research on belladonna root (2). The results on yellow cinchona are in accord with the results of Büchi and Feinstein (3) who found that slight variations of the evacolation process gave results on cinchona which were inferior in some cases and equal in other cases to the results obtained by ordinary percolation.

Loss of Menstruum.—It is to be expected that in using vacuum there will be a greater loss of menstruum than occurs at atmospheric pressure and this expectation is borne out by the experimental data. Because of this loss of menstruum and the expense of operating vacuum pumps it is clearly inadvisable to use vacuum in drug extraction, particularly since no real advantage can be demonstrated relative to efficiency of extraction.

SUMMARY.

Experiments show that the application of vacuum in several ways does not increase the efficiency of extraction of yellow cinchona by maceration or percolation. Various authors have presumed that vacuum maceration increases the proportion of menstruum imbibed by the drug but quantitative studies show that this process does not increase imbibition in the case of yellow cinchona. These results are in accord with earlier results by the present authors on belladonna root. Taken together, these results indicate rather conclusively that the application of vacuum is of no general advantage in drug extraction. Disadvantages of vacuum methods are the greater loss of menstruum and the cost of operating the vacuum pumps.

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PREPARATION OF ALPHA-PHENYLALKANOIC ACIDS AND A STUDY OF THEIR BACTERICIDAL AND PHYSICAL PROPERTIES.*

BY R. H. GOSHORN AND ED. F. DEGERING.[1]

Many organic acids are known to possess some antiseptic and germicidal power, and attempts have been made at various times to correlate both the structure and the physical properties of some of these acids with their bactericidal action. Kuroda and others (1) have found that certain acids were effective antiseptics in dilute solutions if the p_H was below 4.5, but were ineffective in the neutral range. Daniel and Lyons (2) found that, in an homologous series of acids, there was some correlation between bactericidal power and oil-water distribution, solubility and adsorption on charcoal.

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It was the purpose of this work to study the effect of hydrogen-ion concentration on the antiseptic and bactericidal action of a series of phenylalkanoic acids, and to investigate the relation of structure and certain physical properties to the bactericidal action.

EXPERIMENTAL PART.

A series of alpha- and omega-phenylalkanoic acids, from benzoic acid to the alpha- and delta-phenylvaleric acids, was prepared according to methods found in the literature.

Preliminary bacteriostatic tests (by the agar plate method) were run on an incomplete series of the acids. The results obtained were of doubtful comparative value since some of the agar solutions failed to coagulate. Other tests on the same acids whose solutions were brought to a p_H of approximately seven (by the addition of sodium hydroxide solution) indicated that the antiseptic action was greatly reduced by the addition of the alkali.

In order to obtain comparative values for the different acids, bactericidal tests were run using phenol as a standard, and Bacterium coli and Staphylococcus aureus as test organisms. The results are shown in Table I. Since it was thought that the acidity of the solutions might play an important part in determining the amount of dilution necessary to produce a medium in which the bacteria could survive, the bactericidal tests were repeated and the p_H of the solutions at their maximum effective dilutions were determined.[1] The maximum effective dilutions (in Gm./ml.) with the corresponding p_H values are shown in Table II.

TABLE I.—BACTERICIDAL TESTS ON ALPHA- AND OMEGA-PHENYL SUBSTITUTED ACIDS.*

	Alpha Series.		Omega Series.	
Acid Tested Against.**	Colon.	Aureus.	Colon.	Aureus.
Phenol	1/150	1/100	1/150	1/150
Benzoic	1/900	1/700	1/900	1/1000
Phenylacetic	1/800	1/700	1/500	1/700
Phenylpropionic	1/950	1/650	1/1000	1/1200
Phenylbutyric	1/1600	1/1600	1/1300	1/1400
Phenylvaleric	1/2000	1/2300	1/2500	1/2500

* Bactericidal tests were made by Professor P. A. Tetrault.

