TOXICITY OF ORGANIC COMPOUNDS TO THE SPORES OF PHYTOPHTHORA COLOCASIAE RAC.¹

By B. N. UPPAL

Research Fellow, Iowa Agricultural Experiment Station ²

INTRODUCTION

In recent years a large amount of work has been done on spore germination in relation to chemical and physical stimuli, but there has been comparatively little study of the relationship between the chemical constitution of a compound and its physiological action. It is not known, for instance, whether the toxicity of furfural is due to the aldehyde group which it contains, or to the presence of the unsaturated oxygen atom in the furan nucleus; whether there is always a similar physiologic response of the spores to the action of compounds, closely related chemically, such as aromatic and aliphatic aldehydes and alcohols; or whether the physiological response is regularly related to the physicochemical behavior, a conception held by those who believe there is a fundamental unity in the physiologic responses of the living and the nonliving systems. These and a multitude of other matters suggest themselves, but the problem is so complex and its scope so large that it has here been possible to study only certain phases.

This investigation was undertaken to throw light on the group or radical in aldehydes and alcohols to which the toxicity of these compounds may be attributed. Simultaneously, a detailed and careful study of the toxicity of organic acids was made in order that knowledge on the comparative toxicity of these three classes of organic compounds might be gained, for any work that throws light on these problems will materially advance our knowledge of the physiological action of fungicides.

METHODS AND MATERIALS

ORGANISM

After experimentation with 20 species of the genus Phytophthora, cultures of which were obtained from various parts of this country and abroad,³ Phytophthora colocasiae Rac. was selected as the species most suitable for this work, as it makes a fairly rapid growth on oatmeal agar and produces sporangia in abundance.

¹ Received for publication Oct. 14, 1925; issued June, 1926. Published with the approval of the Director of the Iowa Agricultural Experiment Station.

² The writer is grateful to I. E. Melhus, Professor of Plant Pathology, Iowa State College, under whom this work was done, for many suggestions and criticisms. He is also indebted to R. M. Hixon, of the Department of Chemistry, Iowa State College, for critical reading of the manuscript.

³ The writer expresses his thanks to William MacRae, Imperial Mycologist to the Government of India, to Leon H. Leonian of the West Virginia Agricultural Experiment Station, and to J. B. Kendrick of the Purdue University Agricultural Experiment Station, for supplying cultures of Phytophthora.
Phytophthora colocasiae Rac. is the fungus which parasitizes the leaves of Colocasia antiquorum Schott., and also causes a dry rot of the corm during the storage. The fungus produces both sexual and asexual spores. The asexual spores are very large. When formed on the leaf they measure from 38 to 60 by 18 to 26 μ in diameter. In culture, however, the range of variation is much wider. The sporangia germinate readily in distilled water, either by putting out a germ tube or by the formation of zoospores, which in turn germinate by a hypha. These two modes of germination are entirely controlled by the effect of temperature on the spores. Temperatures ranging from 10° to 25° C. favor the formation of zoospores in the sporangia, which, if the culture is not older than 7 days, germinate as high as 95 to 100 per cent. At high temperatures, from about 25° to 32° C., germination of the sporangia occurs exclusively by a hypha, which may form a mycelium or give secondary and sometimes tertiary sporangia after a short growth. Under the most favorable conditions of higher temperatures, from 29° to 32°, the sporangia will produce germ tubes in water cultures in three hours or less.

Two stock cultures of the fungus were made every day, and these were allowed to grow at room temperature for a week. Occasionally, when the temperature of the room fell below 22° C., the growth of the fungus was slowed down and the stock cultures were not ready for use until after eight, occasionally nine, days. At this time the sporangia were fully mature and were produced in great abundance. To obtain uniformity, it has been the custom to use spore material from a single tube in making an entire series of cultures. Direct inoculation of the culture drop was effected by removing a little mycelium with a thin flat-pointed platinum needle and shaking it gently in the drop. The spores fall off very readily, if the culture is not older than 7 days.

**STOCK SOLUTIONS OF CHEMICALS**

The organic compounds used were of the highest purity obtainable.

Stock solutions of the chemicals were prepared in molar (normal in the case of acids) concentrations, the strength of the stock solutions depending upon the degree of solubility of the compounds in water. In preparing stock solutions chemicals were weighed in a clean pyrex glass beaker on a chainomatic balance, which corrected the weight of the chemicals to the fourth decimal place. Most of the organic compounds do not go into solution in cold water readily, and they were dissolved in hot water, care being taken that the temperature of the hot water always remained far below the boiling point of the compound. The volumetric flasks containing the dissolved chemicals were immersed in water at 20° C., and enough water was added to the flasks to make the required volume. The water used in making the stock solutions was doubly distilled over a hard glass condenser from a solution of potassium permanganate in a hard glass flask.

In the case of alcohols, percentage solutions were made, and hence molar stock solutions were not prepared. As some of the alcohols were expensive, a method was worked out whereby fresh experimental solutions of any alcohol could be prepared by pipetting out 0.1 c. c. of a certain alcohol and then adding distilled water.
In this way not more than 3 or 4 c. c. of each alcohol were required for a sufficiently large number of experiments to determine fairly exactly the limits of toxicity.

**METHOD OF CULTURE**

The various dilutions of the compounds to be tested were made in pyrex glass beakers of 50 c. c. capacity. Each beaker was provided with a glass rod having a round, blunt point. Special care was taken to make the blunt points on the rods as nearly alike as possible, for the reason that variable shapes may give culture drops of variable size, thus giving erratic results.

For making cultures, Van Tieghem cells were used, which were cemented to ordinary slides by means of refined beeswax. All cultures were made in duplicate. A drop of the solution to be tested was placed on a clean cover glass, which was then sealed on to the top of the cell with petrolatum. One to two drops of the same solution were placed in the bottom of the cell. The slides were then ready to be removed to an incubator.

For making cultures of alcohols, watch glasses of a type devised by the Bureau of Plant Industry, United States Department of Agriculture, were used, since in hanging-drop cultures the low surface tension of the alcoholic solutions used tended to spread the drop on the cover glass. In cross section these watch glasses are similar to the Syracuse watch glasses, but much smaller in size, viz, 27 mm. diameter by 8 mm. high, and have, unlike the Syracuse glasses, ground beveled edges. In making experimental cultures, 6 drops of the alcoholic solution to be tested were placed in the center of the watch glass and charged with spores.

In attempting to determine exactly the concentration of a compound which would inhibit germination of the spores, the procedure was as follows: 1 c. c. of the stock solution was pipetted out in a beaker, to which 9 c. c. of distilled water was added. The solution thus obtained was in turn diluted to one-tenth of its strength, and the process was repeated until a solution which contained 1 part of the stock solution to 100,000 parts of water was obtained. All these dilutions were charged with spores, and after an incubation period of 18 hours the cultures were examined for the lowest concentration of the compound that inhibited spore germination. Let this be 0.001 M. Then a set of 10 cultures, ranging from 0.001 M to 0.0001 M, was made in duplicate. This made it possible to determine the concentration at which spore germination was inhibited. Suppose the concentration of inhibition was found to be 0.0006 M. Another set of five cultures, ranging from 0.0004 M to 0.0008 M, was prepared. This was repeated twice, and if the concentration of inhibition remained the same, the findings were taken as correct.

**CLEANING GLASSWARE**

After the cultures were examined, the Van Tieghem cells, cover glasses, and microscopic slides were boiled in two changes of water to remove beeswax and petrolatum, and then soaked in xylol for 10 to 15 minutes. They were then washed in much the same manner as that described by Clark (3), except that distilled water was always used in washing cover glasses. All pipettes were washed by
forcing tap water through them for two hours and then washing them in distilled water.

The watch glasses were soaked in xylol for 15 minutes to remove petrolatum, then washed freely under the tap for the same length of time, and finally rinsed in 95 per cent alcohol. They were then washed in several changes of distilled water, wiped, and sterilized in an oven for 30 minutes.

EXAMINATION OF CULTURES

In determining the effect of various organic compounds upon the sporangia, the best index was the occurrence or nonoccurrence of germination after periods of 6 and 18 hours. In the case of indirect germination, any renewed activity in protoplasm, besides the formation of true zoospores, was regarded as germination. The renewed activity of protoplasm, under the influence of deleterious agents, manifests itself in the streaming out of the intersporangial protoplasm, which finally breaks up into small granular masses. Direct germination occurs by the production of a germ tube, although in some cases the mycelium from a germ tube may give secondary and tertiary sporangia. Under the debasing influence of toxic agents, germ tubes become very slender and appear stunted in growth.

The concentrations of toxic agents in which the sporangia failed to germinate were divided into two classes: (1) The critical concentration, or that lowest concentration of a deleterious agent which made germination doubtful or which had an inhibiting influence without the least visible signs of plasmolysis or other abnormalities in the sporangia; (2) the lethal concentration, or that lowest concentration of a toxic agent which brought about slight or pronounced plasmolysis of a few or many sporangia, a fact that was taken as a sure index of the death of the spores. In considering results on the lethal concentrations of various organic compounds, a difficulty was encountered in that resistance of the spores showed great variation; for instance, a concentration of a toxic agent will at one time produce the plasmolyzed condition of the sporangia, while at another time there will be no visible sign of any abnormality. Caution should therefore be exercised in accepting these results, for the boundary line between the critical and lethal concentrations is so narrow that a slight alteration in the resistance of the spores may throw the results from one concentration to the other.

The cultures for direct germination were kept in an electric incubator, in which the temperature was maintained at 30° to 31° C. For indirect germination, the cultures were kept in an automatically controlled refrigerator, cooled by sulphur dioxide. This refrigerator had various compartments, which registered temperatures varying from 4° to 16°; in fact, still lower temperatures could be obtained by regulating the cooling machine. The cultures were kept in a compartment registering 11° to 12°.

COMPARATIVE TOXICITY OF ALCOHOLS

Before proceding with the discussion of the experimental data, a brief resume of the important literature on the toxicity of alcohols will show the present state of knowledge of this subject.

