THE ISOLATION AND CHEMICAL STUDY OF COMPOUNDS

PRODUCED BY A STRAIN OF

PENICILLIUM NOTATUM

APPROVED:

Price Truitt
Major Professor

G.H. Biddle
Minor Professor

James L. Carius
Director of the Department of Chemistry

Joes Johnson
Chairman of the Graduate Council
THE ISOLATION AND CHEMICAL STUDY OF COMPOUNDS
PRODUCED BY A STRAIN OF
PENICILLIUM NOTATUM

THESIS

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By

James Hollis Jones, B.S.

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CHAPTER I

INTRODUCTION AND HISTORICAL ASPECTS

Introduction to Problem

The scientist has always been confronted by molds, yeasts and Ag-
tinomycetes (or Fungi). Many of these are but contaminants in the study
and culture of bacteria, but an increasing number of fungi are found to
be of equal importance with bacteria in the organic products of industry.
Fungi (a part of the subdivision of the plant kingdom known as
Thallophyta) are found not only to be the cause of disease processes in
man and animals, but also to be used in the treatment of certain infec-
tions. That the growth of certain bacteria could be stopped or prevented
by the accompanying growth of another was observed as long ago as 1877.
Pasteur and Joubert suggested this phenomenon of antibiotics might be of
use in treatment of disease.¹ This inhibition of growth is now known to
be due to the products of certain micro-organisms; these products possess
definite chemical and biologic properties. Recently the term
"antibiotic" has been applied to these products with either inhibitory or
lethal properties.

A mold discovered growing on Porto Rico sweet potatoes in the
Gilbert C. Wilson Laboratories, located in Pittsburg, Texas, was trans-
ported to North Texas State College in 1944 by win A. Isaacs and
J.K.G. Silvey. This particular mold was cultivated and found to produce

¹ Pasteur and Joubert as quoted by W.E. Herrell, Penicillin and
Other Antibiotic Agents, p. 1.
substances with antibiotic properties as shown in Table 1. It is the purpose of this paper to present data on the isolation and study of the chemical nature of compounds produced by this strain of mold which resembles *Penicillium notatum* Nestling. 3

**Table 1**

**ANTIBIOTIC ACTIVITY OF MOLD CULTIVATED AT NORTH TEXAS STATE COLLEGE**

<table>
<thead>
<tr>
<th>Organisms inhibited</th>
<th>Organisms not inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Aerobacter aerogenes</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>Torula sp.</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td></td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td></td>
</tr>
<tr>
<td>Bacterium radiobacter</td>
<td></td>
</tr>
<tr>
<td>Acetobacter suboxydans</td>
<td></td>
</tr>
<tr>
<td>Clostridium pasteurianum</td>
<td></td>
</tr>
<tr>
<td>Clostridium butylicum</td>
<td></td>
</tr>
<tr>
<td>Clostridium beijerinckii</td>
<td></td>
</tr>
<tr>
<td>Bacillus butylicus</td>
<td></td>
</tr>
</tbody>
</table>

Procedure: Sample sterilized by passage through Berkefeld candle. Inhibitory activity tested at level of 2 mg. pigment per ml. in yeast extract-peptone-glucose broth.

*Source: Merck and Company, Incorporated, Research Laboratory, Rahway, New Jersey, private communication.

Historical Aspects of Mold Activity

In order to clarify the background of the problem, some historical aspects of mold activity will be noted, along with the use of mold products in industry and some of the characteristics of mold.

Molds are a variety of fungi, belonging mainly to two classes, *Fungi imperfecti* (or Ascomycetes) and *Fungi imperfecti* (or Ascomycetes). They are all heterotrophic.

2 J.K.G. Silvey, Biology Department, North Texas State College, Denton, Texas, personal research files.

5 K.L. Raper, Northern Regional Research Laboratory, United States Department of Agriculture, Peoria 5, Illinois, private communication.
organizes, some of which are parasitic to plants and animals, while others are widely distributed, especially in soil. Molds are seldom capable of fermentation, but if so, fermentative activity is very slight. Consequently, the products formed by mold activity differ from those formed by bacterial and yeast fermentation. The usual acids produced are often polybasic, for instance, oxalic, citric, gluconic, and gallic acid. Molds prefer slightly acidic media and can tolerate much higher acidities than bacteria.