** Maximum effective dilution is expressed in Gm. per ml.

TABLE II.—p_H VALUES OF THE ACIDS AT THEIR MAXIMUM EFFECTIVE BACTERICIDAL DILUTION.*

Acid Tested Against.	Colon at p_H.		Aureus at p_H.	
Benzoic	1/800	3.00	1/1100	3.10
Phenylacetic	1/500	3.10	1/500	3.10
Alpha-phenylpropionic	1/1100	3.32	1/1200	3.33
Beta-phenylpropionic	1/700	3.33	1/900	3.38
Alpha-phenylbutyric	1/1200	3.28	1/1200	3.28
Beta-phenylbutyric	1/1000	3.40	1/750	3.35
Gamma-phenylbutyric	1/1100	3.48	1/1100	3.48
Alpha-phenylvaleric	1/2000	3.38	1/1400	3.30
Delta-phenylvaleric	1/2200	3.75	1/1400	3.62

* Bactericidal tests were made by Professor P. A. Tetrault. The tests on beta-phenylbutyric acid were conducted with a different culture of bacteria than that used in testing the other acids, hence the results are only approximate.

Benzoic acid solutions were prepared at p_H values of 3.5, 4.0, 5.0, 6.0, 7.0 and 8.0 in dilutions of 1/400, 1/250, 1/20, 1/10, 1/10 and 1/5 (Gm./ml.), respectively. Distilled water which

[1] A glass electrode, used in conjunction with a Leeds and Northrup Dual Galvanometer, was employed in measuring p_H. The electrode was checked against buffers of known hydrogen-ion concentration before and after each period of use. The entire assembly was later checked with an electron tube glass electrode outfit.