Stadler (18), using *Staphylococcus aureus*, *Bacterium coli*, and *Bacillus pyocyaneus*, and Morgan and Cooper (13), working with
Staphylococcus pyogenes aureus and Bacillus typhosus, have shown that the toxicity of the normal alkyl alcohols increases as the homologous series is ascended, with the exception that methyl and ethyl alcohols, according to the latter writers, showed the same toxicity. However, Vandevelde (19) and Förster (4) contend that the failure of methyl alcohol to behave in accordance with the rule of increasing molecular weight is largely due to impurities. The conclusions of Stadler (18) concerning bacteria are further supported by the work of Vernon (20) with the tortoise, Kuno (8) with the rabbit, and Macht (11) with the rabbit and the frog. Kamm (7), testing the toxicity of various normal primary alcohols upon paramecia, has found that, with the exception of methyl alcohol, if the toxicity of ethyl alcohol be taken as unity, the successive members may be arranged as to toxicity according to the following geometrical progression: 3, $3^2$, $3^3$, $3^4$, etc.; in other words, the molar toxicity of any member in a homologous series is three times that of the succeeding member.

Vernon (20) and Macht (11), in the case of normal propyl, normal butyl, and normal amyl alcohols, and Morgan and Cooper (13) in the case of normal propyl and normal butyl alcohols, showed that the normal alcohols were more toxic than their corresponding isomers. Moreover, Vernon (20) found that secondary and tertiary butyl alcohols were toxic in this order of mention, but both were less toxic than isobutyl.

Carlier (1, 2) studied the effects of allyl isothiocyanate, allyl sulphide, allyl acetate, and allyl alcohol, upon respiration and blood pressure in the rabbit and the frog, and concluded that the allyl compounds owe their toxicity to the allyl radical.

Ruediger (17, p. 475-477), working with the anthrax organism, found that glycerol exerts more feeble germicidal action at 15° C. than at 30° to 35°. Glycerol also exerts a specific toxic influence on cholera, diphtheria, and plague organisms. Winslow and Holland (23), using varying concentrations of glycerol on Bacterium coli, concluded that a 9 per cent solution of the alcohol did not exercise any appreciable influence on the viability of Bacterium coli, but solutions between 28 and 100 per cent strength showed progressively increasing toxic action on the organism.

Practically no work has been done on the toxicity of alcohols in relation to plant pathogens, especially the parasitic fungi.

**TOXICITY TRIALS**

Typical alcohols from the aliphatic and the aromatic series were investigated. In the present case only indirect germination of the sporangia of Phytophthora colocasiae was employed as a criterion of the relative toxicity of the alcohols.

The toxic action of the 13 alcohols of the aliphatic series has been summarized in Table I. The first group is that of the monohydric alcohols. It will be seen from the table that a 7.9 per cent solution of methyl alcohol prevents the liberation of zoospores, while ethyl alcohol of 6.3 per cent strength exerts the same effect. These results were a little surprising, since it was expected that methyl alcohol, like first members of the homologous series, would stand out by itself in its physiological action, and would prove more toxic to the spores than is indicated in the table.
TABLE I.—Toxic effect of alcohols on the spores of Phytophthora colocasiae Rac.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of experiments</th>
<th>Number of cultures</th>
<th>Indirect germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Aliphatic alcohols:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Monohydric alcohols—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Saturated alcohols—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl alcohol</td>
<td>5</td>
<td>32</td>
<td>7.9</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>5</td>
<td>11</td>
<td>6.3</td>
</tr>
<tr>
<td>Normal propyl alcohol</td>
<td>5</td>
<td>28</td>
<td>3.1</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>5</td>
<td>38</td>
<td>6.5</td>
</tr>
<tr>
<td>Normal butyl alcohol</td>
<td>3</td>
<td>18</td>
<td>1.5</td>
</tr>
<tr>
<td>Isobutyl alcohol</td>
<td>7</td>
<td>36</td>
<td>1.8</td>
</tr>
<tr>
<td>Secondary butyl alcohol</td>
<td>6</td>
<td>30</td>
<td>3.2</td>
</tr>
<tr>
<td>Tertiary butyl alcohol</td>
<td>5</td>
<td>24</td>
<td>6.3</td>
</tr>
<tr>
<td>Normal amyl alcohol</td>
<td>3</td>
<td>16</td>
<td>1.2</td>
</tr>
<tr>
<td>Isoamyl alcohol</td>
<td>4</td>
<td>14</td>
<td>1.0</td>
</tr>
<tr>
<td>II. Unsaturated alcohol—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allyl alcohol</td>
<td>7</td>
<td>34</td>
<td>.0005</td>
</tr>
<tr>
<td>b. Dihydric alcohol—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>7</td>
<td>28</td>
<td>6.5</td>
</tr>
<tr>
<td>c. Trihydric alcohol—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>3</td>
<td>14</td>
<td>6.2</td>
</tr>
<tr>
<td>B. Aromatic alcohols:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Saturated alcohol—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>3</td>
<td>14</td>
<td>.2</td>
</tr>
<tr>
<td>II. Cyclo-olefine—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furfuralcohol</td>
<td>9</td>
<td>46</td>
<td>.2</td>
</tr>
</tbody>
</table>

* To correspond with critical concentrations in per cent, molarity of these solutions is calculated approximately on the basis of the specific gravity of different alcohols at 20°C, unless otherwise stated.

Normal propyl alcohol is about twice as toxic as ethyl alcohol, inhibiting the formation of zoospores in 3.1 per cent solution. On the other hand, isopropyl alcohol, which prevents indirect germination of the spores in 6.5 per cent solution, is a little less toxic than ethyl alcohol. In this connection it is interesting to note how the physiological action of a compound is modified on account of a change in its structural constitution. A satisfactory explanation of such facts would, no doubt, materially advance our knowledge of physiological chemistry.

The behavior of normal butyl alcohol and its isomers is still more interesting. A 1.5 per cent solution of normal butyl alcohol will prevent the formation of zoospores; that is, normal butyl alcohol has 4.2 times the toxicity of ethyl alcohol. Isobutyl alcohol, though still rather toxic, has its poisonous effect reduced to about three-fourths that of normal butyl, or its toxic action is only 3.5 times that of ethyl alcohol. Secondary butyl alcohol shows a still greater fall in toxic value. It is approximately one-half as toxic as normal butyl alcohol, the strength of the alcohol inhibiting indirect germination of the spores being 3.2 per cent. The toxicity of tertiary butyl alcohol is so much reduced that it exactly corresponds with that of ethyl alcohol. Thus, isobutyl alcohol is less toxic than normal butyl, secondary butyl less than isobutyl, and tertiary butyl less than secondary butyl.

Normal amyl alcohol and its isomer were next investigated. A 1.2 per cent solution of normal amyl alcohol prevents the liberation of zoospores, while a 1 per cent solution of isoamyl exerts the same effect. The writer does not place much reliance in these results, as it was not possible to obtain even a 1 per cent solution of normal
amyl alcohol, although it is reported soluble to the extent of 2.72 gm. in 100 c. c. of water at 22° C. Even after a gentle warming, normal amyl alcohol did not go into solution, but floated on the surface of water as tiny droplets, which could be seen easily by holding the beaker against the light.

Allyl alcohol, an unsaturated monohydric alcohol, is the most toxic of the alcohols investigated. A 0.0005 per cent solution of the alcohol prevents the formation of zoospores. Compared with ethyl alcohol, it is 12,600 times more toxic. The presence of a double bond leads to an increased activity of the alcohol, and it seems that this chemical activity accelerates the toxic action of the compound. Moreover, it has been shown by Carlier (2) that the toxicity of the alcohol is due to the allyl radical that it contains.

Ethylene glycol, or glycol, is the simplest glycol known, and corresponds with ethyl alcohol. It is of about the same toxicity as ethyl alcohol, the critical concentration for indirect germination of the spores being 6.5 per cent. Glycerol, the simplest trihydric alcohol, is also not very toxic, its critical concentration being 6.2 per cent.

Only two alcohols from the aromatic series were investigated. Benzyl alcohol prevents the liberation of zoospores in 0.2 per cent solution. It has about 31 times the toxicity of ethyl alcohol. The toxicity of benzyl alcohol is mostly due to the benzene nucleus. Furfuralcohol, like benzyl alcohol, inhibits indirect germination of the spores in 0.2 per cent solution. Its toxicity may be due to the unsaturated oxygen atom in the furyl radical.

COMPARATIVE TOXICITY OF ALDEHYDES

Formaldehyde is one of the most important fungicides, but very little is known regarding its action. So it seemed desirable to investigate a number of aldehydes, both aliphatic and aromatic, in order to throw light on the group or radical in the aldehyde molecule to which the toxicity of aldehydes in general may be attributed. A review of the literature shows that comparatively little study has been made of this problem, especially in its relation to the fungi.

Stadler (18), working with Staphylococcus aureus, Bacterium coli, and Bacillus pyocyaneus, showed that in aliphatic aldehydes the lower homologues decreased in toxicity with increasing molecular weight. However, Vernon (20), using depression of the heart beats of the tortoise as a criterion of toxicity, found that the toxicity of the four aldehydes investigated had no relationship to molecular weight. He has given the relative toxicity of the aldehydes as follows: Propionaldehyde, 1.0; acetaldehyde, 1.2; isobutyraldehyde, 1.8; and formaldehyde, 40.

Warburg (22) tested the action of aldehydes in modifying the oxidative processes of the young goose erythrocytes, and found that the following molar concentrations caused a 30 to 70 per cent reduction in oxygen consumption: Formaldehyde, 0.001; acetaldehyde 0.013; propionaldehyde, 0.01; normal butyraldehyde, 0.008; isobutyraldehyde, 0.01; isovaleraldehyde, 0.0035; furfural, 0.003.

Loeb (9) observed that the subcutaneous injection of rabbits with the aliphatic aldehydes, formaldehyde, acetaldehyde, normal valeraldehyde, enanthaldehyde, and citral, resulted in arterial necrosis, which was not produced by furfural and aromatic aldehydes.
Verzár and Felter (21) found that formaldehyde, glyoxal, and acrolein always produced the typical veratrine contraction of the transversely striated muscles, while acetaldehyde gave the same reaction three out of five times, paraldehyde four out of six, and glyceryl aldehyde two out of five. All other aldehydes failed to give the reaction.

Moore (12) tested a number of organic compounds for toxicity to the house fly, and found that of all the aldehydes used, salicylic aldehyde was the most toxic. Its toxicity was 19 times that of furfural and 3.5 times that of benzaldehyde.

McGuigan (10) investigated the action of furfural on bacteria, yeasts, and goldfish. He found that the phenol coefficient of furfural, measured from its bactericidal action, was 0.26. A 2 per cent solution of furfural entirely inhibited the action of yeasts on dextrose. The toxicity of furfural to goldfish was one-half that of phenol and about one-third that of formaldehyde.

