staph. aureus* the differences in susceptibility were by no means indicative of bactericidal specificity.

Notes on the Preparation of Halogen Hydroxydiphenylmethane Derivatives.—In continuing our studies on related problems, we devoted

[1] Klarmann and Von Wowern, THIS JOURNAL, 51, 605 (1929).

our attention to the substitution by halogen of the 2- and 4- monohydroxydiphenylmethanes and of some of their alkyl derivatives, and to the antibacterial action of the compounds thus obtained. The two groups of compounds studied may be represented schematically by the following structural formulas (where X stands for the halogen)



Group I
Derived from 2-hydroxydiphenylmethane

Group II
Derived from 4-hydroxydiphenylmethane

In addition, a number of dihalogen substituted derivatives were prepared which may be regarded as combinations of the types within each group. The alkyl homologs studied contain the substituting alkyl radicals in the nucleus bearing the hydroxyl group.

The different compounds were obtained by condensation of a benzyl or halogen benzyl derivative with a phenol derivative substituted by halogen only or by halogen and alkyl groups. In the preparation of these substances advantage was taken of the following facts: condensation in the presence of "acid" agents, such as anhydrous zinc chloride, sulfuric acid, etc., leads predominantly to substitution in para-position to the phenolic hydroxyl group if the para-position is open, and to substitution in ortho-position if it is not; on the other hand, condensation according to Claisen leads primarily to substitution in ortho-position to the hydroxyl group.[2]

The 3-methyl-5-chloro-2-hydroxydiphenylmethane was obtained by benzylation of *o*-cresol according to Claisen and subsequent chlorination.

Benzylation of 4-chloro-*m*-cresol (6-chloro-3-hydroxy-1-methylbenzene) according to Claisen might yield two ortho-substituted derivatives, *viz.*, 5-chloro-4-methyl-2-hydroxydiphenylmethane or 5-chloro-6-methyl-2-hydroxydiphenylmethane; possibly a mixture of both is formed. We have made no attempt as yet to establish the position of the methyl group in this product which will be referred to hereafter as 5-chloro-4[6]-methyl-2-hydroxydiphenylmethane.

It is evident that some of the compounds under discussion should permit preparation by either method of condensation. This was actually found to be the case in a number of instances whereby additional evidence was obtained in support of the constitution attributed to the compounds studied.

[2] L. Claisen, *Ann.*, **442**, 210 (1925).

Action on Bacteria

(a) **Bactericidal Effects.**—The researches on the relation between the chemical constitution and the bactericidal action of organic compounds heretofore published point to the great usefulness of testing the effect of the chemicals upon as many microörganisms as possible. In the present investigation four bacterial species were used, *viz.*, two representatives of the Gram-positive and two of the Gram-negative microörganisms. Thus *Staphylococcus pyog. aureus* and *Streptococcus* (hemolytic strain) are representatives of the former, *Eberthella typhi* and *Eberthella paradysenteriae* (Flexner) of the latter class. This procedure appears desirable in view of the findings obtained with other related groups of organic compounds which seem to point distinctly to a class specificity for Gram-positive bacteria in a number of instances.[3] The data reported in the present paper indicate that most of the compounds studied show a similarly specific action; while practically all the compounds under consideration may be regarded quite generally as potent germicides, several of them are many times more effective against Staphylococcus and Streptococcus than against the bacilli of typhoid and paradysentery. It is of interest that similar effects have been observed by several investigators in the case of the inhibitory action of certain basic dyes of high molecular weight, particularly in the triphenylmethane series.

Table I gives a list of the compounds studied, the minimum average concentrations which are capable of killing the four microörganisms at 37° in five, ten and fifteen minutes, respectively, and the "phenol coefficients" calculated from a comparison of this effect with that produced by phenol under the same experimental conditions.

Table I indicates that this series of compounds comprises some very powerful bactericides. The action upon the Gram-positive microbes appears to be generally considerably stronger than upon the Gram-negative. Within the two classes of germs, *E. typhi* is somewhat more resistant than *E. paradysenteriae*, and staphylococcus more so than streptococcus.

When considering the two groups of monohalogen compounds studied, *viz.*, those derived from 2-hydroxydiphenylmethane and those derived from 4-hydroxydiphenylmethane, one cannot say that generally one or the other group is distinguished by greater antibacterial potency. However, certain regularities of behavior toward the microbes are distinctly evident, such as: that the 5-halogen-2-hydroxydiphenylmethanes are more potent than the 3-halogen-4-hydroxydiphenylmethanes, or that the monobromo derivatives of both the 2- and the 4-hydroxydiphenylmethanes are less effective than the corresponding monochloro derivatives against the two Gram-negative microörganisms, but more so against the Gram-positive. The 4′-chloro-2-hydroxydiphenylmethane is less effective than the 4′-chloro-4-hydroxydi-

[3] Klarmann, Gates and Shternov, THIS JOURNAL, **54**, 1204 (1932).