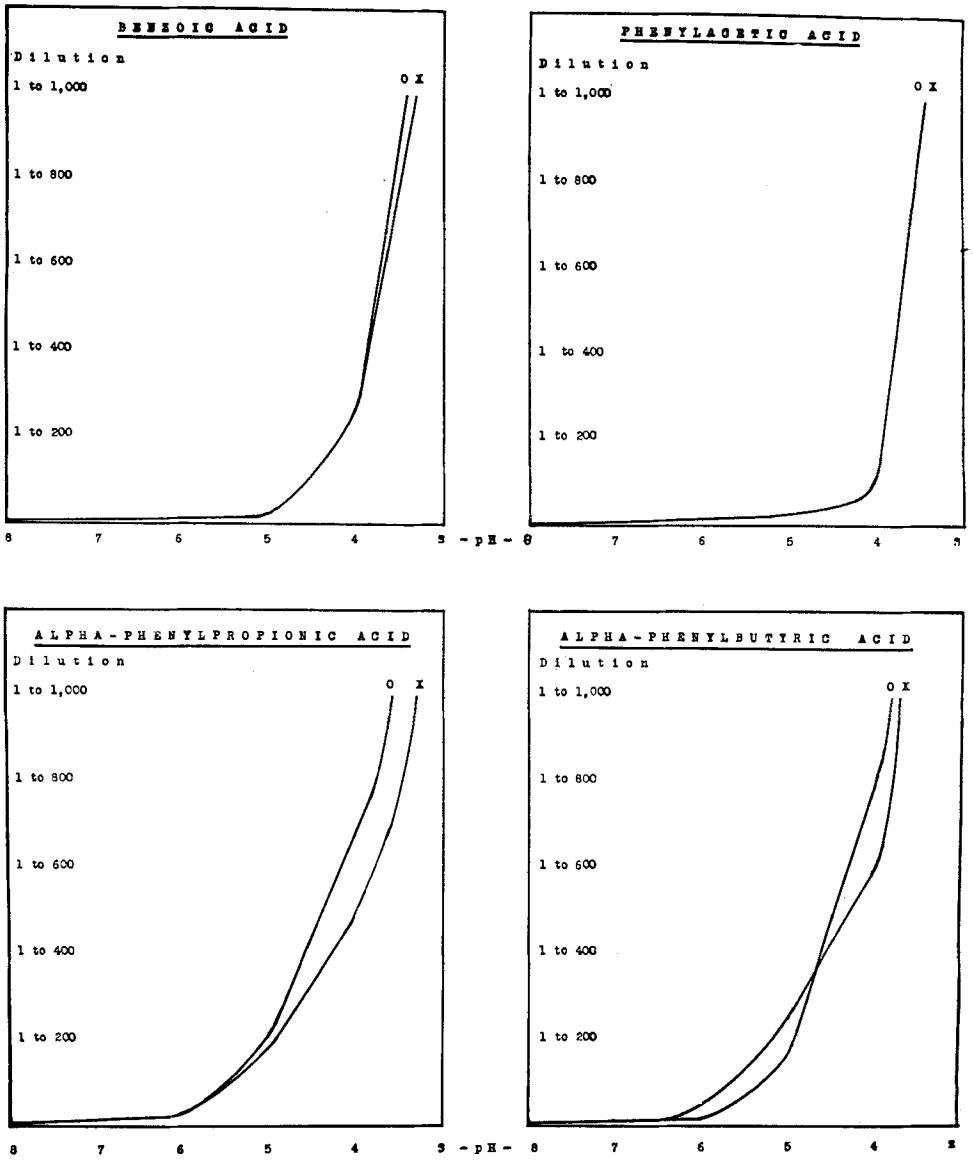

Fig. 1.—Bactericidal tests, showing maximum effective dilution *vs.* p_H values.*

* Concentrations are expressed in Gm./ml. The curves marked x show the results obtained with Bacterium coli, those marked o with Staphylococcus aureus. The tests were made by Professor P. A. Tetrault, Biology Department, Purdue University.

had been boiled to remove CO_2 was used in making up the samples. The required amount of acid was weighed into a 150-ml. beaker and 1 ml. less than the calculated equivalent amount of 4.78 normal sodium hydroxide solution was added. The mixture was diluted to a total volume of 60–75 ml. with water. The solution was stirred with a mechanical stirrer for a few minutes and the electrodes were inserted. Sodium hydroxide solution (approximately one normal) was added dropwise to maintain the acid solution near the desired p_H. When all the acid had dissolved, the final adjustment of the p_H was made by using a very dilute sodium hydroxide solution, 1/100

normal or less.[1] The solution was transferred to a 100-ml. volumetric flask and the beaker, electrodes, and stirrer were rinsed well with water of the same p_H as that of the solution and the rinsings were added to the volumetric flask. The flask was then filled to the mark with water of the proper p_H[2] and the contents thoroughly mixed. The solution was poured into a clean dry beaker and the p_H was checked, a drop or two of alkali solution being added if necessary.

By a similar method solutions of phenylacetic acid, α-phenylpropionic acid, and α-phenylbutyric acid were prepared. The results of bactericidal tests on these solutions are given in Table III and Fig. 1.

TABLE III.—CORRELATION OF BACTERICIDAL PROPERTIES WITH p_H. THE MAXIMUM EFFECTIVE DILUTION (GM./ML.) FOR A GIVEN p_H VALUE.*

Acid Tested at a p_H of.	3.5.	4.0.	5.0.	6.0.	7.0.	8.0.	Strain.
Benzoic	1/800	1/250	1/20	1/10	1/10	1/10	B.C.
	1/900	1/250	1/20	1/10	1/10	1/5	S.A.
Phenylacetic		1/100	1/60	1/24	1/16	1/12	B.C.
		1/100	1/60	1/8	1/4	1/3	S.A.
Alpha-phenylpropionic		1/500	1/175	1/25	1/10	1/7	B.C.
		1/700	1/200	1/25	1/10	1/5	S.A.
Alpha-phenylbutyric		1/600	1/250	1/50	1/10	1/5	B.C.
		1/800	1/150	1/12.5	1/10	1/5	S.A.