TOXICITY TRIALS

Eleven aldehydes from the aliphatic and the aromatic series have been investigated. Direct and indirect germination of the sporangia of Phytophthora colocasiae was used as a criterion of the relative toxicity of aldehydes.

It will be seen in Table II that formaldehyde* exerts a greater inhibitory effect on the formation of zoospores than on the production of germ tubes. It inhibits the liberation of zoospores in 0.0009 M solution, while the critical concentration for direct germination is 0.002 M.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Number of experiments</th>
<th>Number of cultures</th>
<th>Indirect germination</th>
<th>Direct germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Critical concentration, molar</td>
<td>Lethal concentration, molar</td>
<td>Critical concentration, molar</td>
</tr>
<tr>
<td>Aliphatic aldehydes:</td>
<td></td>
<td></td>
<td>0.0009</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>H.CHO</td>
<td>8</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>CH₃.CHO</td>
<td>5</td>
<td>95</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>Propionaldehyde</td>
<td>C₃H₇.CHO</td>
<td>3</td>
<td>60</td>
<td>0.67</td>
<td>0.34</td>
</tr>
<tr>
<td>Normal butyraldehyde</td>
<td>C₄H₉.CHO</td>
<td>3</td>
<td>50</td>
<td>0.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Glyoxal</td>
<td>CHO.CHO</td>
<td>4</td>
<td>50</td>
<td>0.05</td>
<td>0.005</td>
</tr>
<tr>
<td>Aromatic aldehydes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>C₆H₅.CHO</td>
<td>2</td>
<td>16</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>m-Nitrobenzaldehyde</td>
<td>NO₂.C₆H₆.CHO</td>
<td>4</td>
<td>38</td>
<td>0.005</td>
<td>0.008</td>
</tr>
<tr>
<td>o-Nitrobenzaldehyde</td>
<td>NO₂.C₆H₅.CHO</td>
<td>4</td>
<td>40</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>p-Hydroxybenzaldehyde</td>
<td>O.H.C₆H₅.CHO</td>
<td>4</td>
<td>40</td>
<td>0.003</td>
<td>0.004</td>
</tr>
<tr>
<td>Protocatechic aldehyde</td>
<td>(OH)₂.C₆H₅.CHO</td>
<td>3</td>
<td>24</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Furfuraldehyde</td>
<td>C₆H₅.O.CHO</td>
<td>4</td>
<td>80</td>
<td>0.04</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Sporangia germinated.

The critical concentrations of acetaldehyde for direct and indirect germination of the spores are 0.06 M and 0.05 M, respectively. In other words, for indirect germination the toxicity of formaldehyde is

---

* The formaldehyde used in these experiments was analyzed in the Physical Chemistry Laboratories at Ames, Iowa, by F. Schulze, and was found to contain 37.7 per cent of formaldehyde by volume and 34.08 per cent by weight.
about 55.5 times that of acetaldehyde, while for direct germination its action is only 30 times stronger. The toxicity gradient for acetaldehyde showed an abrupt decline, for while a 0.06 M solution of the aldehyde permits the production of germ tubes, the sporangia are badly plasmolyzed in 0.07 M concentration. A similar condition occurs in the case of indirect germination.

Propionic aldehyde acts very much like acetaldehyde, although it is a little weaker than the latter. Its critical concentration for direct and indirect germination is 0.07 M. There is a gradual rise in the toxicity of the aldehyde, and the sporangia do not exhibit any plasmolysis until a 0.1 M concentration of the aldehyde is reached.

Normal butyraldehyde, like formaldehyde, shows a greater inhibitory action to the formation of zoospores than to the production of germ tubes. Its inhibitory concentration for direct and indirect germination is 0.05 M, while the production of germ tubes is retarded at 0.08 M. The toxicity of the aldehyde increases gradually with concentration, so that there is a gradation from true germination through inhibition to plasmolysis of the sporangia. Stock solutions deteriorate on long standing at room temperature, and it is therefore advisable not to prepare stock solutions long before they are to be tested.

Glyoxal or glyoxaldehyde, contains two aldehyde groups without having any alkyl radical attached to them. It is about 10 times more toxic than any other aliphatic aldehyde, except formaldehyde. Its critical concentrations for direct and indirect germination of the spores are very nearly the same, being 0.006 M and 0.005 M, respectively. Glyoxal is about one-half as toxic as formaldehyde. In the case of direct germination, the toxicity curve shows a rather sharp decline, as the concentrations for inhibition of germination and death of the spores coincide. Hence, correctly speaking, there is no critical concentration for direct germination in this case.

Of the aromatic series, six aldehydes were investigated for toxicity, the results being summarized in Table II. Benzaldehyde is reported soluble to the extent of 0.33 gm. in 100 c. c. of water. In other words it is possible to prepare only a 0.025 M solution. However, the stock solution prepared was of 0.02 M strength, a concentration which allowed both direct and indirect germination.

Meta- and ortho-nitrobenzaldehydes were next investigated. These two aldehydes are very nearly as toxic as glyoxal, and are 10 times more toxic than any aliphatic aldehyde, except formaldehyde. These aldehydes exert a greater inhibitory action on indirect than on direct germination. Paranitrobenzaldehyde could not be investigated, as it is only slightly soluble in hot water. Parahydroxybenzaldehyde is about twice as toxic as meta- or ortho-nitrobenzaldehyde. Its critical concentrations for direct and indirect germination are 0.005 M and 0.003 M, respectively. Protocatechuic aldehyde, which has its critical concentration at 0.003 M, shows about the same toxicity as parahydroxybenzaldehyde. Evidently, then, the presence of two hydroxyl groups in protocatechuic aldehyde does not enhance the toxicity of the compound to any great extent. It should also be noted that in all of the four substituted benzaldehydes investigated the toxicity curve shows a rather sharp fall similar to that found in glyoxal.
Furfural is not very toxic to the spores, the concentrations inhibiting direct and indirect germination being 0.05 M and 0.04 M, respectively. Furfural shows the same toxicity to the spores as do the aliphatic aldehydes, except formaldehyde. It also shows a gradual killing effect, increasing steadily with concentration.

COMPARATIVE TOXICITY OF ORGANIC ACIDS

The toxic effect of organic acids on fungous spores has not been studied extensively. In fact, there is very little information on this subject in the literature, except the work of Kahlenberg and True (6), who investigated the toxicity of a number of inorganic and organic compounds to the seedlings of *Lupinus albus* L.

TOXICITY TRIALS

### Summary of Table III

From Table III it appears that formic acid is the most toxic acid of the fatty acid series to the sporangia of *Phytophthora colocasiae*. Its critical concentration for direct germination of the spores is 0.0025 N, which is about one-third the critical concentration of acetic acid, the second in the order of toxicity. With regard to indirect germination of the spores, a similar relationship holds, although in this case the critical concentration of formic acid is one-seventh that of acetic acid and one-fifth that of isovaleric acid, which here happens to be the next in the order of toxicity.

### Table III.—Toxic effect of the monocarboxylic fatty acids and the substituted acids on the spores of *Phytophthora colocasiae* Rac.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of experiments</th>
<th>Number of cultures</th>
<th>Indirect germination</th>
<th>a at 10°C.*</th>
<th>H⁺+R⁻</th>
<th>HR *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Critical concentration</td>
<td>Lethal concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated carboxylic acids:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formic acid</td>
<td>4</td>
<td>50</td>
<td>0.001</td>
<td>0.002</td>
<td>5.4 (9.2)</td>
<td>0.00378</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>6</td>
<td>64</td>
<td>0.007</td>
<td>4.02(6.9)</td>
<td>0.00321</td>
<td>0.00767</td>
</tr>
<tr>
<td>Propanoic acid</td>
<td>6</td>
<td>72</td>
<td>0.008</td>
<td>4.42(9.4)</td>
<td>0.00353</td>
<td>0.00764</td>
</tr>
<tr>
<td>Normal butyric acid</td>
<td>3</td>
<td>40</td>
<td>0.008</td>
<td>4.00</td>
<td>0.00386</td>
<td>0.00864</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>3</td>
<td>32</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal valeric acid</td>
<td>3</td>
<td>36</td>
<td>0.008</td>
<td>5.5(15)</td>
<td>0.00275</td>
<td>0.00472</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>3</td>
<td>36</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsaturated carboxylic acids:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crotonic acid</td>
<td>3</td>
<td>28</td>
<td>0.003</td>
<td>7.6(12)</td>
<td>0.00228</td>
<td>0.00277</td>
</tr>
<tr>
<td>Halogen-substituted acetic acids:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monochloroacetic acid</td>
<td>4</td>
<td>46</td>
<td>0.001</td>
<td>67</td>
<td>0.0067</td>
<td>0.00333</td>
</tr>
<tr>
<td>Dichloroacetic acid</td>
<td>5</td>
<td>78</td>
<td>0.001</td>
<td>99.9</td>
<td>0.00099</td>
<td>0.00001</td>
</tr>
<tr>
<td>Trichloroacetic acid</td>
<td>4</td>
<td>58</td>
<td>0.001</td>
<td>99.9</td>
<td>0.00099</td>
<td>0.00001</td>
</tr>
<tr>
<td>Monobromacetic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyacetic acids:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycollic acid</td>
<td>3</td>
<td>44</td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>6</td>
<td>64</td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE III.—Tonic effect of the monocarboxylic fatty acids and the substituted acids on the spores of Phytophthora colocasiae Rac.—Continued