TABLE I

BACTERICIDAL ACTION

Derivatives of hydroxydiphenylmethane	*Staph. aureus*				*Strept. (hemol.)*				*E. typhi*				*E. paradysenteriae*			
	5 min.	10 min.	15 min.	Phenol coeff.	5 min.	10 min.	15 min.	Phenol coeff.	5 min.	10 min.	15 min.	Phenol coeff.	5 min.	10 min.	15 min.	Phenol coeff.
3-Chloro-4-	1: 9,000	1:10,000	1:10,000	125	1:12,000	1:13,000	1:14,000	165	1: 5,000	1: 5,000	1: 5,500	36	1: 7,000	1: 7,000	1: 7,000	54
3-Bromo-4-	1:13,000	1:14,000	1:15,000	170	1:13,000	1:15,000	1:16,000	185	1: 2,500	1: 2,500	1: 3,250	19	1: 5,000	1: 5,000	1: 5,000	37
3-Chloro-2-	1: 5,500	1: 5,500	1: 6,000	71	1: 7,000	1: 8,500	1: 9,000	94	1: 3,250	1: 3,750	1: 3,750	24	1: 3,750	1: 5,000	1: 5,000	36
4'-Chloro-4-	1:11,000	1:12,000	1:14,000	170	1:12,000	1:15,000	1:16,000	165	1:12,000	1:12,000	1:14,000	83	1:13,000	1:13,000	1:14,000	98
5-Chloro-2-	1:15,000	1:16,000	1:18,000	215	1:18,000	1:18,000	1:22,500	245	1: 9,000	1:10,000	1:13,000	74	1:11,000	1:12,000	1:13,000	92
5-Bromo-2-	1:20,000	1:22,500	1:25,000	295	1:25,000	1:27,500	1:27,500	310	1: 3,500	1: 3,500	1: 4,000	26	1: 6,500	1: 7,000	1: 8,000	55
4'-Chloro-2-	*1:14,000*	*1:15,000*	*1:15,000*	190	*1:14,000*	*1:14,000*	*1:18,000*	175	*1: 7,000*	*1: 8,000*	*1: 8,000*	57	*1:11,000*	*1:12,000*	*1:15,000*	90
3,4'-Dichloro-4-	1:20,000	1:25,000	1:30,000	345	1:15,000	1:18,000	1:22,500	175	1: 5,500	1: 6,500	1: 7,000	41	1:14,000	1:15,000	1:15,000	110
3-Chloro-4'-bromo-4-	Indefinite over 10,000			200	1:40,000	1:40,000	1:50,000	565	1: 2,500	1: 2,500	1: 2,500	17	1: 3,000	1: 3,000	1: 3,250	24
3,5-Dichloro-2-	1: 5,000	1: 6,000	1: 6,000	65	1:20,000	1:22,500	1:22,500	250	1: 3,000	1: 3,000	1: 3,250	22	1:11,000	1:12,000	1:12,000	85
5-Chloro-3-methyl-2-	1:18,000	1:18,000	1:20,000	245	1:27,500	1:30,000	1:30,000	300	1: 2,000	1: 2,500	1: 2,500	16	1: 3,250	1: 3,500	1: 3,750	25
4'-Chloro-3-methyl-2-	1:16,000	1:20,000	1:20,000	240	1:20,000	1:25,000	1:27,500	260	1: 2,000	1: 2,250	1: 2,500	16.0	1: 3,500	1: 3,750	1: 3,750	26
5-Chloro-4[6]-methyl-2-	*1:30,000*	*1:30,000*	*1:32,500*	405	*1:35,000*	*1:35,000*	*1:45,000*	455	*1: 2,250*	*1: 2,750*	*1: 2,750*	17	*1: 3,500*	*1: 4,500*	*1: 5,000*	34
5-Chloro-4,6-dimethyl-2-	1:70,000	1:80,000	1:80,000	920	1:55,000	1:70,000	1:80,000	785	1: 3,750	1: 4,500	1: 5,000	31	1: 2,750	1: 3,000	1: 3,250	22
4'-Bromo-4,6-dimethyl-2-	1:30,000	1:35,000	1:35,000	420	1:50,000	1:60,000	1:70,000	600	.....	.....	.....	..	.....	.....	.....	..
5-Chloro-3-isopropyl-6-methyl-2-	1: 2,250	1: 3,000	1: 3,250	35	1: 2,750	1: 3,000	1: 3,250	38	1: 2,000	1: 2,250	1: 2,500	16	1: 2,000	1: 2,000	1: 2,500	15
Phenol (control)	1: 70	1: 80	1: 80	1	1: 80	1: 90	1: 90	1	1: 130	1: 140	1: 160	1	1: 120	1: 130	1: 150	1