* Bactericidal tests were made by Professor P. A. Tetrault, Biology Department, Purdue University. Hydrochloric acid solution was effective against both organisms at a p_H of 2. B.C. is *Bacterium coli*, S.A. is *Staphylococcus aureus*.

Antiseptic tests on the benzoic acid solutions gave considerably higher results than were obtained in the bactericidal tests, and no growth was obtained at a p_H of 4.0 even when the benzoic acid was not present.

The solubilities of the acids at 25 ± 0.2° C. were measured in the usual manner by shaking three samples (one of them supersaturated with acid and all containing excess acid) in a constant temperature bath until check results were obtained. The amount of acid in a weighed sample of the saturated solution was determined by titrating with 0.005 normal sodium hydroxide solution[3] using phenolphthalein indicator. The results expressed in Gm. of acid dissolved in 100 Gm. of water are shown in Table IV. The normalities of the saturated solutions are also shown.

TABLE IV.—CORRELATION OF BACTERICIDAL PROPERTIES WITH OIL-WATER DISTRIBUTION AND SOLUBILITY.

Acid Tested.	Solubility.*	Normality.**	Oil/Water.
Benzoic	0.346	0.028	3.977
Phenylacetic	1.810	0.133	1.853
Alpha-phenylpropionic	1.171	0.078	5.640
Beta-phenylpropionic	0.822	0.055	6.660
Alpha-phenylbutyric	0.317	0.019	14.417
Beta-phenylbutyric	0.568	0.035	11.270
Gamma-phenylbutyric	0.189	0.012	14.800
Alpha-phenylvaleric	0.076	0.0043	26.410
Delta-phenylvaleric	0.061	0.0035	28.830

* Solubility is expressed in Gm. dissolved in 100 Gm. of water.

** Normality of a saturated solution.

[1] To obtain accurate adjustment to p_H values of 7 and 8, it is particularly desirable to use very dilute sodium hydroxide solution.

[2] The p_H of water was adjusted by adding dilute sodium hydroxide or hydrochloric acid solution.

[3] The sodium hydroxide solution was standardized against a solution of the organic acid of known normality.

The oil-water distributions were determined using U. S. P. cottonseed oil and 1/200 normal solutions of the acids. Equal volumes of oil and acid solution were shaken at room temperature for twenty hours and let stand to separate. Samples of the water layer were then pipetted out and titrated with 0.005 normal sodium hydroxide solution using phenolphthalein indicator. From the results of the titration and the known normality of the original acid solution, the distribution was calculated. The values are given in Table IV.

Adsorption on activated charcoal was measured using freshly opened Norit A.[1]

Samples of the phenyl substituted acids were made up at strengths corresponding to the minimum bactericidally effective concentrations. To 25 ml. of each solution, in an oil-sample bottle, 0.1 Gm. of Norit was added. The mixture was shaken for 20 minutes in a mechanical shaker, removed and filtered. A 10-ml. sample of the filtrate was titrated with a solution of sodium hydroxide, somewhat less than 0.01 normal, standardized against the organic acid solution of known concentration. Phenolphthalein indicator was used and nitrogen was bubbled through the solutions during the titrations. The amount of acid adsorbed was calculated from the known concentration of the original solution, and the results obtained in titrating the 10-ml. sample after treatment with activated carbon.

In Table V are listed the grams and mols of each acid adsorbed.

TABLE V.—CORRELATION OF BACTERICIDAL PROPERTIES WITH ADSORPTION ON CHARCOAL.

