Bacteriostatic Properties of Phenylacetic Acid

Effect of Ortho-Substituents on*

By C. F. Feasley and B. H. Gwynn, with Ed. F. Degering and P. A. Tetrault

The use of acids as antiseptics is a natural consequence of the fact that the growth of bacteria *in vitro* is self-limited and that organic acids, produced from the medium in which bacteria are growing are one of the limiting factors. This was indicated by Taylor (28) as early as 1917.

Mineral acids have bacteriostatic action, which is inversely proportional in general to the p_H of the solution, but organic acids exhibit a high degree of specificity. There are some rather definite correlations between structure, chemical and physical properties, and bacteriostatic and bactericidal activity. These have been considered in some detail in previous papers, but are briefly summarized in the following paragraph.

Bacteriostatic and bactericidal activity is found to parallel rather closely with (1) an increase in the hydrogen ion concentration of the medium (5, 12, 14, 31), (2) an increase in the oil-water distribution coefficient (3, 4, 10, 15), (3) an increase in adsorption on activated charcoal (3, 10, 15) and (4) a lengthening of the carbon chain (3, 6, 10, 15, 20).

The effect of unsaturation has not been clearly established. In some few cases, particularly with the phenols, unsaturation definitely enhances the bacteriostatic properties (16). With acids this effect is not so apparent. In this series unsaturation may even show slight inhibition on the effectiveness of the bacteriostatic agent. This may be attributed in part to the susceptibility of bacteriostatic and bactericidal reagents to unstable organic compounds (8).

Closely allied to this is the relation between chemical stability and bacteriostatic activity. This has been investigated in the case of maleic and fumaric acids, and the stabler isomer (fumaric acid) has been found to be a more effective bacteriostatic agent (8).

The effect of varying a substituent in an aromatic nucleus, as well as varying the substituent in any given position on the nucleus (4, 19) has been studied but not generally enough to permit of many definite conclusions. In general, however, *p*-substituted compounds have been found to be more effective against bacteria than have the corresponding *o*-substituted isomers.

In this research a study has been made of certain *o*-substituted phenylacetic acids, and the bacteriostatic properties of these compounds compared with those of the corresponding members of the *p*-series (4). A few other acids have been included in the group in an effort to obtain further information about the effect of unsaturation, position isomerism, and *cis-trans* isomerism on bacteriostatic activity.

EXPERIMENTAL

A number of *o*-substituted phenylacetic acids have been prepared by variations in methods available in the literature. *o*-Nitrophenylacetic acid was prepared by a modified Reissert method (22, 24) in which ethyl oxalate was condensed with *o*-nitrotoluene in the presence of sodium ethoxide to give the sodium salt of *o*-nitrophenylpyroracemic acid. This product was oxidized with hydrogen peroxide, and the reaction mixture acidified to give *o*-nitrophenylacetic acid.

o-Aminophenylacetic acid was obtained by the reduction of *o*-nitrophenylacetic acid with hydrogen, in the presence of colloidal platinum and ethanol, in an Adams reductor (1). The general procedure follows the method described by Neber (23).

o-Hydroxyphenylacetic acid was prepared from the methyl ether of salicylaldehyde (17). This ether was converted to the bisulfite addition compound, and then treated with a saturated aqueous solution of potassium cyanide. The resulting cyanohydrin was extracted with ether, the extracts dried, decolorized with Norite and the ether recovered.

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This is the eighth of a series of papers by Ed. F. Degering and collaborators on the effect of structure and the p_H of the media on the bacteriostatic and bactericidal properties of alkanoic acids and commercial antiseptics. The others appeared in: *Ind. Eng. Chem.*, 30 (1938), 646, JOUR. A. PH. A., 27 (1938), 865, *Ind. Eng. Chem.*, 31 (1939), 742, JOUR. A. PH. A., 28 (1939), 514, *Ind. Eng Chem.*, 32 (1940), 996, *J. Chem. Educ.*, in publication, *Proceedings Ind. Academy of Science*, 49 (1940), 42.