phenylmethane against the Gram-negative, but it is somewhat more effective against the Gram-positive germs. The 3-chloro-2-hydroxydiphenylmethane in which the halogen is in ortho-position to the hydroxyl group is considerably less effective than the corresponding 5-chloro derivative, *i. e.*, one in which the chlorine and the hydroxyl groups are in para-position to each other. The germicidal superiority of the para- over the ortho-halogen substituted compounds has been observed previously in the case of simple phenol derivatives,[4] both *p*-chloro- and *p*-bromophenol being more effectively bactericidal for *E. typhi* and *Staph. pyog. aureus* than the corresponding ortho derivatives.

Little regularity is discernible in the antibacterial behavior of the dihalogen derivatives. They also appear to be strong germicides; however, in one instance no definite bacteriological data could be obtained, in some others the results will be of a somewhat limited significance as the relative insolubility of these compounds necessitated the use of comparatively large quantities of alcohol in the preparation of dilutions; the presence of alcohol in excessive proportions, as will be shown later, may affect the outcome of the experiment either by impairing or by promoting the antibacterial action of the solutions.

The introduction of alkyl groups into the nucleus of phenol and of simple phenol homologs is known to produce an increase of bactericidal potency. The presence of alkyl groups in the compounds of considerable molecular weight under discussion produces no uniform effect on the bactericidal action. The presence of one or two methyl groups increases the germicidal potency against staphylococci and streptococci, the dimethyl derivatives in particular showing extraordinarily high phenol coefficients. The presence of one methyl and one isopropyl group, however, does not have any such effect, the compound in question (5-chloro-6-methyl-3-isopropyl-2-hydroxydiphenylmethane) actually being the least effective in the series. The bactericidal action upon the typhoid and paradysentery germs generally is not intensified by the presence of alkyl groups in the nucleus bearing the hydroxyl group.

In order to obtain an idea of the effect of temperature several chloro derivatives were allowed to act upon *Staph. aureus* at 20°. Table II shows

TABLE II

GERMICIDAL ACTION UPON *Staph. Aureus* AT 20°

Derivatives of hydroxydiphenylmethane	5 min.	10 min.	15 min.	Phenol coeff.
5-Chloro-2-	1:10,000	1:10,000	1:12,000	200
4'-Chloro-4-	1:10,000	1:10,000	1:11,000	195
5-Chloro-4[6]-methyl-2-	1:20,000	1:20,000	1:20,000	380
5-Chloro-4,6-dimethyl-2-	1:40,000	1:40,000	1:40,000	810

[4] Klarmann, Shternov and Von Wowern, *J. Bact.*, **17**, 423 (1929).

that somewhat higher concentrations are necessary at the lower temperature to produce a germicidal effect; the numerical values of the phenol coefficients compare with those obtained at 37°.

(b) **Bacteriostatic Effects.**—The data given in Table I indicate the germ-killing action of the compounds prepared. To supplement our knowledge of the antibacterial behavior of this class of chemicals we determined the inhibitory effect of a selected number of them upon the growth of *Staphylococcus aureus*. With this we combined the determination of the status of viability of the germs after different times of exposure to the inhibitory concentrations, ascertaining thereby the concentrations and times at which the bacteria are still capable of development when brought under favorable conditions. In Table III we give a complete record of data obtained with 5-chloro-4,6-dimethyl-2-hydroxydiphenylmethane in order to illustrate the procedure.

This table shows that the growth of staphylococcus in nutrient broth is prevented by a concentration of 1:150,000 or 0.00066% of 5-chloro-4,-6-dimethyl-2-hydroxydiphenylmethane. This result is apparent after twenty-four hours of incubation, and does not change on keeping the set of culture tubes at 37° for another two days. Nevertheless the microbes are not actually dead or incapable of growth for many hours after having been implanted in broth containing this or even stronger concentrations of the compound.

When a transfer to fresh media is made fifteen minutes after planting, the concentration of 1:40,000 appears to be actually germicidal, while one of 1:50,000 is not. The longer the time allowed to elapse between planting and transfer, the lower is the concentration which kills the bacteria. But even after long exposure to the minimum bacteriostatic concentration of 1:150,000. there seem to be individual organisms which when transferred to fresh media are capable of producing growth.

Thus the minimum bacteriostatic concentration for *Staphylococcus aureus* is about twice lower than the minimum bactericidal concentration (given in Table I). It is also noteworthy that by this method the minimum bactericidal concentration after fifteen minutes appears to be 1:40,000, while by the direct method a concentration of only 1:80,000 was found. Indubitably this is due to the presence in the broth of organic matter in the bacteriostatic test, which interferes with the antibacterial action of the chemical.