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of experiments</th>
<th>Number of cultures</th>
<th>Direct germination</th>
<th>Critical concentration, normal</th>
<th>Lethal concentration, normal</th>
<th>a at 30° C. a</th>
<th>H⁺ + R⁻ b</th>
<th>HR c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated carboxylic acids:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formic acid</td>
<td>4</td>
<td>50</td>
<td>0.0025</td>
<td>0.003</td>
<td>35.4 (25)</td>
<td>0.000885</td>
<td>0.00161</td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>6</td>
<td>64</td>
<td>0.007</td>
<td>0.01</td>
<td>5.4</td>
<td>0.000378</td>
<td>0.0062</td>
<td></td>
</tr>
<tr>
<td>Propionic acid</td>
<td>6</td>
<td>72</td>
<td>0.008</td>
<td>0.009</td>
<td>4.02</td>
<td>0.000521</td>
<td>0.0076</td>
<td></td>
</tr>
<tr>
<td>Normal butyric acid</td>
<td>3</td>
<td>40</td>
<td>0.008</td>
<td>0.01</td>
<td>4.24</td>
<td>0.000339</td>
<td>0.0096</td>
<td></td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>3</td>
<td>32</td>
<td>0.009</td>
<td>0.01</td>
<td>3.7</td>
<td>0.000333</td>
<td>0.0096</td>
<td></td>
</tr>
<tr>
<td>Normal valeric acid</td>
<td>3</td>
<td>36</td>
<td>0.008</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>3</td>
<td>36</td>
<td>0.009</td>
<td>0.009</td>
<td>4.07</td>
<td>0.000366</td>
<td>0.0063</td>
<td></td>
</tr>
<tr>
<td>Unsaturated carboxylic acids:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crotonic acid</td>
<td>3</td>
<td>28</td>
<td>0.003</td>
<td>0.004</td>
<td>7.68</td>
<td>0.00023</td>
<td>0.0027</td>
<td></td>
</tr>
<tr>
<td>Halogen-substituted acetic acids:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monochloracetic acid</td>
<td>4</td>
<td>46</td>
<td>0.002</td>
<td>0.003</td>
<td>56.3</td>
<td>0.00112</td>
<td>0.0068</td>
<td></td>
</tr>
<tr>
<td>Dichloracetic acid</td>
<td>5</td>
<td>73</td>
<td>0.002</td>
<td>0.003</td>
<td>96.5</td>
<td>0.00107</td>
<td>0.0063</td>
<td></td>
</tr>
<tr>
<td>Trichloracetic acid</td>
<td>4</td>
<td>58</td>
<td>0.002</td>
<td>0.003</td>
<td>99.3</td>
<td>0.00198</td>
<td>0.0062</td>
<td></td>
</tr>
<tr>
<td>Monobromacetic acid</td>
<td>4</td>
<td>60</td>
<td>0.0005</td>
<td>0.0007</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyacarboxylic acids:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycollic acid</td>
<td>3</td>
<td>44</td>
<td>0.001</td>
<td>0.002</td>
<td>31.8</td>
<td>0.000318</td>
<td>0.00682</td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>6</td>
<td>64</td>
<td>0.001</td>
<td>0.002</td>
<td>30.6</td>
<td>0.000306</td>
<td>0.00694</td>
<td></td>
</tr>
</tbody>
</table>

a The value of a is the percentage of an acid ionized at its critical concentration.
b H⁺ + R⁻ represents the concentration of the H⁺-ions and the anions at the critical concentration.
c HR Represents the concentration of the undissociated molecules at the critical concentration.
d The number in parentheses represents the temperature on the centigrade scale, at which the value of a is determined.

It is evident from Ostwald's dilution law and formula that of two acids the one which is more highly ionized has a greater constant. Thus formic acid, which is the most highly ionized of the fatty acids, is stronger than any other in the series. Acetic acid, the second in the order of toxicity as well as the extent of ionization, is a little stronger than propionic acid. With regard to direct germination, there seems to be but little difference in the critical concentrations of all the normal fatty acids, except formic; but for indirect germination the critical concentration of acetic acid is a little higher than that of isovaleric acid, which in the case of direct germination appears to be one of the least toxic; otherwise the differences among other acids are not great enough to be of significance. This is, however, in accordance with what would have been expected, considering that these acids are ionized to approximately the same degree, and their alkyl radicals do not appear to differ markedly in chemical nature.

The relatively greater toxic effect of formic acid may also be due to its structural peculiarity. According to its structure, formic acid contains an aldehyde group; in other words, it is the only organic acid that has in the molecule an unsaturated hydrogen atom, which is very sensitive to oxygen. This, coupled with the fact that formic acid at its critical concentrations is more highly ionized than any other saturated monocarboxylic fatty acid, may account for its relatively greater toxicity.

According to Ostwald's (15) determination, 1 gram molecular weight of acetic acid in 1,024 liters of water is 12.66 per cent ionized; but in the case of direct and indirect germination the critical concentrations of this acid contain only 1 gram molecular weight of it in about 143 liters of water; that is, at these concentrations the acid is about 5.4 per cent ionized (by interpolation). It therefore follows that since
such a small portion of the acid is ionized, its toxic properties can not
be due entirely to the influence of the H-ions or to that of the anions.
Hence it is the undissociated molecules which partly seem to be
responsible for the toxic effects of acetic acid.

For direct and indirect germination of the spores, the critical con-
centration of propionic acid is 0.008 N. According to Jones's (5,
p. 87-144) determination, the acid at the critical concentration is
about 4 per cent ionized. Hence the toxicity of the acid does not
seem to be determined entirely by the poisonous action of the H-ions
or the anions, but is due, in part, to the effect of the undissociated
molecules.

The electrolytic dissociation of normal butyric and isobutyric acids
is very nearly the same, which is characteristic of a large number of
isomeric compounds. The poisonous action of these two acids cor-
responds with the degree of their electrolytic dissociation, normal
butyric acid, which is slightly more ionized than its isomer, being more
toxic than isobutyric. It is of interest to note in this connection that
a change in the structural constitution of the two acids has not
modified their physiological action, except for a slight difference in the
electrolytic dissociation. However, it is to be seen later that this
does not hold true in all cases of isomerism. The concentrations of
normal butyric and isobutyric acids which do not permit direct and
indirect germination of the spores are 0.009 N and 0.008 N, respec-
tively. According to Jones's (5, p. 87-144) determination, normal
butyric and isobutyric acids, at their critical concentrations, are about
4 per cent ionized. A study of their sodium salts will determine the
extent to which the anions and the undissociated molecules influence
the toxicity of the acids. However, the effect of the H-ions in contrib-
uting to the total toxicity is about the same as in acetic acid.

Unlike normal butyric acid, the ionization constant of normal
valeric acid is less than that of isovaleric acid. It is to be expected,
then, that normal valeric acid will be less poisonous to the spores
than its isomer. This is true in the case of indirect germination,
where the critical concentration of normal valeric acid is 0.008 N,
and that of its isomer 0.005 N; but the order of the toxicity of the
two acids is reversed in the case of direct germination. Here normal
valeric acid inhibits germination at 0.008 N, while its isomer exerts
the same effect at 0.009 N. Interpolating Jones's (5, p. 87-144)
figures, the electrolytic dissociation of isovaleric acid at 15° C. and
at the dilution 200' (the critical concentration for indirect germina-
tion) is about 5.5 per cent, and at 30° C. about 5.2 per cent, a signifi-
cant difference considering the amount of the acid ionized. But it
is very doubtful whether this difference in the degree of ionization
accounts for the decrease in the strength of the acid at 30° C., which
is a little less than one-half of its strength at 15°. The experiment
has not been repeated a sufficient number of times to exclude all
chances of error.

Of the unsaturated carboxylic fatty acids, crotonic acid was in-
vestigated. The critical concentration for direct and indirect germi-
nation of the spores is 0.003 N. According to Jones's (5, p. 87-144)
determination, the acid at its critical concentrations is about 7.6
per cent ionized. It seems that the effect of the H-ions in determin-
ing the toxicity of the acid at its critical concentrations is not very
great, since only about one-thirteenth of the total amount of the acid
present in the solution is ionized. The presence of a double bond in the acid seems to accelerate its toxicity.

The three chloro-acetic acids differ greatly from acetic acid as to toxicity, in that they are about seven times more toxic than the latter. This difference, however, is due to the fact that the introduction of chlorine or any other halogen into the positive methyl radical of acetic acid renders the radical negative, and brings about a marked change in its properties. This change manifests itself partly in an increase in the ionization constants of these acids. For instance, the replacement of one hydrogen atom in acetic acid by one chlorine atom increases the constant of the acid 86 times, while the constant of dichloracetic acid is about 33 times as great as that of monochloracetic acid, and that of trichloracetic acid 23.5 times as great as that of the disubstituted acid. Monobromacetic acid has about 80.6 times the constant of acetic acid. It seems reasonable, then, to assume that the order of toxicity would be tri-, di-, and mono-chloracetic acid, followed closely by monobromacetic acid. But such a condition is obtained only when the toxicity of these acids is solely dependent upon the concentration of the H-ions in the solution.

Since the introduction of a halogen into the methyl radical of acetic acid also results in the increased toxicity of the undissociated molecules, it seems that the toxic properties of the halogen-substituted acetic acids are due to the combined effects of the H-ions and the undissociated portion. Moreover, as ionization advances, the toxic influence is exerted by a greater concentration of the H-ions, more than by the presence of the undissociated molecules; conversely, the lower the ionization, the greater the toxic effect due to the undissociated portion. Clark (3), using potassium salts of the three chloro-acetic acids, worked out the toxic values of their undissociated molecules, showing that the replacement of one hydrogen atom in the methyl radical of acetic acid doubles the toxic properties of the un-ionized portion, while the replacement of two hydrogen atoms trebles its toxicity, and the replacement of all three hydrogen atoms increases its toxic value about five times.

That the three chloro-acetic acids behave alike as respects their toxic action now seems easy to explain. In the case of indirect germination, monochloracetic acid, which is about 67 per cent ionized at its critical concentration (0.001 N), has about 33 per cent more of the un-ionized portion than either of the other two chloro-acetic acids, which are about 99.5 per cent ionized. In other words, the toxicity of monochloracetic acid is determined by the combined effects of the H-ions and the undissociated molecules; whereas in di- and tri-chloracetic acids the influence of the H-ions alone seems to account for their toxic properties, for at this dilution (0.001 N) the two acids are so completely ionized that the amount of the dissociated portion is rather too small. Thus the slight toxicity of monochloracetic acid due to low degree of electrolytic dissociation is so supplemented by the toxic properties of the undissociated portion that the resultant of these two factors gives a toxic value equal to that for the other two acids.

The concentrations of the chloro-acetic acids inhibiting the production of germ tubes is 0.002 N, which is twice the concentration which prevents the liberation of zoospores. Moreover, it should be noted that at the critical concentrations of the acids, both for direct
and indirect germination, most of the sporangia were badly plas-
molyzed, and that it was only a very few sporangia that germinated.