The colorless cyanohydrin residue was refluxed for 2 hours with 20 ml. of 57% hydrogen iodide in the presence of red phosphorus, and the mixture poured into a solution of sodium bisulfite. The excess phosphorus was removed by filtration, washed with ether, and the filtrate extracted with ether to obtain the crude acid. This was purified by extraction with a 10/1 chloroform-ether mixture.

The *o*-bromophenylacetic, the *o*-chlorophenylacetic and the *o*-methylphenylacetic acids were all prepared by treating the appropriate benzyl halides with sodium cyanide to give the corresponding substituted benzyl cyanides. The cyanides were then converted to the corresponding acids. It was found that acid hydrolysis gave better yields of the *o*-bromophenylacetic and *o*-chlorophenylacetic acids, whereas alkaline hydrolysis gave the best yields of the *o*-methylphenylacetic acid.

Cinnamic acid was prepared according to Knoevenagel (18).

Phenylpropiolic acid was obtained by converting cinnamic acid to the dibromide (9) and subsequent removal of two equivalents of hydrogen bromide (21).

Hydrocinnamic acid was prepared by the reduction of sodium cinnamate with sodium amalgam, acidification, isolation and crystallization from petroleum ether (9).

The *cis-o*-methoxycinnamic and the *trans-o*-methoxycinnamic acids were obtained by the method of Reychler (25).

The *o*- and *p*-nitrocinnamic acids were prepared by the method of Tiemann and Opperman (30), except that mixed acid was used in the place of fuming nitric acid.

m-Nitrocinnamic acid was synthesized by the method of Thayer (29).

Furylacrylic acid was prepared similarly (29) but the mixture was heated at 165–170° for seven hours.

Furylpropanoic acid was obtained by the reduction of furylacrylic acid with sodium amalgam in water (9).

2-Iodo-4-nitrophenylacetic acid, a new compound, was prepared by dissolving 6 g. (0.033 mols) of *p*-nitrophenylacetic acid in 50 ml. of glacial acetic acid in a 250-ml. three-necked round bottom flask equipped with a dropping funnel, a reflux condenser and a mechanical stirrer (11, 26). To this was added 5 g. of iodine and then dropwise, during the period of refluxing, 12.5 ml. of nitric acid (sp. gr. 1.41). Refluxing was continued until there was no evolution of the oxides of nitrogen or evidence of iodine vapors.

The reaction mixture was poured into ice water to precipitate a yellow solid, which was filtered off. This product was dissolved in 95% alcohol, decolorized with Norite, and recrystallized three times to give 3 Gm. of a light yellow crystalline powder, which melted at 236°.

The determination of a neutral equivalent on this acid clearly indicated that it contained only one iodine atom per molecular weight. Since both of the initial groups in the ring were directing to the 2-position, it is assumed that the product obtained was 2-iodo-4-nitrophenylacetic acid.

2-Iodo-4-aminophenylacetic acid, a new compound, was obtained by dissolving 3 g. of 2-iodo-4-nitrophenylacetic acid in 150 ml. of 95% ethyl alcohol in an Adams reductor, and then reducing with hydrogen in the presence of colloidal platinum. After reduction, the catalyst was filtered off, the solution decolorized by treatment with Norite and the solvent removed by vacuum distillation at room temperature. The residue was recrystallized twice from hot 95% ethyl alcohol to give a fine, crystalline solid with a m. p. of 184°.

The Development of a New Test Technique.—Because of the limited solubility of these acids in pure distilled water, no satisfactory bacteriostatic tests could be obtained. A need immediately arose for a new non-toxic medium in which the acids were sufficiently soluble.

The medium that filled the needs more closely than others tried was a dilute solution of 1,4-dioxan. One part of 1,4-dioxan in 10 parts of water prevents growth, but 1 part of 1,4-dioxan to 15 parts of water allows growth of both *Staphylococcus aureus* and *Escherichia coli*.

It has been used with success on various acids that were not sufficiently soluble in pure distilled water to give tests. The bacteriostatic tests of a few of these acids are given in Table II along with the bacteriostatic tests of certain acids in both distilled water and in the 1,4-dioxan medium.

Preparation of Solutions for the Bacteriostatic Tests.—Since previous works in this laboratory have shown that the hydrogen-ion concentration is an important factor in the bacteriostatic property displayed by any solution, it was necessary to make the solutions up to a definite p_H.