Similar tests were carried out with a series of four compounds, *viz.*, 5-chloro-2-hydroxydiphenylmethane, 4′-chloro-4-hydroxydiphenylmethane, 5-chloro-4[6]-methyl-2-hydroxydiphenylmethane and 5-chloro-4,6-dimethyl-2-hydroxydiphenylmethane. In Table IV, instead of the complete records only the minimum concentrations are given at which no growth has occurred, and the phenol coefficients of bacteriostatic action.

TABLE III

BACTERIOSTATIC ACTION OF 5-CHLORO-4,6-DIMETHYL-2-HYDROXYDIPHENYLMETHANE

	Time in hours				Transfer to fresh media after							
	24	48	72	96	15 min.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	72 hrs.
1:40,000	−	−	−	−	−	−	−	−	−	−	−	−
1:50,000	−	−	−	−	+	−	−	−	−	−	−	−
1:60,000	−	−	−	−	+	−	−	−	−	−	−	−
1:70,000	−	−	−	−	+	+	+	−	−	−	−	−
1:80,000	−	−	−	−	+	+	+	+	−	−	−	−
1:100,000	−	−	−	−	+	+	+	+	+	−	−	−
1:150,000	−	−	−	−	+	+	+	+	+	+	−	−
1:200,000	−	+	+	+	+	+	+	+	+	+	+	+
1:250,000	−	+	+	+	+	+	+	+	+	+	+	+
1:300,000	+	+	+	+	+	+	+	+	+	+	+	+
1:400,000	+	+	+	+	+	+	+	+	+	+	+	+
1:500,000	+	+	+	+	+	+	+	+	+	+	+	+

TABLE IV

BACTERIOSTATIC ACTION UPON *Staphylococcus Aureus*

Derivatives of hydroxydiphenylmethane	Minimum inhibitory concentration (96 hrs.)	No growth in subcultures from the following concentrations after						Bacteriostatic phenol coefficient
		15 min.	1 hr.	6 hrs.	24 hrs.	48 hrs.	72 hrs.	
5-Chloro-2-	1: 70,000	1: 7,000	1:15,000	1:20,000	1: 30,000	1: 50,000	1: 70,000	230
4'-Chloro-4-	1: 50,000	1:10,000	1:15,000	1:20,000	1: 20,000	1: 30,000	1: 50,000	165
5-Chloro-4[6]-methyl-2-	1:100,000	1:30,000	1:30,000	1:50,000	1: 70,000	1:100,000	1:100,000	330
5-Chloro-4,6-dimethyl-2-	1:150,000	1:40,000	1:60,000	1:80,000	1:100,000	1:150,000	1:150,000	500
Brilliant green	1:2,000,000	1: 5,000	1:50,000	.....	1:2,000,000	1:2,000,000	1:2,000,000	6600
Phenol	1:300	1:100	1:120	1:150	1:200	1:200	1:300	1

Table IV indicates that the bacteriostatic action upon *Staph. aureus* of the four compounds tested is symbat with their bactericidal action upon the same microörganism. Thus in both cases the presence of the halogen in the nucleus bearing the hydroxyl group makes a more effective bacteriostatic agent than its presence in the other nucleus. The presence of one methyl radical increases the inhibitory capacity while the second methyl group brings about a further increase to more than double the value of the non-alkylated compound.

A parallel test with brilliant green (tetraethyldiaminotriphenylmethane) was included in order to demonstrate that the compounds of the class under discussion are typical bactericidal rather than typical bacteriostatic agents. Thus in three cases the maximum bacteriostatic dilution is only three to five times greater than the bactericidal (in fifteen minutes), while in one case it is ten times greater. In contrast to this, the maximum inhibitory dilution of brilliant green is about four hundred times its maximum germicidal dilution (in fifteen minutes), *i. e.*, the divergence of bactericidal and bacteriostatic concentrations is very considerable; brilliant green is a typical bacteriostatic agent.

The interesting antibacterial properties of the compounds described in this communication have suggested an extension of our investigations to include the alkyl and other alpharyl derivatives of halogenated phenols, work on which is in progress at this time.

Experimental Part

(a) **Chemical.**—The two methods used in the preparation of the compounds under consideration are illustrated in the following two examples.

Method I. Preparation of 3-Chloro-4-hydroxydiphenylmethane, Cl(3)OH(4)-$C_6H_3CH_2C_6H_5$.—*o*-Chlorophenol (51.4 g.) is mixed with 50.6 g. of benzyl chloride and 10 g. of anhydrous zinc chloride is added. The mixture is heated with stirring to 60° and kept at this temperature for two hours, then the temperature is raised to 90° and heating discontinued. Water is added and the mixture shaken with ether. The aqueous portion is withdrawn and the ethereal solution washed with dilute hydrochloric acid and finally with water. The ethereal solution is then shaken repeatedly with dilute potassium hydroxide. The alkaline extracts are washed with ether, then combined and acidified with hydrochloric acid. An oil precipitates which is shaken out with ether, and washed free from acid and salt. After drying and evaporation of the solvent, the oil is subjected to repeated distillation *in vacuo*. The fraction distilling at 155–160° at 5 mm. is isolated.