For direct germination of the spores, monobromacetic acid is
four times as toxic as the chloro-acetic acids, while for indirect
germination it is only about two and one-half times as toxic. Data
on the electrolytic dissociation of the acid for dilutions higher than
1,024 were not available, although it seems quite safe to assume
that the dissociation of the acid at the dilutions 2,500 and 2,000 is
not far from complete. Hence the toxic effects at these high dilu-
tions are exerted more by the concentration of the H-ions and the
anions in the solution than by that of the undissociated portion.
However, it appears hardly likely that the H-ions will exert any
great effect at these high dilutions. For an explanation of the
toxicity of the acid the aid of the sodium salt of the acid must be
invoked, in order to show the extent to which the anions influence
the total toxicity.

Passing to the hydroxycarboxylic fatty acids, we come to glycollic
acid, which is a hydroxyacetic acid. The introduction of a hydroxyl
group into acetic acid increases the dissociation constant of the acid
seven and seven-tenths times. The liberation of zoospores is in-
hibited at 0.0007 N, which is about one and one-half times less than
the concentration which allows a few sporangia to germinate by
germ tubes. For direct germination there does not seem to be a
critical concentration of the acid, since no concentration was found
to inhibit germination of the spores without showing signs of
plasmolysis.

Lactic acid is a hydroxypropionic acid, and has about 9.7 times
the dissociation constant of propionic acid, due to the presence of
a hydroxyl group. The critical concentrations of this acid are
exactly the same as those of glycollic acid. In addition, the physio-
logical response of the sporangia at these concentrations is very
nearly the same.

**SUMMARY OF TABLE IV**

Eight dicarboxylic acids of the aliphatic series were investigated
for their toxic action upon germination of the sporangia of *Phytoph-
thora colocasiae*. The results are summarized in Table IV.

**Table IV.—Toxic effect of di- and tri-carboxylic fatty acids on the spores of *Phytophthora colocasiae* Rac.**

| Compound                  | Number of experiments | Number of cultures | Indirect germination | Lethal concentration, normal | Critical concentration, normal | $a$ at $10^\circ$ C. | $H^+ + R^-$ | HR * | Saturated dicarboxylic acids:
|---------------------------|-----------------------|--------------------|----------------------|-----------------------------|--------------------------------|-------------------|------------|-------| Oxalic acid          | 3 | 46 | 0.0015 | 0.004 | 42.48(4.9) | 100 | 0.0015 | 0.0       |
| Malonic acid              | 3                     | 46                 | .003                 | .004                        | 42.48(4.9)                    | 100               | 0.0015    | 0.00127 |
| Succinic acid             | 3                     | 44                 | .002                 | 10                          | 10 (5.7)                      | .00032            | .00015    | .000127 |
| Unsaturated dicarboxylic acids:
| Maleic acid              | 4                     | 40                 | .0015                | .003                        | 90.86(12)                    | .00136            | .00014    | .000014 |
| Fumaric acid             | 3                     | 36                 | .0009                | 65.26(12)                   | .000387                       | .000413           | .00015    | .000014 |
| Hydroxydicarboxylic acids:
| Malic acid               | 3                     | 34                 | .002                 | .007                        |                                |                   |           |       |
| Tartaric acid            | 4                     | 42                 | .006                 | 38.91                       | .000972                       | .000153           |           |       |
| Hydroxytricarboxylic acid: Citric acid | 3 | 38 | 0.0025 | 38.91 | .000972 | .000153 |           |       |

* $a$ at $10^\circ$ C. indicates the temperature at which the critical concentration was determined.
* $H^+ + R^-$ represents the lethal concentration of the acid.
* HR * denotes the hydrogen ion concentration required for the lethal effect.
### TABLE IV.—Toxic effect of di- and tri-carboxylic fatty acids on the spores of Phytophthora colocasiae Rac.—Continued

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of experiments</th>
<th>Number of cultures</th>
<th>Direct germination</th>
<th>Critical concentration, normal</th>
<th>Lethal concentration, normal</th>
<th>a at 35° C.</th>
<th>( H^+ + R^- )</th>
<th>HR*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saturated dicarboxylic acids:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>3</td>
<td>46</td>
<td>0.002</td>
<td>0.003</td>
<td>100</td>
<td>0.002</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Malonic acid</td>
<td>3</td>
<td>46</td>
<td>.004</td>
<td>.005</td>
<td>40.71</td>
<td>.00162</td>
<td>.00238</td>
<td></td>
</tr>
<tr>
<td>Succinic acid</td>
<td>3</td>
<td>44</td>
<td>.015</td>
<td>.062</td>
<td>6.05</td>
<td>.000007</td>
<td>.0141</td>
<td></td>
</tr>
<tr>
<td><strong>Unsaturated dicarboxylic acids:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malic acid</td>
<td>4</td>
<td>40</td>
<td>.003</td>
<td>.004</td>
<td>81.62</td>
<td>.00244</td>
<td>.00652</td>
<td></td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>3</td>
<td>36</td>
<td>.004</td>
<td></td>
<td>37.06</td>
<td>.00148</td>
<td>.00252</td>
<td></td>
</tr>
<tr>
<td><strong>Hydroxydicarboxylic acids:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malic acid</td>
<td>3</td>
<td>34</td>
<td>.006</td>
<td>.007</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>4</td>
<td>42</td>
<td>.006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hydroxytricarboxylic acid:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>3</td>
<td>38</td>
<td>.008</td>
<td></td>
<td></td>
<td>28.3</td>
<td>.00226</td>
<td>.00574</td>
</tr>
</tbody>
</table>

* The value of \( a \) is the percentage of an acid ionized at its critical concentration.  
* \( H^+ + R^- \) represents the concentration of the H-ions and the anions at the critical concentration.  
* HR represents the concentration of the undissociated molecules at the critical concentration.  
* The number in parentheses represents the temperature on the centigrade scale at which the value of \( a \) is determined.

Oxalic acid has about the same critical concentration for direct and indirect germination of the spores. In the case of direct germination, the toxicity gradient shows a sharp decline, for while in a 0.002 N solution of the acid there was slight germination, the sporangia were badly plasmolyzed in a 0.003 N solution. The ionization constant of the acid at 25° C. is approximately 63 times that of malonic acid, and 1,503.7 times that of succinic acid. Hence, we have another rule that "the nearer the substituent is to the carboxyl group, the greater is its effect on the ionization constant" (14). Moreover, this is also in accord with the general principle that "in a homologous series of organic acids the lower members of the series are the stronger" (5, p. 87-144). However, it should be remembered that the strength of an acid, expressed in terms of its ionization constant, does not necessarily imply a corresponding increase in its toxic action; for in the expression of the latter some other factors, such as the poisonous actions of the anions and the undissociated molecules, are involved. At its critical concentration the acid is practically completely dissociated. Hence, its toxic action at this dilution is due mainly to the effect of the H-ions. A study of the sodium salts showed that the anions do not appreciably influence the total toxicity of the acid.

For direct and indirect germination of the spores, malonic acid appears to exert about the same poisonous action. However, as in the case of oxalic acid, there appears an abrupt rise in the toxic value of the acid for direct germination, for while a 0.004 N solution of the acid allows germination of the sporangia, they show plasmolysis in 0.005 N concentration. The acid at the critical concentrations for direct and indirect germination of the spores is about 40.71 and 42.48 per cent ionized, respectively. Its toxicity to the spores is one-half that of oxalic acid, which exerts its toxic action mainly through the medium of the H-ions. From Table IV it will be seen that the toxic action of malonic acid also is due mostly to the H-ions.
that the solution contains, although the effects of the anions and the undissociated portion on the toxicity have not been investigated.

Succinic acid shows a very peculiar behavior toward the two types of germination. While a 0.002 N solution of the acid inhibits the liberation of zoospores, the production of germ tubes is not prevented until a solution about seven times as concentrated as the one which just inhibits indirect germination is obtained. The remarkable specific toxic action of succinic acid in preventing the formation of zoospores is noteworthy. The experiment was repeated for a number of times at two different periods, and gave essentially similar results; fresh stock solutions of the acid were prepared each time. Considering indirect germination of the spores, the acid is a little more toxic than malonic acid, although the electrolytic dissociation of the latter at its critical concentration is about three times that of succinic acid. Evidently, then, the specific toxicity of succinic acid is due to the poisonous action of the anions or to the undissociated portion.

Of the unsaturated dicarboxylic acids, maleic and fumaric acids were investigated. It has already been noticed in the case of the carboxylic fatty acids that ordinary isomeric acids have very nearly the same electrolytic dissociation; but maleic and fumaric acids present a different case. There is a marked difference between the values of the ionization constant of the two acids, which appears to be the result of the position of two hydroxyl groups with respect to each other. The stereo-chemical influence is thus worthy of note. According to Ostwald's determination, the electrolytic dissociation of maleic and fumaric acids at the dilution 1,024 is 92.8 and 63.9 per cent, respectively. If the toxicity of the two acids were due to the effect of the H-ions in the solutions, one should expect maleic acid to be more toxic than fumaric. This, however, is not the case, since for indirect germination of the spores a 0.0015 N solution of maleic acid exerts the same toxic effect as does a 0.0009 N solution of fumaric acid. The greater toxicity of fumaric acid to the formation of zoospores may be due to the relatively greater poisonous action of the anions or the undissociated portion, or it may be due to the fact that the trans form is physiologically more active than the cis form. A study of the sodium salts will decide as to which of these two explanations is tenable. But when we consider the relative toxicity of the two acids to the production of germ tubes, the suggestion that the trans form may be more toxic than the cis form does not seem to hold. In this case maleic acid (cis form) is slightly more toxic than fumaric. Evidently, then, the explanation has to be sought in the specificity of the action of the acids toward the two types of germination.

It should be pointed out that from a practical chemical view point the variation between the critical concentrations of maleic and fumaric acids is well within the range of experimental error, considering that the organic compounds were not tested for purity or for alkali content of water distilled in glass apparatus. This would tend to show that the toxicity of the two acids is determined chiefly by the H-ions, and that the anions and the undissociated portions have a rather slight influence. Moreover, it should be noted that a highly dissociated acid will not have the same killing effect as an acid solution of the same pH but of lower dissociation will have; for it is almost impossible to keep the acidity of a highly dissociated acid solution constant over a period of time. However, the variation in
the toxic values of the acids for a number of trials is so small that it seems somewhat doubtful whether the criticisms offered have any value.

Passing to the hydroxydicarboxylic acids, we come to malic and tartaric acids. Malic acid exerts a specific inhibitory influence on the formation of zoospores. A 0.002 N solution of the acid prevents the liberation of zoospores, while the production of germ tubes is retarded in 0.006 N solution. According to Ostwald's (15) determination, the electrolytic dissociation of the acid at the dilution 500, which is the critical concentration for indirect germination, is about 36 per cent. It hardly seems possible, therefore, that the toxic action is solely dependent upon the H-ions in the solution. At the critical concentration for direct germination the acid is much less ionized; hence the anions and the undissociated portion may have some effect in determining the total toxicity.

