

SCIENTIFIC SECTION

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THE RELATION OF SOME PHYSICAL PROPERTIES TO BACTERICIDAL ACTION OF SOME α -PHENYLSUBSTITUTED ACIDS.

BY L. H. BALDINGER AND J. A. NIEUWLAND.

Duggan (1) has shown that in the fatty acid series with increasing length of side chain there is a decrease in the antiseptic action. Using *Bacillus subtilis* as the test organism, he found that seven per cent formic acid, nine per cent acetic acid and twelve per cent propionic acid were required to restrain the growth of the organism. Siegler and Popenoe (2), (3) while investigating the action of fatty acids on green apple aphid found that the toxicity of the aliphatic acids increased with the molecular weight, reaching a practical toxicity at the C_8 acid. Tattersfield (4), using a different organism, later reported an increasing toxicity in the fatty acid series up to undecylic acid. According to his work, some correlation was shown between certain physical properties and toxicity. Loeb (5), while investigating the action of acids on the eggs of the sea urchin, found that the permeability of the cell membrane varied with the chemical constitution of the acid, thereby accounting for the varying toxicity. He also reported that the undissociated molecule of the acid appeared to constitute the toxic agent against the organism used. Similar relationships as to the disinfectant action of undissociated molecules have been shown by Ishiwara (6), Halvorson and Cade (7) and Levine, Peterson and Buchanan (8).

Laws (9), using a series of phenylsubstituted acids, observed that the converse of Duggan's results held for this type of acid. He found that phenylacetic acid showed a bactericidal action more than twice as strong as phenol, β -phenylpropionic acid three times that of phenol and γ -phenylbutyric acid five times that of phenol. Daniels and Lyons (10) prepared a series of ω -phenylsubstituted acids and determined certain physical constants of solutions of these acids, correlating these constants with the relative bactericidal action of the acids using *B. coli* and *B. typhosus* as test organisms. They found that the type of curve presented by the solubilities, the distribution coefficients, adsorption on activated charcoal, quite closely paralleled the curve presented by the bactericidal action. They found the surface tension lowered constantly with increase in molecular weight.

Having available a fairly convenient method for the preparation of a series of α -phenylsubstituted acids, it was decided to determine the relative bactericidal power of each of the acids and to compare this with some physical properties of their solutions, namely, solubility, adsorption on animal charcoal, distribution coefficient between oil and water and surface tension. While benzoic acid cannot, strictly speaking, be considered a member of this series, it was included in the tests. In the preparation of the graphs throughout this work the following abbreviations have been used:

B	= Benzoic acid	V	= α -Phenylvaleric acid
A	= Phenylacetic acid	C	= α -Phenylcaproic acid
P	= α -Phenylpropionic acid	H	= α -Phenylheptoic acid
Bu	= α -Phenylbutyric acid		

EXPERIMENTAL.

Preparation of the Acids.—The nitriles of the acids, with the exception of benzoic and phenylacetic, were prepared by the sodium-liquid ammonia method (11). The nitriles were then hydrolyzed by a slightly modified procedure of Pickard and Yates (12), which gave much better results than the alkali hydrolysis as recommended by Bodroux and Taboury (13). The procedure was as follows:

The nitrile was refluxed for eight or nine hours with moderately strong sulphuric acid (3:2). In order to prevent violent bumping of the mixture a glass tube, drawn into a capillary, was inserted through the stopper of the reaction flask so that the capillary dipped into the lower acid layer. A fine stream of dry air was passed through the mixture during the refluxing. The mixture was diluted with water and extracted with ether. The organic acid was then removed from the ether by extraction with ten per cent potassium hydroxide solution. This alkaline solution was then acidified with dilute sulphuric acid and extracted with ether to remove the organic acid. After drying the combined ethereal extracts over calcium chloride, the ether was distilled over a water-bath, and the acid distilled under reduced pressure.

All of the freshly distilled acids were clear, viscous liquids with a manure-like odor. The α -phenylbutyric, valeric and heptoic acids, upon cooling and standing, solidified to masses of crystals. The first two members of the series to be examined, benzoic acid, m. p. 121.5–122° C. (corr.) and phenylacetic acid, m. p. 77.2–77.7° C. (corr.), were Eastman Kodak products, recrystallized from hot water. When cooled in liquid ammonia, the liquid members of the series solidified but did not crystallize. The α -phenyl derivatives of propionic, butyric and valeric acids have been prepared by previous workers by the hydrolysis of the corresponding nitriles. The boiling points and analyses of the acids prepared in our work are given in Table I.

