

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXI

APRIL, 1942

NUMBER 4
CONSECUTIVE NO. 7

The Fungistatic Properties of Pyridine Carboxylic and Aminobenzoic Acids, a Resonance Effect*

By Charles Hoffman, T. R. Schweitzer and Gaston Dalby

The relationship of fungistatic properties to molecular structure is of considerable interest. The effect of many chemical substances on bacteria and mold varies with the *pH* of the media. Bacteria in general prefer the neutral *pH* ranges, but mold will grow readily from *pH* 2 to *pH* 8 thus affording a wide range of *pH* values for a study of fungistatic properties. The relationship of structure to mold-inhibiting power often is not apparent unless the effect of *pH* is considered.

Hoffman, *et al.* (1), studied the fungistatic properties of benzoic acid and related compounds. The introduction of a polar group into the benzene ring nucleus markedly affects the fungistatic properties of the molecule. If, for example, a hydroxyl group is introduced into the *ortho* position to form salicylic acid, the power of the molecule as a fungistat is decreased. If the hydroxyl is introduced into either the *meta* or *para* positions, the fungistatic properties are eliminated entirely. An analogous situation was shown to exist in the dihydroxybenzene compounds. Catechol is

more powerfully fungistatic than resorcinol, and resorcinol more powerful than hydroquinone. Hoffman, *et al.* (1), therefore suggested the hypothesis that if the polar groups are concentrated in one area of the molecule, a more powerful fungistat results than if the polar groups are spaced around the non-polar nucleus.

The introduction of an amino group into the benzene ring nucleus of benzoic acid forms three isomeric aminobenzoic acids, one of which, *p*-aminobenzoic, has been recognized as a vitamin of the B-complex. The three isomeric pyridine carboxylic acids (including the important vitamin, nicotinic acid) have a skeleton structure similar to benzoic acid, with a nitrogen replacing a carbon in the ring. Since such a nitrogen is capable of forming a pyridonium ion, it acts as a polar group. These six compounds form, therefore, an interesting group of substances for the study of the relationship of structure to fungistatic properties.

Hoffman, *et al.* (2), reported a method for the determination of fungistatic properties. This method was used exactly as originally reported. The concentrations in the table and graph represent the minimum molar

* From the Ward Baking Company, New York, N. Y.

Table 1.—Molar Concentrations of Benzoic and Substituted Benzoic Acids Necessary to Inhibit Mold for 48 Hours at 37.5° C. at Various pH Values

	pH 2	pH 4	pH 5	pH 6	pH 7	pH 8
Benzoic acid	0.0016	..	0.0074	0.0532	(Ineffective above pH 6)	
<i>Ortho</i> (α) substitution						
Anthranilic acid	0.0058	..	0.0146	0.051	(Ineffective above pH 6)	
Picolinic acid	0.063	(Ineffective above pH 3.8)				
<i>Meta</i> (β) substitution						
<i>m</i> -Aminobenzoic acid	0.161	(Ineffective above pH 3)				
Nicotinic acid	(Ineffective over pH range 2 to 8)					
<i>Para</i> (γ) substitution						
<i>p</i> -Aminobenzoic acid	0.0365	..	0.051	0.132	(Ineffective above pH 6)	
Isonicotinic acid	(Ineffective over pH range 2 to 8)					

concentrations at each pH value necessary to inhibit all visible mold growth for 48 hrs. at 37.5° C. The spores used for seeding were from a mixed culture of common molds occurring on foodstuffs, and consisted of representatives of the usual types such as *Aspergillus niger*, *Aspergillus glaucus*, *Rhizopus nigricans*, *Penicillium frequentans*, etc. A temperature of 37.5° C. has been found to serve excellently for these tests. On the plates without inhibitor (media plus buffer) there was always a vigorous growth even in 24 hrs., and in 48 hrs. sporulation was heavy. The media consisted of nutrient agar containing 1% sucrose, and the buffers were mixtures of citric acid and disodium phosphate.

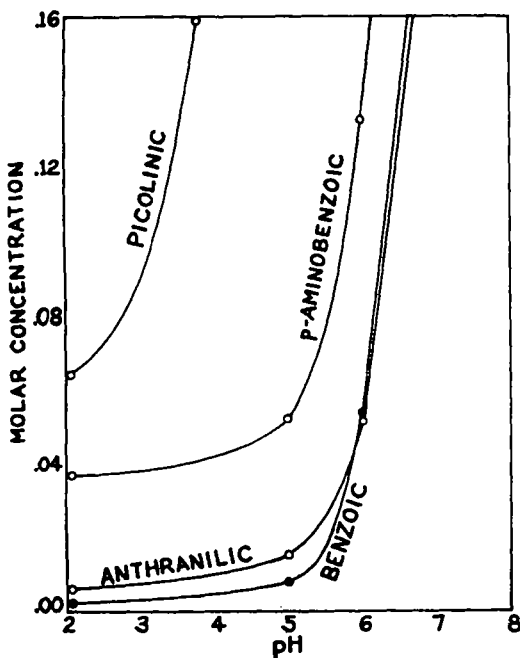


Fig. 1.—Molar Concentrations of the Indicated Acids at Various pH Values Necessary to Inhibit Mold for 48 Hours at 37.5° C.