Tartaric acid is not a very strong acid, the concentration inhibiting direct and indirect germination of the spores being 0.006 N. At this concentration the acid is approximately 33 per cent ionized. The extent to which the anions and the undissociated portion influence the toxicity of the acid does not seem to be appreciably great.

Citric acid, a hydroxytricarboxylic acid, exerts a specific toxic action on the formation of zoospores. It inhibits the liberation of zoospores at 0.0025 N, while the production of germ tubes is prevented in 0.008 N solution. According to Jones's (5, p. 87–144) determination, the electrolytic dissociation of the acid at the critical concentrations for direct and indirect germination of the spores is approximately 28 and 38 per cent, respectively. The toxicity of the acid is due mostly to the effect of the H-ions in the solution.

**SUMMARY OF TABLE V**

Fifteen acids from the aromatic series were tested for their toxicity to the sporangia of *Phytophthora colocasiae*. By referring to Table V it will be seen that benzoic acid has a specific toxic action upon the liberation of zoospores. While a 0.0006 N solution of the acid prevents the formation of zoospores, it is only when a 0.002 N concentration is reached that the production of germ tubes is inhibited. In other words, the inhibitory effect of benzoic acid is about 3.3 times more effective in preventing indirect germination than in retarding the production of germ tubes. According to Jones's (5, p. 87–144) determination, benzoic acid at its critical concentrations for direct and indirect germination is about 16.69 and 27.39 per cent ionized, respectively; in other words, the difference between the electrolytic dissociation at the critical concentrations for direct and indirect germination is approximately 11 per cent. Moreover, it will be seen from Table VI the toxic values of the anions and the undissociated molecules in retarding the production of germ tubes are very nearly the same. However, inspection of Tables VI and VII will point out the fact that the value of the undissociated molecules in preventing the liberation of zoospores is more than four times the value calculated for direct germination. Also, the toxic value of the anions in inhibiting indirect germination is about three times that calculated for direct germination. Putting all these facts together, it will be easy of explanation as to how benzoic acid exerts a specific inhibitory action upon indirect germination of the spores.
### Table V.—Toxic effect of aromatic acids on the spores of Phytophthora colocasiae Rac.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of experiments</th>
<th>Number of cultures</th>
<th>Indirect germination</th>
<th>Critical concentration, normal</th>
<th>Lethal concentration, normal</th>
<th>(a) at 10° C.</th>
<th>(H^+ + R^-)</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Monocarboxylic acid:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Saturated—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Benzoic acid</td>
<td>3</td>
<td>28</td>
<td>0.0006</td>
<td>0.0025</td>
<td>16.69</td>
<td>0.00033</td>
<td>0.00167</td>
<td></td>
</tr>
<tr>
<td>b. Substituted acids—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>3</td>
<td>28</td>
<td>0.0002</td>
<td>0.002</td>
<td>28.55</td>
<td>0.00057</td>
<td>0.00143</td>
<td></td>
</tr>
<tr>
<td>(o)-hydroxybenzoic acid</td>
<td>3</td>
<td>26</td>
<td>0.0007</td>
<td>0.0008</td>
<td>67.31</td>
<td>0.00047</td>
<td>0.00229</td>
<td></td>
</tr>
<tr>
<td>(m)-hydroxybenzoic acid</td>
<td>3</td>
<td>26</td>
<td>0.0001</td>
<td>0.002</td>
<td>24.44</td>
<td>0.00024</td>
<td>0.000756</td>
<td></td>
</tr>
<tr>
<td>(p)-hydroxybenzoic acid</td>
<td>3</td>
<td>40</td>
<td>0.0001</td>
<td>0.002</td>
<td>13.50</td>
<td>0.00015</td>
<td>0.000649</td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>5</td>
<td>34</td>
<td>0.0003</td>
<td>0.0007</td>
<td>7.08</td>
<td>0.00045</td>
<td>0.000651</td>
<td></td>
</tr>
<tr>
<td>(o)-nitrobenzoic acid</td>
<td>5</td>
<td>46</td>
<td>0.0009</td>
<td>0.001</td>
<td>84.5</td>
<td>0.00076</td>
<td>0.00014</td>
<td></td>
</tr>
<tr>
<td>(m)-nitrobenzoic acid</td>
<td>3</td>
<td>28</td>
<td>0.0004</td>
<td>0.0005</td>
<td>84.38</td>
<td>0.00026</td>
<td>0.00074</td>
<td></td>
</tr>
<tr>
<td>(p)-nitrobenzoic acid</td>
<td>3</td>
<td>30</td>
<td>0.0003</td>
<td>0.0007</td>
<td>75.75</td>
<td>0.00045</td>
<td>0.000146</td>
<td></td>
</tr>
<tr>
<td>Dinitrobenzoic acid (3,5)</td>
<td>2</td>
<td>24</td>
<td>0.0005</td>
<td>0.0007</td>
<td>20.08</td>
<td>0.0004</td>
<td>0.00056</td>
<td></td>
</tr>
</tbody>
</table>

| II. Unsaturated— | | | | | | | | |
| Cinnamic acid | 4 | 34 | 0.0007 | 0.0008 | 61.09 | 0.00064 | 0.00038 |

| B. Dicarboxylic acids: | | | | | | | | |
| \(o\)-phthalic acid | 3 | 28 | 0.0015 | 0.002 | 64.09 | 0.00024 | 0.00064 |

| C. Amino acids: | | | | | | | | |
| Aspartic acid | 6 | 64 | 0.05 | 0.06 | 0.0008 | 0.0008 | 0.0008 |

| D. Sulphonic acids: | | | | | | | | |
| Sulphanilic acid | 2 | 24 | 0.003 | 0.004 | 36.67 | 0.0007 | 0.0007 |

| E. Tannic acid | 4 | 46 | 0.006 | 0.008 | 7.08 | 0.00045 | 0.000146 |

---

* The value of \(a\) is the percentage of an acid ionized at its critical concentration.

* \(H^+ + R^-\) represents the concentration of the H-ions and the anions at the critical concentration.

* HR represents the concentration of the undissociated molecules at the critical concentration.

* The number in parentheses represents the temperature on the centigrade scale at which the value of \(a\) is determined.

* Molar concentration.
June 1, 1926  
**Toxicity of Organic Compounds to Phytophthora Spores**

**TABLE VI.**—Relative toxic properties of the undissociated molecules as measured by their inhibiting power on the direct germination of the spores of Phytophthora colocasiae Rac.

<table>
<thead>
<tr>
<th>Agent</th>
<th>m-Nitrobenzoic acid</th>
<th>Salicylic acid</th>
<th>Hippuric acid</th>
<th>Benzoic acid</th>
<th>Hydrochloric acid</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.004 N</td>
<td>0.0007 N</td>
<td>0.002 N</td>
<td>0.002 N</td>
<td>0.002 N</td>
<td>0.002 N</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>285</td>
<td>100</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>67.3</td>
<td>28</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>67.3</td>
<td>28</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td>0.66</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>68.3</td>
<td>28.66</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>439</td>
<td>217.7</td>
<td>71.34</td>
<td>83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>32.7</td>
<td>72</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.9</td>
<td>6.65</td>
<td>.99</td>
<td>.98</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Critical concentration.  
Relative toxic value of HCl = 100.  
Per cent ionized at critical concentration.  
Toxic value of cation in terms of ionic H⁺.  
Toxic value of anion in terms of ionic H⁺.  
Total, cation + anion.  
Residual units.  
Per cent un-ionized.  
Value of un-ionized molecules in terms of ionic H⁺.

Hippuric acid, like benzoic acid, is relatively weakly dissociated. For direct and indirect germination the acid has the same critical concentration, viz, 0.002 N. Its electrolytic dissociation at this concentration is approximately 28 per cent, while at the same dilution benzoic acid is only about 16 per cent ionized but exerts the same toxic effect. It will be seen from Table VI that the relative toxic value of the anions of benzoic acid for direct germination is about one and one-half times that for hippuric acid, which, however, has the same toxic value of the undissociated molecules as benzoic acid. It therefore follows that the slight toxicity of benzoic acid due to low degree of electrolytic dissociation is so supplemented by the specific toxic action of the anions that the resultant of these factors gives a toxic value equal to that of hippuric acid, in the case of which the concentration of the H-ions make up any deficiency in toxicity due to relatively lower toxic value of the anions. With regard to indirect germination, the electrolytic dissociation of the two acids at their critical concentrations is approximately the same, although the relative toxic values of the anions and the undissociated portion of benzoic acid are greater than those of hippuric acid.

Turning to the substituted benzoic acids, we first come to the hydroxybenzoic acids. The three hydroxybenzoic acids differ greatly in regard to their toxicity to the spores. It will be seen from Table V that o-hydroxybenzoic acid (salicylic acid) prevents the liberation of zoospores in 0.0006 N solution, while m- and p-hydroxybenzoic acids exert the same effect at 0.0009 N and 0.001 N, respectively.
In the case of direct germination the order of toxicity is essentially the same, except that \( m- \) and \( p- \) hydroxybenzoic acids have the same critical concentration, viz, 0.001 N. The introduction of a hydroxyl group in benzoic acid and its position relative to the carboxyl group have a marked influence on the ionization constant of these acids. For instance, the substitution of the hydroxyl group in the position ortho to the carboxyl group in benzoic acid increases the constant of the acid 17 times, while the constant of \( m- \) hydroxybenzoic acid is only one and one-half times as great as that of benzoic acid. The case of \( p- \) hydroxybenzoic acid is quite peculiar; its dissociation constant, instead of increasing, decreases to one-half that of benzoic acid. The physiological action of the acids must be proportional to the degree of their electrolytic dissociation, if the toxicity of these acids is, to a large extent, determined by the effect of the H-ions. This is true, since the order of the toxicity of the acids is ortho, meta, and para. Since the sodium salts of the hydroxybenzoic acids, except sodium \( o- \) hydroxybenzoate, were not available, there was no means of comparing the relative toxic values of the anions and the undissociated portion. However, a glance at Tables VI and VII will show the relative toxic values of the anions and the undissociated molecules of \( o- \) hydroxybenzoic acid for direct and indirect germination.