TABLE I.

α -Phenyl- Derivative of.	B. P. (corr.).	Formula.	Carbon, %.		Hydrogen, %.	
			Calc.	Found.	Calc.	Found.
<i>n</i> -Propionic acid	132–34 ₈	C ₉ H ₁₀ O ₂	71.9	72.4	6.67	6.59
<i>n</i> -Butyric acid	141–44 ₈	C ₁₀ H ₁₂ O ₂	73.2	73.1	7.32	7.34
<i>n</i> -Valeric acid	153–57 ₇	C ₁₁ H ₁₄ O ₂	74.2	73.8	7.86	7.66
<i>n</i> -Caproic acid	161–65 ₇	C ₁₂ H ₁₆ O ₂	74.8	74.7	8.32	8.25
<i>n</i> -Heptoic acid	179–82 _{11.5}	C ₁₃ H ₁₈ O ₂	75.7	76.0	8.73	8.55

Solubilities of the Acids.—The solubilities of the acids were determined at 30° C. and 40° C., using a rotating apparatus, similar to that described by Noyes (14), carrying four sixty-cubic centimeter wide-mouth bottles. The entire apparatus was immersed in a thermostat, capable of temperature control to within five-hundredths degree. Fifty cubic centimeters of boiled distilled water were placed in each bottle together with an excess of acid, the solubility of which was to be determined. The bottles were rotated from five to six hours at the desired temperature and then placed in an upright position in the bath to permit clarification. A pipette, to which was attached a cotton-filled bulb for filtration, was used to remove portions of the solution from each bottle. These portions were run into dry, tared, glass-stoppered weighing bottles and weighed. The contents of each bottle were titrated with *N*/100 sodium hydroxide, using phenolphthalein as an indicator. Before titration dry oxygen was bubbled through the solution to displace carbon dioxide. See Table II and Fig. 1.

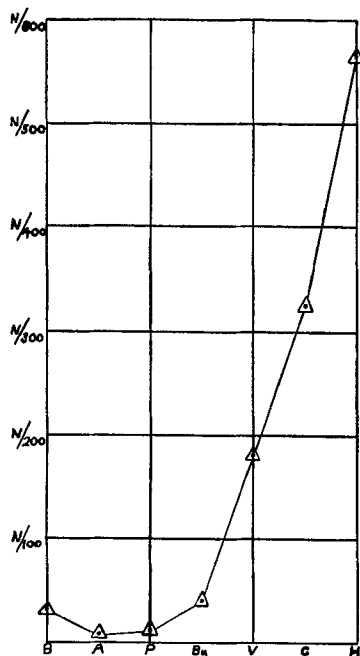


Fig. 1.—Solubilities of acids expressed as molalities at 30° C.

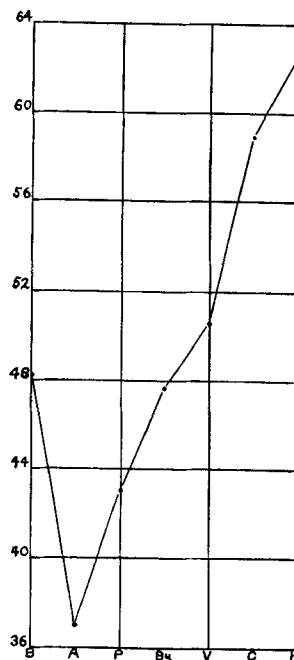


Fig. 2.—Distribution coefficients, C_2/C_1 , of acids between animal charcoal and water, using $N/600$ solutions.

TABLE II.—SOLUBILITIES OF ACIDS EXPRESSED IN GRAMS OF ACID DISSOLVED IN ONE HUNDRED GRAMS OF WATER.

Acid.	30° C.	40° C.
Benzoic	0.408	0.552
Phenylacetic	2.068	3.334
α -Phenylpropionic	1.2154	1.3325
α -Phenylbutyric	0.4230	0.4445
α -Phenylvaleric	0.0978	0.1854
α -Phenylcaproic	0.0591	0.0633
α -Phenylheptoic	0.0365	0.0416

Adsorption of Acids on Bone Black.—The adsorption of the acids on animal charcoal was determined using $N/100$ solutions of the acids as starting concentration wherever possible and approximately saturated solutions in other cases. The high dilutions of acids used rendered duplication of values very difficult, but by determining the coefficients of all the acids at one time under approximately constant conditions, values were obtained from which could be plotted a curve showing the variation in adsorption with increase in molecular weight. It will be observed that the ratio of the concentrations, *i. e.*, C_2/C_1 , is not a constant but increases with decrease in concentration. From the data obtained, it appears that the adsorption process follows an equation similar to that of Freundlich (15), $\ln C_2 = \ln k + \frac{1}{n} \ln C_1$. Widely varying values for the constants k and n were obtained when the data observed was substituted in the adsorption formula.