The alpha pyridine carboxylic acid, picolinic, shows fungistatic properties but to a much less degree than benzoic acid. The beta and gamma compounds, nicotinic and isonicotinic,¹ are without mold-inhibiting properties. The action of these compounds is in agreement, therefore, with the previously suggested hypothesis.

The *o*-aminobenzoic acid, anthranilic, is slightly less powerful than benzoic acid, but the decrease in activity is not as much as might be expected from the analogy to salicylic or picolinic acid. *m*-Aminobenzoic acid is a very weak fungistat. At pH 2, it inhibits at 0.161 *M*, but even this slight inhibiting power is lost when the pH approaches 4. *p*-Aminobenzoic acid, however, presents an interesting contradiction to the expected non-inhibiting nature of such a molecule, since in this compound the two polar groups are present in as widely separated positions as possible.

The fact that *o*-aminobenzoic and *p*-aminobenzoic acids show greater fungistatic properties than would be expected because of the usual effect of the introduction of a polar group into benzoic acid, and because *m*-aminobenzoic acid shows the expected non-inhibiting action, suggests that a condition of resonance masks the polar nature of the amino group in the *ortho* and *para* positions. Buswell, *et al.* (3), state that "one of the important structures in the resonance theory is the quinoid form in which the nitrogen forms a double bond with the carbon of the ring and thereby becomes positive. A nitrogen atom in this condition may readily furnish an acidic hydrogen but

¹ Sample of isonicotinic acid by courtesy of the Upjohn Company.

cannot act as an electron donor since it has no electrons to donate."

The demonstration that the biological activity of a molecule is related to its structural ability to exist as a resonance hybrid is of interest from many points of view. Sulfanilamide, for example, is *p*-aminobenzenesulphonamide. The isomeric *meta* and *ortho* compounds are therapeutically inactive.

REFERENCES

- (1) Hoffman, Charles, Schweitzer, T. R., and Dalby, Gaston, *Ind. Eng. Chem.*, 33 (1941), 749-751.
- (2) Hoffman, Charles, Schweitzer, T. R., and Dalby, Gaston, *Food Research*, 4 (1939), 539-545.
- (3) Buswell, A. M., Downing, J. R., and Rodebush, W. H., *J. Am. Chem. Soc.*, 62 (1940), 2759-2765.

A New Method of Testing Enteric Coatings

By K. Lark-Horovitz* and Herta R. Leng†

It is sometimes necessary to prevent the reaction of medicaments with the gastric juice in the stomach because the substance administered may produce either irritation of the mucous membrane or the preparation may be destroyed by the chemical reaction. To avoid these effects, protective coatings, so-called enteric coatings, are used which are supposed to protect the preparation in the stomach and let it dissolve after a definite length of time in the small intestines. According to Jordan (1) 3.3% of the medicinal preparations on the market are administered enterically, therefore methods to test the efficacy of enteric coatings are of great interest.

To test the coatings in the test tube with solutions artificially reproducing the fluids in the stomach is not very satisfactory or convincing. To investigate the coatings *in vivo*, it is necessary either to make the preparations opaque, so as to produce shadowgraphs with X-rays (2, 3) or to make them self-radiating and to detect their location and behavior by following the radiation. This latter procedure has been proposed by Lark-Horovitz (4). The method is ideally suited for the problem since only a substance which is used in our everyday diet, sodium chloride, is introduced and

since the amounts of radiating material are so small as to cause no harm; the lifetime of the radioactive material is so short that the same person can be used again for a test after three or four days.

EXPERIMENTAL

Sodium chloride in the form of a crystal is activated by the impact of heavy hydrogen nuclei, deuterons, which are accelerated to several million volts by one of the modern devices available in the nuclear physics laboratories, a cyclotron or high-tension machine. Both the sodium and the chlorine in the salt become radioactive. The lifetime of the chlorine is so short (half-life¹ about one-half hour) that practically one is dealing only with sodium of mass 24, which has a half-life of 14.8 hrs.

The radioactive sodium emits fast electrons and gamma (γ) rays. It is detected mostly by the effect of its γ -rays. For the detection one uses so-called counter tubes which consist of a metallic cylinder and a coaxial wire in a vapor atmosphere, usually argon and a trace of alcohol, at a few centimeters pressure. We have been using Geiger counters consisting of a Dow-metal² tube of 11 cm. length, 1.5 cm. width, and a thickness of about 0.01 cm. The counters are filled with an argon-alcohol mixture at about 7 cm. pressure (1 cm. alcohol, 7 cm. argon).

¹ Radioactive substances decay with a certain probability and it is customary to characterize this probability by the time after which half the number of original atoms is still present. Thus radioactive chlorine of mass 38 with a half-life of 37.5 min. has a greater chance to disintegrate than sodium of mass 24 with a half-life of 14.8 hrs.

² Since this metal and also aluminum are difficult to obtain at present it is quite feasible to use brass counters with a thin mica window of 0.3 mm. thickness mounted over a grill so that the window will stand the difference between the atmospheric pressure and the vacuum inside.

* Head of the Department of Physics, Purdue University, Lafayette, Ind.

† Amelia Henry Reinhardt International Fellow of the American Association of University Women.