In making up the solutions, the proper amount of the acid was transferred to a 25-ml. volumetric flask where it was dissolved in 15 ml. of a 1,4-dioxan-water mixture. The solutions were then adjusted to a p_H of 5 by the addition of dilute hydrochloric acid or dilute sodium hydroxide. A Universal potentiometer assembly designed by Goodhue (13) and modified by Swank (27) was used in adjusting the solutions.

Accurately prepared buffer solutions were used in checking the glass electrode both before and after each period of use. The quinhydrone glass electrode and the calomel cell were rinsed well with distilled water and dried before insertion into a new acid solution.

After adjusting the solutions, they were returned to the volumetric flask, the electrodes washed well with water adjusted to a p_H of 5, the washings being added to the solution and the flask diluted to the 25-ml. mark with distilled water adjusted to the desired p_H of 5. The p_H of this solution was rechecked, a drop or two of acid or base being added

as needed and the solution returned to the flask and stoppered.

Interpretations of the Bacteriostatic Tests.—The results of the bacteriostatic tests for the ortho-substituted phenylacetic acids and a few related compounds are given in Table I.

None of the simple ortho-substituted phenylacetic acids were more effective than the parent compound, phenylacetic acid. Four related compounds were, however, more effective than phenylacetic acid. These acids were diphenylacetic acid, *N*-methyl-*p*-amino-phenylacetic acid, 2-iodo-4-nitrophenylacetic acid and 2-iodo-4-aminophenylacetic acid.

The members of the *ortho*-series tested in this study fall in the decreasing order of hydroxy, chloro, bromo, methyl and nitro. In the *para*-series the order is bromo, iodo, chloro, ethyl, nitro and hydroxy (4). Oxindole is much more specific against *Escherichia coli* than against *Staphylococcus aureus*. In the former case it has an activity a little less than that of the *o*-hydroxy-isomer, while in the latter case it was the least active of the compounds tested in this series.

It was found that replacement of an α-hydrogen by a hydroxyl group decreases bacteriostatic properties. Benzilic acid is less effective than diphenyl-

Table I.—Bacteriostatic Tests in 1,4-Dioxan Solution

Name of Compound	p_H ±0.1	Highest Killing Dilution[b] *Staph. aureus* Weight	*Staph. aureus* Normality	*Es. coli* Weight	*Es. coli* Normality
2-Iodo-4-nitrophenylacetic acid[c]	5	1/2658	0.00123	1/4430	0.000737
2-Iodo-4-aminophenylacetic acid[c]	5	1/736	0.00490	1/920	0.00393
Phenylacetic acid	5	1/1200	0.00612	1/1200	0.00612
o-Hydroxyphenylacetic acid[d]	5	1/669	0.00984	1/892	0.00737
o-Chlorophenylacetic acid	5	1/396	0.01472	1/594	0.009875
o-Bromophenylacetic acid	5	1/312	0.01495	1/468	0.00994
o-Methylphenylacetic acid	5	1/448	0.01485	1/560	0.01188
o-Nitrophenylacetic acid	5	1/376	0.01467	1/470	0.0172
Oxindole	5	1/381	0.0196	1/889	0.00844
N-benzoyl, *o*-aminophenylacetic acid[c]	5	[a]		[a]	
Benzilic acid	5	1/446	0.009825	1/1092	0.00402
Diphenylacetic acid	5	1/1377	0.003422	1/1838	0.002565
Furylacrylic acid	5	1/315	0.02281	1/630	0.0115
Furylpropionic acid	5	1/543	0.01315	1/1086	0.00656
p-Methylphenylacetic acid[e]	5	1/540	0.01232	1/540	0.01232
N-methyl-*o*-aminophenylacetic acid[e]	5	1/1239	0.00490	1/2065	0.00294

[a] The lowest possible dilution did not kill the bacteria.
[b] A control of the 1,4-dioxan solution gave growth in every dilution comparable to test solutions.
[c] These compounds were prepared in part by Carl Gochenour.
[d] This compound was prepared with Julian Dorskey.
[e] These compounds were prepared by D. Herman.