Gallic acid, a trihydroxybenzoic acid, has an interest of its own. The introduction of three hydroxyl groups in benzoic acid, instead of increasing, decreases the constant of the acid to a little over one-half that of benzoic acid; but considering direct germination of the spores, the toxicity of gallic acid is one-third that of benzoic acid. The concentration of gallic acid which prevents the liberation of zoospores is 0.003 N, while the production of germ tubes is not inhibited until a 0.007 N concentration is reached, the latter concentration being twice the critical concentration for indirect germination. Evidently, then, gallic acid, like benzoic acid, exerts a specific toxic action on the formation of zoospores, the concentration of the acid preventing germination by the production of hyphae being twice that which exerts the same effect on indirect germination.

The other interesting group of the substituted benzoic acids is that of nitrobenzoic acids. By referring to Table V it will be seen that the order of the toxicity of these acids is not the same as that of the hydroxybenzoic acids. For the substitution of a nitro group in the position para to the hydroxyl group in benzoic acid increases the constant of the acid 100 times, while the constant of \( m- \) nitrobenzoic acid is 16 times that of benzoic acid, and that of \( p- \) nitrobenzoic acid about 6.6 times that of the latter. Thus we arrive at another general rule: “The relation between the dissociation constant of the para acid and that of ortho or meta acid varies with the nature of the substituent” (14). In other words, \( p- \) nitrobenzoic acid is about three times more ionized at the dilution 1,024 than \( p- \) hydroxybenzoic acid, for the nature of the nitro group is quite different from that of hydroxy. The critical concentrations of the three nitrobenzoic acids do not differ in regard to direct and indirect germination; that is, for each acid the concentration at which the liberation of zoospores is inhibited is the same as that which prevents the production of germ tubes. Considering indirect germination of the spores, \( o- \) nitrobenzoic acid retards germination in 0.0009 N solution, while \( m- \) and
p-nitrobenzoic acids exert the same effect at 0.0004 N and 0.0006 N, respectively. Since the order of the poisonous action of these acids does not correspond with the degree of their electrolytic dissociation, it follows that the effect of the H-ions alone is not responsible for the total toxicity, but the anions and the undissociated portion have a toxic action of their own. It was not possible to determine the extent to which the anions and the undissociated portion contribute to the toxicity of their respective acids, since the sodium salts of the two nitrobenzoic acids were not available for experimentation. Tables VI and VII will, however, show the relative toxic values of the anions and the undissociated portion of m-nitrobenzoic acid. It will be seen that the relative toxic value of the undissociated molecules of this acid is the greatest as compared with those of other acids in the table.

For direct and indirect germination of the spores, the critical concentration of 1-, 3-, 5-dinitrobenzoic acid is 0.0006 N. At this concentration the acid is about 75 per cent ionized, according to Jones’s (5, p. 87-144) determination (by interpolation). Evidently the toxic action is determined chiefly by the effect of the H-ions at this dilution. Since the sodium salt of the acid was not available, no data as to the extent to which the anions and the undissociated molecules influence its toxicity are at hand.

Cinnamic acid is the best-known unsaturated acid of the aromatic series. At a concentration of 0.0007 N of the acid, there is inhibition of direct and indirect germination of the spores. According to Jones’s (5, p. 87-144) determination, the electrolytic dissociation of the acid at its critical concentration is approximately 20 per cent. Hence the toxic effect of the H-ions is, indeed, not very great, since only one-fifth of the acid is ionized at these concentrations. Obviously, then, an explanation of the toxic action of the acid has to be sought in the poisonous action of the anions and the undissociated portion. It is also possible that the presence of an ethylenic linkage and a conjugated system in the acid may have to do materially in increasing its toxic action.

Phthalic acid is a dicarboxylic acid, and, according to Jones (5, p. 87-144), is about 65 per cent ionized at its critical concentrations. The introduction of a second carboxyl group has increased the strength of the acid, for its dissociation constant is 18.33 times that of benzoic acid. However, an increase in the degree of electrolytic dissociation of the acid has not enhanced its toxic action, since it inhibits germination of the spores at 0.001 N solution.

Aspartic acid is a monamino-dicarboxylic acid. The dissociation of the acid is not given in Ostwald’s (15) or Jones’s (5, p. 87-144) tables. The acid inhibits the liberation of zoospores in 0.004 N solution, while the production of germ tubes is not retarded until a 0.05 N concentration is reached; in other words, like benzoic acid, this acid shows a specific inhibitory action to the formation of zoospores. It will be of interest to know the extent to which the anions and the undissociated molecules are responsible for the toxicity of the acid. The electrolytic dissociation of the acid at its critical concentrations is certainly not very great.

For direct and indirect germination of the spores, sulphanilic acid has critical concentrations at 0.002 N and 0.003 N, respectively. According to Jones’s (5, p. 87-144) determination, the electrolytic dissociation of the acid at its critical concentrations is approximately
36 per cent. Whether the poisonous action at these concentrations is due entirely to the effect of the H-ions or partly to that of the anions and the undissociated molecules could not be determined, as the sodium salt of the acid was not available for experimentation. Sulphanilic acid, however, is not a very strong acid, for the presence of an amino group lowers its strength.

For indirect germination of the spores, tannic acid has a critical concentration at 0.0005 M, while the production of germ tubes is not retarded until 0.006 M concentration of the acid is reached. Thus tannic acid exerts a specific inhibitory action against the formation of zoospores.

DISCUSSION

ALCOHOLS

Very few cases are known in which the physiological action of organic compounds can be predicted from a knowledge of their chemical constitution. This is probably due to the complex nature of many such substances, which makes any prediction as to their physiological action a rather difficult matter. Instances are known, however, of simple substances whose physiological effects can be foretold by their chemical and physical properties. A case of this kind is furnished by the so-called Richardson's law, which states that the toxicity of the normal aliphatic alcohols increases in proportion to their molecular weight.

Kamm (7) has recently brought forward facts to show that the physiological effects of the members of the normal aliphatic alcohols also admit of mathematical interpretation, which is a real contribution to our knowledge of the physiology of these compounds. For instance, he has found that in a homologous series of aliphatic alcohols the toxicity of a member was three times that of the preceding one. On this basis he has developed a mathematical relationship for determining the toxicity of any member in the series, having been given the toxicity of ethyl alcohol. Using his formula and expressing the toxicity of ethyl alcohol as 6.3, as found for the sporangia of Phytophthora colocasiae, the following figures are obtained for normal propyl and normal butyl alcohols:

(a) For normal propyl alcohol:

\[
1 \times 3 = 3
\]

\[
\frac{6.3 \times 1.3}{3} = 2.73 \text{ (uncorrected for specific gravity)}.
\]

(b) For normal butyl alcohol:

\[
1 \times 3 \times 3 = 9
\]

\[
\frac{6.3 \times 1.6}{9} = 1.12 \text{ (uncorrected for specific gravity)}.
\]

By reference to Table I, it will be seen that there is a very close correspondence between the values calculated by the above method and those experimentally determined for Phytophthora colocasiae. For instance, normal propyl alcohol was found to prevent the formation of zoospores in a 3.1 per cent solution, which compares
favorably with the calculated value of 2.73. Normal butyl alcohol has experimental and calculated values of toxicity of 1.5 and 1.2 per cent, respectively. However, it should be remembered that the calculated values are not corrected for the specific gravity of the corresponding alcohols, which may explain the slight difference between the experimental and the calculated values.

It has been found, as shown by various investigators, that for bacteria and mammals the toxicity of the normal fatty alcohols increases as the homologous series is ascended; that is, the alcohols having higher boiling point, specific gravity, and heat of combustion, and lower solubility have the greater toxic value. In other words the phenomenon of toxicity is a function of the chemical and physical properties of these substances.

According to the writer’s results, methyl alcohol has not proved an exception to the general rule that the toxicity of the normal aliphatic alcohols increases with molecular weight. These results are in agreement with the findings of Vernon (20), Macht (11), and Kuno (8), in the case of mammals; and Stadler (18), in the case of bacteria. These results lead the writer to conclude with Vandeveldt (19) and Förster (4) that any failure of methyl alcohol to behave in accordance with the rule of increasing molecular weight is due to impurities in the alcohol. Moreover, Macht (11) has pointed out that the toxic effects of methyl alcohol, when taken by mouth into the alimentary canal, are due to its decomposition products, especially formic acid and formaldehyde. However, such conditions are not obtained in a drop of methyl alcohol in which fungous or bacterial spores are sown, for methyl alcohol, under these conditions, does not change into formic acid or formaldehyde, with the result that the toxicity of the drop is not altered. Therefore, methyl alcohol as such is not more toxic to the spores than ethyl alcohol.

The isomeric alcohols were found to be less toxic than the corresponding normal alcohols, thus showing that a change in the structural constitution of an alcohol was accompanied by a change in its toxicity, although the molecular weight remained the same. In the case of butyl alcohols, Overton (16, p. 101,) has shown that of isomeric alcohols those having the least-branched carbon chain have the strongest narcotic action, while those with the most-branched carbon chain have the weakest action. However, Vernon (20) found, in the case of the depressant action of alcohols on the heart beats of the frog, that the behavior of secondary butyl alcohol was contrary to the rule laid down by Overton for the toxicity of isomeric butyl alcohols. The writer's results are in agreement with those of Vernon, and show that Overton’s rule does not hold good for indirect germination of the sporangia of Phytophthora colocasiae.

The high toxic value of allyl alcohol may be due to the allyl radical that it contains. The presence of a double bond has also, no doubt, an accelerating influence on the toxicity of the alcohol. Glycerol and ethylene glycol, being the lowest members of the homologous series, are not very toxic. The toxicity of benzyl alcohol is probably determined by the benzene nucleus, while that of furfuralcohol may be due to the unsaturated oxygen atom in the furyl radical.

The close approximation of the results obtained in these investigations with those of other workers emphasizes the important fact that the relative physiological action of alcohols is the same for living
matter—amphibians, mammals, higher plants, bacteria, and fungi. However, it is very doubtful whether alcohols have any desirable features as fungicides, for their high cost and comparatively low toxicity will prohibit their use in any commercial work. Allyl alcohol, however, is about as toxic to the spores as copper sulphate, but it is many times more expensive than the latter. Its strong, irritant odor is also a disagreeable feature.