Besides zinc chloride, aluminum chloride and sulfuric acid were used as condensing agents with success in several instances.

Method II. (Claisen's Reaction) Preparation of 3-Chloro-2-hydroxydiphenylmethane, Cl(3)OH(2)$C_6H_3CH_2C_6H_5$.—*o*-Chlorophenol (128 g.) is dissolved in 500 cc. of toluene and added slowly with constant mechanical stirring to 23 g. of "bird-shot" sodium in 100 cc. of toluene. Violence of reaction may be reduced by occasional immersion of the flask in cold water. When all of the sodium has disappeared the mixture is

cooled to room temperature and benzyl chloride is added very slowly with stirring. The reaction is completed by heating slowly in an oil-bath to 110° and maintaining at this temperature for four hours. The solvent and any uncombined phenol and benzyl chloride are removed by steam distillation. To the residue methyl alcoholic alkali is added and the mixture shaken repeatedly with petroleum ether. The alkaline extract is acidified and, after the usual treatment, the oil is purified by vacuum distillation. The fraction distilling at 144° at 4 mm. is isolated.

Satisfactory results were also obtained when the sodium compounds of the halophenols were prepared by means of sodium hydroxide and subsequent drying of the reaction product.

The following Table V gives the physical constants of the compounds studied, together with a statement of the method and of the initial materials used in their preparation.

TABLE V

HALOGEN DERIVATIVES OF HYDROXYDIPHENYLMETHANE

Derivatives of hydroxydiphenylmethane	Initial materials	Chloride	Method of prepn.	M. p., °C.
3-Chloro-4-	*o*-Chlorophenol	Benzyl	I	
3-Bromo-4-	*o*-Bromophenol	Benzyl	I	
3-Chloro-2-	*o*-Chlorophenol	Benzyl	II	
4′-Chloro-4-	Phenol	*p*-Chlorobenzyl	I	85.5
5-Chloro-2-	*p*-Chlorophenol	Benzyl	I, II	48.5
5-Bromo-2-	*p*-Bromophenol	Benzyl	I	
4′-Chloro-2-	Phenol	*p*-Chlorobenzyl	II	61.5
3,4′-Dichloro-4-	*o*-Chlorophenol	*p*-Chlorobenzyl	I	64
3-Chloro-4′-bromo-4-	*o*-Chlorophenol	*p*-Bromobenzyl	I	65
5-Chloro-3-methyl-2-	*o*-Cresol	Chlorine + benzyl	II	55
4′-Chloro-3-methyl-2-	*o*-Cresol	*p*-Chlorobenzyl	II	48
5-Chloro-4[6]-methyl-2-	4-Chloro-*m*-cresol	Benzyl	II	
5-Chloro-4,6-dimethyl-2-	4-Chloro-symm.-xylenol	Benzyl	I, II	68.5
4′-Bromo-4,6-dimethyl-2-	4-Chloro-symm.-xylenol	*p*-Bromobenzyl	I	101.5
5-Chloro-3-isopropyl-6-methyl-2-	4-Chlorothymol	Benzyl	I	

Formula	B. p., °C.	Mm.	% Halogen Calcd.	% Halogen Found
$Cl(3)OH(4)C_6H_3\cdot CH_2\cdot C_6H_5$	155–160	5	16.22	15.83
$Br(3)OH(4)C_6H_3\cdot CH_2\cdot C_6H_5$	152–154	3	30.38	30.40
$Cl(3)OH(2)C_6H_3\cdot CH_2\cdot C_6H_5$	144	4	16.22	15.64
$OH(4)C_6H_4\cdot CH_2\cdot C_6H_4Cl(4')$	175–177	4	16.22	16.10
$Cl(5)OH(2)C_6H_3\cdot CH_2\cdot C_6H_5$	160–162	3.5	16.22	16.25
$Br(5)OH(2)C_6H_3\cdot CH_2\cdot C_6H_5$	189–192	3.5	30.38	30.42
$OH(2)C_6H_4\cdot CH_2\cdot C_6H_4Cl(4')$	168–171	4	16.22	16.24
$Cl(3)OH(4)C_6H_3\cdot CH_2\ C_6H_4Cl(4')$	160–164	3	28.02	27.62
$Cl(3)OH(4)C_6H_3\cdot CH_2\cdot C_6H_4Br(4')$	182	3	38.79	38.35
$Cl(5)CH_3(3)OH(2)C_6H_2\cdot CH_2\cdot C_6H_5$	147–149	4.5	15.24	14.82
$CH_3(3)OH(2)C_6H_3\cdot CH_2\cdot C_6H_4Cl(4')$	167–172	4	15.24	14.98
$Cl(5)CH_3(4[6])OH(2)C_6H_2\cdot CH_2\cdot C_6H_5$	176–178	4.5	14.38	14.86
$Cl(5)CH_3(4)CH_3(6)OH(2)C_6H\cdot CH_2\cdot C_6H_5$	182–185	4	14.38	14.80
$CH_3(4)CH_3(6)OH(2)C_6H_2\cdot CH_2C_6H_4Br(4')$	194–196	4	27.46	26.98
$Cl(5)C_8H_7(3)CH_3(6)OH(2)C_6H\cdot CH_2\cdot C_6H_5$	175	3	12.91	12.89