Table II.—Bacteriostatic Tests

Name of Compound	p_H ±0.1	Highest Dilution That Inhibits *Staph. aureus* in 10% EtOH[b]	*Staph. aureus* in 1,4-Dioxane	*Es. coli* in 10% EtOH[c]	*Es. coli* in 1,4-Dioxane
cis-o-Methoxycinnamic acid	5	1/532	1/625	1/532	1/625
trans-o-Methoxycinnamic acid	5	[a]	1/3000	[a]	1/4000
trans-Cinnamic acid	5	[a]	1/1000	[a]	1/1250
Phenylpropiolic acid	5	1/386	1/308	1/386	1/385
Hydrocinnamic acid	5	1/576	1/830	1/576	1/820
o-Nitrocinnamic acid	5	[a]	[a]	[a]	[a]
m-Nitrocinnamic acid	5	[a]	[a]	[a]	[a]
p-Nitrocinnamic acid	5	[a]	[a]	[a]	[a]
p-Nitrocinnamic acid, ethyl ester	5	[a]	[a]	[a]	[a]

[a] The lowest possible dilution did not kill.
[b] A control of 10% ethanol gave growth in every dilution comparable to test solutions.
[c] A control of 1,4-dioxan gave growth in every dilution comparable to test solutions.

Table III.—Bacteriostatic Tests[b]

Name of Compound	p_H ±0.1	Highest Dilution That Inhibits *Staph. aureus* in H_2O	*Staph. aureus* in 1,4-dioxane[c]	*Es. coli* in H_2O	*Es. coli* in 1,4-dioxane[c]
Phenylacetic acid	5	1/1100	1/1200	1/1500	1/1200
p-Chlorophenylacetic acid	5	1/3000	1/2000	1/1100	1/1000
p-Bromophenylacetic acid	5	1/5000	1/3000	[a]	1/600
p-Iodophenylacetic acid	5	1/4000	1/648	[a]	1/810
p-Hydroxyphenylacetic acid	5	[a]	1/650	[a]	1/650
p-Ethoxyphenylacetic acid	5	1/1000	1/460	[a]	1/564
N-Acetyl-*p*-aminophenylacetic acid	5	[a]	1/500	[a]	1/625

[a] The lowest dilution gave positive growth because of the limited solubility in water medium.
[b] These compounds were prepared by W. A. Bittenbender (4).
[c] A control of the 1,4-dioxan solution gave growth in every dilution comparable to test solutions.

acetic acid. Either free alcoholic or free phenolic hydroxyl groups have been found by other workers (23*a*, 26*a*) to decrease the bactericidal activity. The replacement of an α-hydrogen by the benzene nucleus was found to increase the effectiveness of the bacteriostat. This is evidenced by the activity of phenylacetic and diphenylacetic acid.

The substitution of an iodine atom in the 2-position of *p*-nitrophenylacetic acid greatly enhanced the bacteriostatic properties against both organisms. The iodine-containing compound is more effective against *Escherichia coli* whereas *p*-nitrophenylacetic acid, which was tested by Bittenbender (4), is more effective against *Staphylococcus aureus*. Both 2-iodo-4-aminophenylacetic acid and *p*-aminophenylacetic acid (4) were more effective against *Escherichia coli*.

In all cases except the hydroxy derivatives of phenylacetic acid, the *para*-isomer is superior to the corresponding *ortho*-isomer. The *p*-halogenphenylacetic acids are more effective against *Staphylococcus aureus* than they are against *Escherichia coli*. The reverse is true in the two *o*-halogenphenylacetic acids studied in this series.

Table III shows the results of several compounds prepared by Bittenbender (4) that have been retested in 1,4-dioxan solution. Some of these acids because of their limited solubility in water medium gave positive growth in the lowest possible dilution against both bacteria while other acids were effective only against one organism. A few acids are also included in Tables *II* and *III* to compare the bacteriostatic properties in the two media. The test organisms used were *Escherichia coli* and *Staphylococcus aureus*.

In most cases studied the bacteriostats were found to be less effective (disregarding solubility) in the 1,4-dioxan medium than in pure water. This is in harmony with the results of many other workers who have found bacteriostats and bactericides less effective in the presence of organic matter. A brief literature survey on this and closely related effects has been made by one of the writers (12*a*).