**ALDEHYDES**

The results of tests on the toxicity of aldehydes show that the toxic properties of aldehydes are due mostly to the aldehyde group which they contain, and that the aromatic or the aliphatic nature of a radical, to which the aldehyde group is united, does not seem to influence the toxicity of the compounds to any marked degree. For example, normal butyraldehyde which contains a propyl (C₉H₂₃) radical directly united to an aldehyde group, in place of a furyl (C₄H₃O) radical as in furfuraldehyde, shows about the same toxicity as the latter. Indeed, it was surprising to find that the unsaturated oxygen atom in the closed chain of furfural did not have any accelerating effect on the toxicity of the compound. Therefore, it is safe to assume that the furyl and the alkyl radicals are equally toxic, if at all. However, these radicals are so different in their chemical properties that their physiological action would hardly be similar, with the result that the aldehydes should show different toxic values. This is, however, contrary to the experimental data, which show that all aldehydes containing one aldehyde group, except the substituted benzaldehydes and formaldehyde, were equally toxic to the spores. It follows, therefore, that the toxic action due to the alkyl radicals must be negligible. It has also been shown that the toxicity of the aliphatic aldehydes, unlike that of the aliphatic alcohols, does not increase with molecular weight.

The seemingly erratic results with formaldehyde need not surprise us at all, for it corresponds with anomalies observed in regard to its chemical and physical properties. For instance, it is known that formic acid, the first member of the fatty acid series, differs markedly in some of its reactions from acetic acid and other members of the series; the same relations have been observed in the first members of other homologous series. Hence, the reactions of formaldehyde are not as representative of the group as are those of acetaldehyde. It is therefore not surprising to find that its toxicity to the spores is different from that of other aldehydes in the series. Moreover, it is also possible that the presence of a hydrogen atom in formaldehyde, which is very sensitive to oxygen, may have an accelerating influence on its toxicity.

The results of tests on the toxicity of glyoxal are very instructive. Glyoxal contains two aldehyde groups without having any alkyl radical united to them. Evidently, then, the toxicity of this compound must be an expression of the physiological action of the aldehyde groups, which is not influenced by any other group or radical in the molecule. Considering that the poisonous action of glyoxal is an expression of the toxic value of its two aldehyde groups, it may be expected that the toxicity of such aldehydes as contain only one aldehyde group must be about one-half that of glyoxal.
However, glyoxal is about 10 times as toxic as any of the aliphatic aldehydes containing only one aldehyde group, excepting formaldehyde. This would seem to indicate that the presence of an alkyl radical, in fact any radical, in aldehydes, instead of increasing, diminishes their physiological action.

Moore (12) generalized that "up to 250° C. the higher the boiling point the more toxic the compound to insects." This, however, does not apply to germination of the sporangia of Phytophthora colocasiae. For glyoxal, which has a boiling point (50°), is the most toxic of the aldehydes used in these experiments, excepting formaldehyde and the substituted benzaldehydes, whereas benzaldehyde with a boiling point of 179.5° is much less toxic than glyoxal. Moreover, Moore thought that the lessened toxicity of furfural was due to its low boiling point, 90°. The furfural used by the writer in these experiments had a boiling point of 160°. However, acetaldehyde, which has a boiling point about one-sixth as high as that of furfural, showed much the same toxicity to the spores as did the latter.

The substituted benzaldehydes show a decided increase in toxicity over that of benzaldehyde. The introduction of the nitro and the hydroxyl groups into the benzene nucleus of benzaldehyde increases the toxicity of the compound to a marked degree. In the two nitrobenzaldehydes investigated, the position of the substituent with respect to the aldehyde group does not make any difference in their physiological action. However, the substitution of a hydroxyl group in the position para to the aldehyde group in benzaldehyde increases the toxicity of the compound over that of \( p \)-nitrobenzaldehyde. The substitution of two hydroxyl groups does not result in any further increase in toxicity, because protocatechuic aldehyde and \( p \)-hydroxybenzaldehyde are about equally toxic. Whether the increase in toxicity of the substituted benzaldehydes is due to the position of the substituent with respect to the aldehyde group or to the nature of the substituent, could not be definitely determined, since \( o \)- and \( m \)-hydroxybenzaldehydes did not lend themselves to experimentation, being very slightly soluble in water. However, it will be seen from Table II that protocatechuic aldehyde and \( p \)-hydroxybenzaldehyde are more toxic than \( m \)- or \( o \)-nitrobenzaldehyde. It seems, therefore, that the substitution of a hydroxyl group in benzaldehyde increases its toxicity more than does that of a nitro group.

**ACIDS**

The results on the toxicity of organic acids to the sporangia of Phytophthora colocasiae are not in agreement with those of Kahlenberg and True (6), who investigated the toxicity of a number of organic and inorganic compounds to the seedlings of Lupinus albus L. All the acids used in these present experiments were found to be toxic to the spores at concentrations higher than those which were found by Kahlenberg and True to kill the lupines. However, it should be noted that, with some exceptions, the order of the toxicity of most of the acids investigated corresponds with that determined for the seedlings of L. albus L. If the acids used by Kahlenberg and True were pure chemically, the differential behavior of the compounds is of great significance in their use as fungicides when applied on the host.
It should be pointed out that the organic compounds used in this research were not tested for purity in the laboratory, and all conclusions are based on the assumption that they were pure chemically. Every effort was made to obtain chemicals of the highest purity, yet they could not be compared for purity with chemicals prepared in the laboratory for experimentation. However, in biological work, the variation in results obtained with compounds of “C. P.” purity and of "absolute" purity is so small that the general conclusions of an experiment would not be affected appreciably.

**SUMMARY**

The results on the toxicity of the aliphatic alcohols substantiate Richardson's law, which states that the toxicity of the normal aliphatic alcohols increases with molecular weight.

The isomeric aliphatic alcohols were found to be less toxic than the corresponding normal alcohols. Normal butyl, isobutyl, secondary butyl, and tertiary butyl alcohols were decreasingly toxic in the order mentioned.

Allyl alcohol was the most toxic of the alcohols investigated, being 12,600 times more toxic than ethyl alcohol, which was toxic at a concentration of 6.3 per cent. The high toxic value of allyl alcohol is due probably to the allyl radical. The presence of a double bond has also an accelerating influence on its toxicity.

Ethylene glycol and glycerol were not very toxic, being the lowest members of the homologous series.

The toxicity of benzyl alcohol and furfuralcohol was 31 times greater than that of ethyl alcohol. The toxicity of benzyl alcohol is due probably to the benzene nucleus, while that of furfuralcohol may be due to the presence of the unsaturated oxygen atom in the furyl radical.

The toxicity of aldehydes is due mostly to the aldehyde group which they contain, and the aromatic or the aliphatic nature of a radical to which the aldehyde group is united seems to have very little influence on their toxicity.

Unlike the normal aliphatic alcohols, the toxicity of aldehydes did not increase as the homologous series was ascended.

The increased toxicity of formaldehyde corresponded with anomalies observed in regard to its chemical and physical properties. It is possible that the presence of a hydrogen atom in formaldehyde which is very sensitive to oxygen may have an accelerating influence on its toxicity.

The toxicity of glyoxal is an expression of the toxic value of the two aldehyde groups which it contains. It was much more toxic than any other aldehyde investigated, excepting formaldehyde and the substituted benzaldehydes.

The substitution of a nitro or a hydroxyl group in benzaldehyde increased its toxicity many times. The substitution of two hydroxyl groups did not result in any further increase in toxicity. The hydroxybenzaldehydes were more toxic than the nitrobenzaldehydes.

The results on the toxicity of organic acids signify that the electrolytic dissociation of an organic acid is not always an index of its physiological action, for in these acids the undissociated molecules and the anions had a distinct toxic action of their own.
Disregarding the toxic action of the undissociated portion and the anions, in a homologous series of organic acids the lower members of the series were stronger. In general, the ordinary isomeric acids had very nearly the same toxicity.

The introduction of a halogen into acetic acid enormously increased its dissociation, and consequently its physiological action, though not to the same extent. The three chloro-acetic acids were found to be equally toxic. The high toxic value of monobromacetic acid is probably due to the action of the anions.

The introduction of a hydroxyl group into acetic and propionic acids increased their electrical conductivity, with a consequent increase in their toxic action.

"The nearer the substituent is to the carboxyl group, the greater is its effect on ionization constant." However, this was not true with respect to the physiological action of oxalic, malonic, and succinic acids, although their electrolytic dissociation is governed in accordance with the above formula.

The stereoisomerism, as illustrated by maleic and fumaric acids, had a marked influence on the physiological action of these acids. Fumaric acid (trans form), which has a lower dissociation than maleic acid (cis form), was more toxic than the latter.

The nature of a substituent in an aromatic acid and its position relative to the carboxyl group generally determine the toxic action of the acid. The nitrobenzoic acids differed in toxicity from the hydroxybenzoic. The order of the toxicity of the hydroxybenzoic acids was ortho, meta, and para, whereas for the nitrobenzoic acids it was meta, para, and ortho.

A hydroxyl or a nitro group in benzoic acid increased the dissociation constant of the acid, with a consequent increase in toxicity. However, in the case of indirect germination, an increase in the dissociation constant of the acid was not always accompanied by an increase in its toxicity. The introduction of three hydroxyl groups in benzoic acid, instead of increasing, decreased its dissociation constant, and consequently its toxicity.

A double bond led to an increased physiological activity of the acid, as in cinnamic acid. It is possible that a conjugated system also accelerated its toxicity.

The introduction of a second carboxyl group into benzoic acid increased its dissociation, but not its toxicity, as noted with o-phththalic acid.

The presence of an amino group lowered the toxicity of sulphanilic acid.

Benzoic, succinic, and tannic acids showed specific inhibitory action against the formation of zoospores in the sporangia of Phytophthora colocasiae Rac.

LITERATURE CITED

(1) Carlier, E. W.

(2) ———
(3) Clark, J. F.

(4) Förster, R.

(5) Jones, H. C.

(6) Kahlenberg, L., and True, R. H.

(7) Kamm, O.

(8) Kuno, Y.

(9) Loeb, O.

(10) McGuigan, H.

(11) Macht, D. I.

(12) Moore, W.

(13) Morgan, G. T., and Cooper, E. A.

(14) Norris, J. F.

(15) Ostwald, W.

(16) Overton, E.

(17) Ruediger, E. H.

(18) Stadler, H.

(19) Vandevenelle, A. J. J.

(20) Vernon, H. M.
Toxicity of Organic Compounds to Phytophthora Spores