Acid Tested.	Gm./Ml.*	Mols/1.	Gm. Adsorbed.	Mols × 10^3.
Benzoic	1/800	0.0102	0.0295	0.242 B.C.**
	1/1100	0.00745	0.0223	0.182 S.A.
Phenylacetic	1/500	0.0147	0.0456	0.335 B.C. & S.A.
Beta-phenylpropionic	1/700	0.00953	0.0336	0.224 B.C.
	1/900	0.00741	0.0270	0.180 S.A.
Alpha-phenylpropionic	1/1100	0.00606	0.0214	0.143 B.C.
	1/1200	0.00556	0.0203	0.135 S.A.
Gamma-phenylbutyric	1/1100	0.00554	0.0224	0.136 B.C. & S.A.
Beta-phenylbutyric	1/1000	0.00610	0.0244	0.149 B.C.
	1/750	0.00813	0.0318	0.194 S.A.
Alpha-phenylbutyric	1/1200	0.00508	0.0203	0.124 B.C. & S.A.
Delta-phenylvaleric	1/2200	0.00255	0.0110	0.062 B.C.
	1/1400	0.00401	0.0175	0.098 S.A.
Alpha-phenylvaleric	1/2000	0.00281	0.0121	0.068 B.C.
	1/1400	0.00401	0.0171	0.096 S.A.

* These values represent the minimum bactericidally effective concentrations.

** B.C. is *Bacterium coli*, S.A. is *Staphylococcus aureus*.

CONCLUSIONS.

It may be noted from observation of Fig. 1 that there is a rather definite p_H at which the organic acid begins to function effectively as a bactericide. These p_H values are 4.3, 4.4, 5.1 and 5.3 for benzoic acid, phenylacetic acid, α-phenylpropionic acid and α-phenylbutyric acid, respectively. The corresponding concentrations (in Gm./ml.) are 1/25, 1/80, 1/170 and 1/100, respectively. By comparing the above p_H values with those at the maximum effective dilutions, Table II, it may be concluded that there is a rough correlation between the point at which the p_H *vs.* acid concentration curve breaks, and the p_H of the acid solution at its maximum effective dilution in pure water. On the other hand it is evident from the above concentrations and the maximum effective dilutions in pure water, Table II, that there is no correlation between these values.

[1] The material gave more consistent results than old Norit A or samples of either old or freshly opened Norit A that had been heated to 900° C. for one hour.

Tables I and IV indicate that for both the alpha and omega series of acids there is a correlation between the relative effectiveness as bactericides and (*a*) the relative solubilities (in mols per liter), and (*b*) the relative distribution coefficients. The alpha-phenyl substituted acids are more soluble and have lower distribution coefficients than the corresponding omega-phenyl substituted acids (see Table IV). This should indicate greater bactericidal effectiveness for the omega acids. However, if phenol coefficients[1] are calculated from the values of Table I, it becomes apparent that the reverse is true in several instances.

As indicated in Table V, the amount of acid adsorbed on activated charcoal, at the maximum bactericidally effective dilution, decreases as the length of the aliphatic chain increases.

The effect of diluting the acids to their maximum effective dilution must, therefore, more than counterbalance the tendency of the higher molecular weight acids to be more completely adsorbed. It may also be noted (Table V) that the difference in the number of mols of each acid adsorbed is nearly proportional to the corresponding difference in concentration (in mols per liter) at the maximum effective dilution. This may indicate that at least the primary step in bactericidal action is one of adsorption.

SUMMARY.

1. Alpha- and omega-phenylalkanoic acids have been prepared and studied.

2. The bactericidal properties of these acids are rather closely correlated with the p_H of the media. The p_H value below which a small decrease in p_H will permit a large increase in the maximum effective dilution lies between 5.3 and 4.3 for this series.

3. A correlation exists between the oil-water distribution coefficients and the bactericidal properties of these acids.

4. Adsorption studies indicated that bactericidal action may be closely related to adsorption.

5. The omega-phenylalkanoic acids are less soluble and have slightly higher oil-water distribution coefficients than do the corresponding alpha-phenylalkanoic acids.

6. The difference in bactericidal power (as compared to phenol) between the omega-phenyl substituted acids and the corresponding alpha-phenyl substituted acids is neither very marked nor very consistent.

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[1] To find the phenol coefficient, the reciprocal of the maximum effective dilution of the acid is divided by the reciprocal of the maximum effective dilution of phenol under the same conditions and against the same organism.