The procedure described by Huston and Eldridge[5] was followed in the preparation of 3,5-dichloro-2-hydroxydiphenylmethane. However, the compound was found to melt at 81° while these authors report a melting point of 77–77.5°.

3-Methyl-5-chloro-2-hydroxydiphenylmethane was obtained by chlorination with chlorine in carbon tetrachloride of the condensation product of the sodium compound of *o*-cresol with benzyl chloride, referred to in the papers of Schorigin[6] and of Huston, Swartout and Wardwell.[7]

(b) **Bacteriological.**—The determination of the bactericidal action upon *E. typhi* and *Staph. aureus* was carried out according to the method used by the Food and Drug Administration of the U. S. Department of Agriculture.[8] In the work with *E. paradysenteriae* the same methods of culturing and testing were used as with *E. typhi*. Streptococcus (a very resistant hemolytic strain) was grown for the test in meat infusion–peptone broth (glucose free) at a P_H 7.2–7.4; for subcultures beef extract broth containing 1% of glucose and adjusted to the same P_H was used.

In the preparation of solutions, varying proportions of alcohol were employed. Because of the efficacy of most of the compounds studied toward staphylococci and streptococci in extremely low concentrations, the antibacterial effect of the alcohol present may be regarded as negligible. In most cases the minimum germicidal concentrations for the cocci in Table I did not contain more than 3% of alcohol, with the following exceptions: the extremely insoluble 3,5-dichloro-2-hydroxydiphenylmethane called for 20 and 10% of alcohol, respectively, and 6-methyl-3-isopropyl-5-chloro-2-hydroxydiphenylmethane required 15% of alcohol in both cases. Larger proportions of alcohol were necessary in the tests with the Gram-negative *E. typhi* and *E. paradysenteriae* because of the higher concentrations employed against these more resistant germs than against the Gram-positive cocci. Accordingly in the tests with monohalogen (non-alkylated) derivatives the minimum germicidal concentrations required not more than 5% of alcohol, in those with the alkyl derivatives not more than 15% (except the 6-methyl-3-isopropyl derivative which called for 20%) while the greatest proportion of alcohol had to be used with the dihalogen derivatives: 3,5-dichloro-2-hydroxydiphenylmethane (20%), 3-chloro-4′-bromo-4-hydroxydiphenylmethane (15%) and 3,4′-dichloro-4-hydroxydiphenylmethane (18% in the test with *E. typhi*, but only 3% with *E. paradysenteriae*).

It was considered important, therefore, to attempt a more complete determination of the effect of alcohol in our experiments. It may be assumed that the addition of a small amount of alcohol, by improving the

[5] Huston and Eldridge, THIS JOURNAL, **53**, 2263 (1931).

[6] Schorigin, *Ber.*, **58B**, 2033 (1925).

[7] Huston, Swartout and Wardwell, THIS JOURNAL, **52**, 4488 (1930).

[8] G. L. A. Ruehle and C. M. Brewer, U. S. Dept. of Agriculture, Circular No. 198, Dec., 1931.

solubility of an antiseptic substance, will make the preparation of a concentration within the bactericidal range possible. On the other hand, a large proportion of alcohol may produce one of two effects: a sufficient alcohol concentration may be present to be germicidal *per se*, or the alcohol concentration may be insufficient to kill bacteria, but sufficient to reduce the effectiveness of the antiseptic substance through a shift of its distribution ratio in favor of the aqueous phase. The following experiments, therefore, were made to ascertain to what extent these assumptions were correct.

Although the antibacterial action of alcohol has been studied by previous investigators,[9] it was deemed necessary to carry out an experiment with alcohol under the exact conditions obtaining in our regular tests. The following table illustrates the results.