Unsaturation is one of the structural changes that has been considered in this report. The saturated isomer is more efficient in the furylacrylic and furylpropionic acid pair. Phenylpropiolic is much less effective than either *trans*-cinnamic acid or hydrocinnamic acid. This, likewise, furnishes evidence that the unsaturated compound is less effective than the saturated compound. Cooper and associates (8) have found that unsaturated acids such as acrylic, crotonic, maleic and fumaric are very susceptible to organic matter. This susceptibility to organic matter may account in part for the lower activity of unsaturated compounds. The position of the double bond is bound to be of importance in any case because of its chance for resonance with the carboxyl group, the benzene nucleus, or both which should change somewhat the properties of the acid being studied.

The phenyl group was found to be more effective than the furyl group in comparing *trans*-cinnamic acid with furylacrylic acid and also hydrocinnamic acid with furylpropionic acid.

SUMMARY

1. Seven *ortho*-substituted derivatives of phenylacetic acid, and 17 closely related compounds were prepared and their bacteriostatic properties tested against *Staphylococcus aureus* and *Escherichia coli*.

2. A solution of 1,4-dioxan was first used as a test medium for antiseptics or bacteriostats. Bacteriostatic tests were completed for seven previously prepared acids that had failed to give tests in other media in which they were less soluble. Oxindole was much more effective against *Escherichia coli* than against *Staphylococcus aureus* while with other compounds the difference was less pronounced.

3. None of the simple *ortho*-substituted phenylacetic acids were as effective as the parent compound, phenylacetic acid. They fell in the following decreasing order: hydroxy, chloro, bromo, methyl and nitro.

4. Phenylpropiolic acid was less effective than either *trans*-cinnamic acid or hydrocinnamic acid. Likewise, furylacrylic acid was less effective than furylpropionic acid.

5. The replacement of an α-hydrogen of diphenylacetic acid by a hydroxyl group was found to decrease the bacteriostatic properties. A phenyl group was shown to be superior to a furyl group and far superior to a hydrogen atom in the α-position.

6. The substitution of an iodine atom in the 2-position of *p*-nitrophenylacetic acid greatly increased its effectiveness.

7. In all cases except the hydroxy derivatives of phenylacetic acid that have been studied the *para*-isomer is superior to the corresponding *ortho*-isomer.

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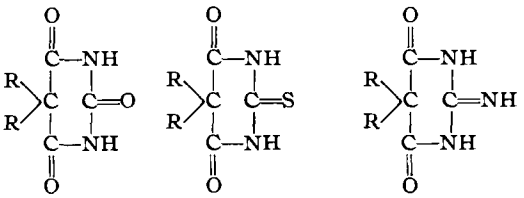
The Synthesis of Several Dialkylmalonylguanidines with a Preliminary Note on Their Pharmacology*

By Orville H. Miller† and Louis Fischer‡

In searching for new therapeutically valuable hypnotics of the barbiturate series, much attention has been devoted to replacing the two hydrogen atoms in the 5,5 position of barbituric acid with a wide variety of alkyl, aryl and other radicals. Recently a series of dialkyl thiobarbiturates (2 thio-5,5 dialkylbarbituric acids) have likewise been prepared. Of these several were found to be hypnotics and better adapted to certain uses than the barbiturates.

A few members of a third series in which the urea oxygen is replaced by an imino group (2 imino-5,5 dialkylbarbituric acids) have also been prepared, chiefly for the purpose of converting to the corresponding barbiturate. The chemical relationship of the three series may be best expressed structurally:



Barbiturate | Thiobarbiturate | Iminobarbiturate (Substituted Malonyl Guanidine)

Aside from an early experiment by Fischer and von Mering (1) there has been little interest indicated in the pharmacology of this iminobarbiturate series. Apparently basing their results on a single administration, Fischer and von Mering found dipropylmalonylguanidine to be devoid of any

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Suggestion of problem and supervision of preliminary pharmacology by Dr. J. M. Dille, Associate Professor of Pharmacology, University of Washington.