To twenty-five cubic centimeters of the diluted acid solutions was added one-tenth Gm. of animal charcoal and the solutions agitated thoroughly at five-minute intervals for twenty minutes, filtered, and twenty cubic centimeters of the filtrate titrated with $N/100$ sodium hydroxide from a microburette using phenolphthalein as an indicator. See Table III and Fig. 2.

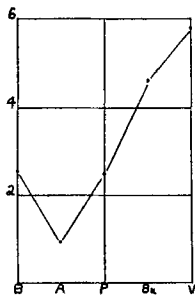


Fig. 3.—Distribution coefficients, C_2/C_1 , of acids between oil and water, using $N/600$ solutions.

Partition Coefficient between Oil and Water.—Twenty-five cubic centimeters of fresh cottonseed oil were added to twenty-five cubic centimeters of a solution of the acid. The same dilutions of acids were used as in the adsorption experiments. The oil and solution mixture was rotated for five hours at room temperature. Twenty cubic centimeters of the aqueous layer were pipetted off and titrated with $N/100$ sodium hydroxide, phenolphthalein as indicator. The last two members of the series were not included in this test. See Table IV and Fig. 3.

Surface Tension of the Acid Solutions.—The surface tensions of $N/600$ solutions, so as to include the entire series, were determined using a du Nouy Tensiometer at 24.5°C . The water used in making the dilutions gave a reading of 73.7 dynes/cm. at this temperature. It was observed that the surface tension

TABLE III.—ADSORPTION ON ANIMAL CHARCOAL EXPRESSED BY THE VALUES OF C_2/C_1 .

C_2 = Millimols of solute per Gm. of animal charcoal.

C_1 = Millimols of solute per milliliter of solution.

Normality.	B.	A.	P.	Bu.	V.	C.	H.
1/100	8.81	7.8	9.44	10.8
1/200	20.05	14	19.65	18.9
1/300	30.85	19.7	22.7	25.2	26.3
1/400	38.4	31	27.5	30.5	35.5	24.5	..
1/500	46.7	36.8	41	34.1	41.6	39.6	..
1/600	48.2	37.1	43.1	47.7	49.6	58.8	62.5

TABLE IV.—PARTITION COEFFICIENT C_2/C_1 BETWEEN OIL AND WATER.

C_2 = Gm. acid per twenty-five cubic centimeters of oil.

C_1 = Gm. acid per twenty-five cubic centimeters of solution.

Normality.	B.	A.	P.	Bu.	V.
1/100	5.55	1.93	4.31	11.14	..
1/200	4.96	1.58	3.9	8.48	..
1/300	3.85	1.55	3.25	5.98	7.12
1/400	3.09	1.24	2.98	5.08	6.61
1/500	3.03	1.15	2.64	4.78	5.96
1/600	2.64	0.96	2.54	4.63	5.77

of the water was lowered by all of the acids but no definite relationship between increasing side chain length and surface tension lowering could be inferred from the data obtained. It is a well-known and recognized fact that surface tension lowering does not alone serve as a criterion for a good antiseptic, but that other factors may enter into the bactericidal action.

Bactericidal Values of the Acids.—Proper dilutions of the acids were made from relatively concentrated solutions. Phenylheptoic acid could not be included

in the tests because of limiting solubility. The test organism used was a 22-26-hour culture of *Bacillus coli* (*Harris strain*), incubated and grown at 37.5° C. on nutrient broth. The stock culture was carried on agar slants of the same composition as the broth medium plus one and one-half per cent agar. When the test organism had not been transferred daily, four or five daily transfers were made before using it for testing purposes. Transfers and inoculations were made with a four-millimeter loop. Five-tenths cubic centimeter of a suspension of *B. coli* was added to four and one-half cubic centimeters of nutrient broth. One-tenth cubic centimeter of this suspension was then added to five cubic centimeters of the acid solutions. The organism was allowed to stand in the acid solution for an hour at 37.5° C. and was then inoculated into nutrient broth and incubated for forty-eight hours at 37.5° C. A test with phenol was added as a comparator for bactericidal action. See Table V and Fig. 4.