TABLE VI

BACTERICIDAL ACTION OF ALCOHOL

	5 min.		10 min.		10 min.	
	Killed	Not killed	Killed	Not killed	Killed	Not killed
Staph. aureus	40 (34.5)	30 (25.9)	40 (34.5)	30 (25.9)	40 (34.5)	30 (25.9)
Streptococcus (haemol.)	40 (34.5)	30 (25.9)	40 (34.5)	30 (25.9)	30 (25.9)	25 (21.6)
E. typhi	25 (21.6)	20 (17.3)	20 (17.3)	15 (13.0)	20 (17.3)	15 (13.0)
E. paradysenteriae	25 (21.6)	20 (17.3)	20 (17.3)	15 (13.0)	20 (17.3)	15 (13.0)

The figures without brackets give the alcohol concentrations in per cent. obtained by diluting 95% alcohol. The bracketed figures have been recalculated on the basis of the fact that the addition of 0.5 cc. of culture to 5 cc. of diluted alcohol brings about a further dilution of the latter; they represent the actual concentrations of alcohol (100%) in which the bacteria are suspended. It appears that *E. typhi* and *E. paradysenteriae* are considerably more sensitive to alcohol than staphylococcus and streptococcus. The germicidal alcohol concentrations for the latter two microorganisms appear to be considerably above the alcohol concentrations used in the tests with the antiseptic substances while with *E. typhi* and *E. paradysenteriae* some alcohol concentrations approach those employed in a few tests with the most insoluble antiseptics; thus in certain isolated instances an interfering influence of the alcohol may be counted with.

Another experiment therefore was run in which the dilutions of an antiseptic (5-chloro-2-hydroxydiphenylmethane) were made with diluted alcohol of different concentrations instead of water. The microörganisms used were *E. typhi* and *Staph. aureus*. The results obtained are given in Table VII.

This table shows the interesting fact that alcohol, if used in concentrations which by themselves are not germicidal, is capable of reducing the germicidal action of an antiseptic substance to a considerable extent

[9] F. W. Tilley and J. M. Schaffer, *J. Agr. Res.*, **65**, 611 (1931).

TABLE VII

THE EFFECT OF DILUTED ALCOHOL ON THE BACTERICIDAL POTENCY OF 5-CHLORO-2-HYDROXYDIPHENYLMETHANE

Alcohol concn., %	*Staph. aureus* Minimum germicidal concn. in 5 min.	10 min.	15 min.	*E. typhi* Minimum germicidal concn. in 5 min.	10 min.	15 min.
< 5	1:14,000	1:15,000	1:16,000	1:6500	1:1000	1:7000
10	1:12,000	1:13,000	1:13,000	1:6500	1:6500	1:7000
15				1:8000	1:8000	1:9000
20	1:8000	1:10,000	1:10,000	Indefinite, about 1:60,000		
30	1:6000	1:1000	1:10,000	All dilutions germicidal		
40	All dilutions germicidal			All dilutions germicidal		

(particularly in the case of *Staph. aureus*), the intensity of the impairment depending upon the alcohol concentrations. The most probable reason for this is the shift of the partition equilibrium of the antiseptic substance between the microörganisms and the aqueous-alcoholic phase, in favor of the latter, owing to an increased solvent capacity for the antiseptic substance.

One finds occasionally in the literature that difficultly soluble phenols are brought into solution for the purpose of bacteriological tests by means of diluted alkalies. We ran a series of experiments with three compounds which permitted the preparation of such solutions, using 0.035% potassium hydroxide for the preparation of the initial 0.1% solutions; the further dilutions were made with water. The results given in Table VIII were obtained.

TABLE VIII

THE EFFECT OF ALKALI USED IN THE PREPARATION OF THE INITIAL SOLUTION UPON THE GERMICIDAL ACTION

Derivatives of hydroxy-diphenylmethane	*Staph. aureus* 5 min.	10 min.	15 min.	Diff. of effectiveness, %	*E. typhi* 5 min.	10 min.	15 min.	Diff. of effectiveness, %
3-Chloro-4-	1:6,000	1:8,500	1:9,000	−18	1:5,000	1:5,000	1:5,500	0
3-Bromo-4-	1:11,000	1:11,000	1:12,000	−20	1:2,000	1:2,500	1:2,500	−15
5-Chloro-2-	1:14,000	1:16,000	1:18,000	− 2	1:11,000	1:12,000	1:12,000	+ 9

This table indicates that the use of very diluted alkali in preparing solutions offers no advantage, as it produces no uniform effect. It also obscures the picture because of the change of P_H to which bacteria are known to be sensitive in varying degrees.

The determination of the bacteriostatic effect upon *Staphylococcus aureus* was carried out according to the following procedure. To nine cc. of broth (of the same composition as used in growing the germs for the tests on bactericidal action), one cc. of the antiseptic solution was added (containing ten times the concentration which appears in the table). A spiral loop (of five turns of 2.18 mm. inside diameter, wire gage 23 B. and S., and holding approximately 0.02 cc.) was used to plant the twenty-four hour

culture in this mixture previously warmed to 37°. The tubes were kept in the incubator for ninety-six hours. Transfers were made into fresh broth after fifteen minutes, 1, 2, 4, 6, 24, 48 and 72 hours with a loop of the same size. Readings of subcultures were made after forty-eight hours' incubation.

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Summary

A number of mono- and di-halogen derivatives of 2- and of 4-hydroxydiphenylmethanes and their alkyl homologs have been synthesized either by direct benzylation of halogen phenols or by halogenation of benzylated phenols. The benzyl group was directed into either the ortho or para position to the hydroxyl group by the choice of suitable condensing agents.

While all the compounds of this series were found to be potent bactericides, some derivatives showed an extraordinarily high efficacy toward the two Gram-positive cocci studied (*Staph. aureus* and *Streptococcus haemol.*). Certain regularities in the relation between the antibacterial action and the chemical composition of the compounds studied were found. Thus halogen in the para position to the hydroxyl group conditioned a greater antibacterial efficiency than halogen in ortho position. The 4'-chloro-2-hydroxydiphenylmethane was more effective than the 4'-chloro-4-hydroxydiphenylmethane against the Gram-positive cocci but less so against the two Gram-negative bacteria (*E. typhi* and *E. paradysenteriae*). The monobromo derivatives of both the 2- and the 4-hydroxydiphenylmethanes were less effective than the corresponding monochloro derivatives against the germs of typhoid and paradysentery but more so against staphylococcus and streptococcus. The dihalogen derivatives were also highly bactericidal.

The presence of one or two methyl groups was found to cause a considerable increase in germicidal potency toward the cocci but not toward the germs of typhoid and paradysentery. A methyl and an isopropyl group however, reduce the bactericidal action upon all four microörganisms.

Experiments were also carried out with several compounds to determine their capacity of inhibiting bacterial growth. While, as was to be expected, much lower concentrations were necessary to produce this effect, than to kill the microörganisms upon short exposure, this class of substances appears to show a typically bactericidal rather than a bacteriostatic behavior, when compared, *e. g.*, with bacteriostatic dyes.

Some attention was devoted also to the effect of alcohol used in the preparation of the solutions employed in the bacteriological tests. It was found that alcohol concentrations not germicidal *per se* impaired the antibacterial action of 5-chloro-2-hydroxydiphenylmethane; an explanation

for this was suggested on the basis of an assumed shift of the partition equilibrium of the dissolved substance between the bacteria and the aqueous-alcoholic phase. The effect of diluted alkali in the preparation of the solutions upon their germicidal action was also studied.

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STUDIES IN THE PHENANTHRENE SERIES. II. PHENANTHRENE CARBOXYLIC ACIDS AND 9-BROMOPHENANTHRENE DERIVATIVES[1]

BY ERICH MOSETTIG AND JACOB VAN DE KAMP

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These investigations have been carried out in order to find a practicable way of preparing the phenanthrene carboxylic acids, whereby an adequate supply of starting material for further syntheses in this series would be assured.

Of the hitherto known preparative methods, that of Pschorr[2] is the best for the phenanthrene-9-carboxylic acid, while the -2- and -3-carboxylic acids were best prepared through the corresponding phenanthrene-2- and 3-sulfonic acids.[3,4] Neither of these methods is suitable however for large scale preparation of the carboxylic acids.

Recently, Mosettig and van de Kamp[5] found a way of preparing phenanthrene-2- and -3-carboxylic acids by oxidizing 2- and 3-acetylphenanthrenes, respectively, with sodium hypochlorite, a method which seemed much simpler than those hitherto employed because of the relatively easy separation of the acetylphenanthrenes on a large scale and the good yields obtained in their oxidation.

Liebermann and Zsuffa[6] described the preparation of a phenanthrene carboxylic acid from phenanthrene with oxalyl chloride and aluminum chloride. These authors claimed for their acid the constitution of phenanthrene-9-carboxylic acid and mentioned that besides this acid another one, containing the carboxyl group "in the nucleus," was found in smaller quantities.

In consideration of the results which we obtained from the acetylation of phenanthrene,[5] where it was shown that the acetyl group enters the

[1] This investigation was supported by a grant from the Committee on Drug Addiction of the National Research Council from funds provided by the Bureau of Social Hygiene, Inc.

[2] Pschorr, *Ber.*, **29**, 496 (1896).

[3] Werner and co-workers, *Ann.*, **321**, 248 (1902).

[4] Fieser, THIS JOURNAL, **51**, 2460 (1929).

[5] Mosettig and van de Kamp, *ibid.*, **52**, 3704 (1930).

[6] Liebermann and Zsuffa, *Ber.*, **44**, 207 (1911).