DISCUSSION.

It will be observed that an unlimited increase in germicidal activity with increasing molecular weight is prevented by the decreasing solubility which also accompanies increasing molecular weight. With this series and the test organism used, the practical bactericidal efficiency appears to reach a maximum at the C₆ acid. All of the series, with the exception of benzoic acid, are expensive or difficult to prepare, and have a disgusting odor, two factors which would limit their use as practical antiseptics.

As pointed out by Tattersfield (4) and Daniels and Lyons (10), no one of the physical properties entirely accounts for the toxicity shown by the fatty acids.

TABLE V.—DILUTIONS OF ACIDS WHICH WILL INHIBIT THE GROWTH OF *B. coli* IN ONE HOUR.

Acid.	Dilution.	Acid.	Dilution.
Phenol	1-180	α -Phenylbutyric	1-1600
Benzoic	1-800	α -Phenylvaleric	1-3000
Phenylacetic	1-600	α -Phenylcaproic	1-5700
α -Phenylpropionic	1-1000		

SUMMARY.

1. By the hydrolysis of the corresponding nitriles, a series of α -phenyl-substituted acids has been prepared, and their solubilities determined in water at 30° C. and 40° C.
2. The curves showing the solubilities of the acids, the adsorption by animal charcoal of the acids from their aqueous solutions and the distribution coefficients between oil and water parallel that of the bactericidal action of the series, using *B. coli* as the test organism.

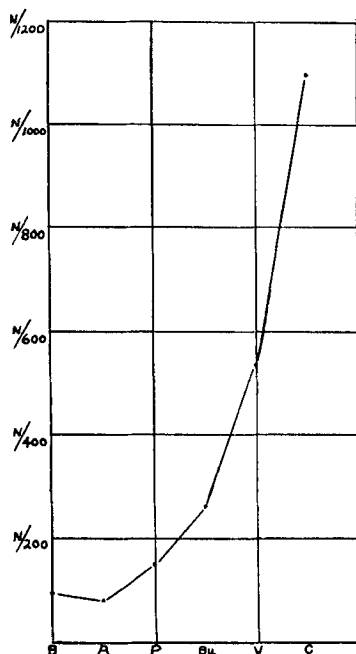


Fig. 4.—Bactericidal values of acids expressed as normalities.

3. The bactericidal action of these acids appears to be a function of solubility, side chain length, adsorption by animal charcoal distribution coefficient between oil and water and surface tension lowering.

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 THE CONSTITUENTS OF WU CHÜ YÜ (*EVODIA RUTÆCARPA*)*

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Wu Chü Yü is the fruit of a shrub and has been used for a long time in Chinese medicine as a drug for the treatment of headache, abdominal pain, dysentery, cholera, worm infestations and postpartum disturbances (1). Botanically, the plant has been identified as *Evodia rutæcarpa*, family rutaceæ (2). The crude drug is easily available from Chinese drug stores. Our supply came from Tientsin, China. Each fruit consists of small black carpels, five in number, with short stalks, weighs on the average 10.3 mg., has an aromatic odor and is hot and bitter to the taste, similar to black pepper.

Chemical studies on Wu Chü Yü have been undertaken by several Japanese investigators. Keimatsu (3) reported the isolation of an indifferent crystalline substance, having the empirical formula $C_{18}H_{22}O_6$, which he named evodin. Asahina and Ishio (4) presented evidence that the formula of evodin was $C_{17}H_{20}O_9$. In addition, Asahina and Kashiwaki (5) succeeded in isolating two alkaloids, evodiamine and rutæcarpine, having the formulas $C_{19}H_{17}ON_3$ and $C_{18}H_{13}ON_3$, respectively. During the following fourteen years, Asahina and his associates published data on the chemical structures of both evodiamine and rutæcarpine, and finally their syntheses (6), (7), (8), (9), (10), (11), (12), (13).

Our chief interest in Wu Chü Yü was to obtain in an amount sufficient for pharmacological study the principles known to be present in the fruit. In the process of separation, we obtained evodiamine and rutæcarpine, and by elementary analyses and molecular weight determinations we confirmed Asahina's formulas. With regard to the non-nitrogenous substance, evodin, our results differ from those

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