Molds are of economic importance because of their enzymes and toxines. They bring about chemical reactions of value to man and are therefore important as industrial fermentations. The enzymes of molds are specific for the species and may be utilized to a certain extent for identification. In addition some of the enzymes of mold are used commercially.

The action of molds has long been made use of in Oriental countries. Koji acid was first isolated in 1807 from cultures of *Aspergillus oryzae* and was used in Japan to ferment rice to produce sake, an alcoholic beverage. It was observed as a metabolic product of other members of the *Aspergillus* genus and by one species of *Penicillium*, *P. dodecar*. The enzyme Takediastase, prepared from *Aspergillus oryzae*, has been produced commercially in the United States as a substitute for malt in hydrolizing starch and is used for the treatment of certain types of indigestion. The proteolytic action of this same mold has been made use of in the preparation of partially digested foods from soy beans in Japan, and in making the dark brown soy bean sauce associated with Chinese food.

---

5 A.t. Henrici, *Nobus Yeasts and Actinomycetes*, p. 44.
8 Henrici, *op. cit.*, p. 45.
Gallic acid and citric acid were first observed as products of molds in 1883. Their formation from sugar is a common characteristic of many species of *Penicillium* and *Aspergillus*. The industrial microbiological production of citric acid from molasses started in the United States in 1926 and developed so rapidly that this country soon became independent of outside sources and even exported sizable quantities.

Gluconic acid is produced by a variety of molds, usually along with citric and oxalic acids. Reduction of phosphate and nitrate salts in the media favors the formation of gluconic acid; research on this reaction indicates the process has commercial possibilities. Calcium gluconate is used as a means of administering calcium to children and in the treatment of cows suffering from milk fever.

Gallic acid is produced commercially by the action of molds on a complex group of substances, gallotannins, occurring in plants. This reaction was reported in 1867 by Sneath. Later investigators have shown that various strains of *Aspergillus* and *Penicillium* produce an enzyme, tannase, which can be freed from mold cells and used to produce gallic acid.

The activity of certain species of *Penicillium* is used in the ripening of Camembert and Roquefort cheeses. The characteristic appearance of Roquefort cheese is due to the presence of a blue-green mold, *Penicillium roqueforti*, and the flavor to the formation of caproic, caprylic, and capric acid liberated from the butter fats by the lipases of the mold.

Many molds are vividly colored due to characteristic pigments, the majority of which are derivatives of toluquinone and of 1-methylanthraquinone.
Too mold pigments have been characterized as xanthones. In certain species of mold, large amounts of water are taken from the medium and then given off into the air, or moisture may condense in droplets on the surface of the plant. These droplets are frequently colored by pigments excreted by the mold.

As could be assumed, the characteristics of the species of mold, such as the color, are helpful in the identification along with the determination of the individual chemical and biological properties of the mold. New molds and mold products are continually being investigated for their possible value in chemotherapy and the industrial world.

The Chemical Nature of Antibiotic Substances

Penicillin is an antibiotic of great value in chemotherapy; it has been developed in the last few years from a mold belonging to the genus Penicillium. Alexander Fleming reported the discovery of penicillin in 1929, then the original mold was identified as Penicillium notatum by Charles Thom, mycologist in the U.S. Department of Agriculture. The possible usefulness of penicillin in the chemotherapy field was discovered in 1940 by a group of investigators at Oxford, England, led by Howard W. Florey.

It is the chemical nature of various antibiotics which is of interest in this paper for comparison with the chemical nature of the antibiotic produced by the mold secured from decaying Porto Rico sweet potatoes. Penicillin is a logical choice as an example because the mold from the sweet potato has been identified by Kenneth B. Raper as being similar to strain NRRL 653. A 26 of Penicillium notatum by.ling. Therefore some brief statements of chemical data will be presented.

13 Ibid., p. 468. 14 Henrik, op. cit., p. 129.
15 Herrell, op. cit., p. 2-4 16 Raper, op. cit.
Chemical data of penicillin were obtained in 1843 by Abraham and Chain using a preparation of the barium salt of penicillin containing approximately 500 units per milligram. Analysis of barium penicillin revealed 45.6 per cent carbon, 4.85 per cent hydrogen, and about 29 per cent barium with apparently no phosphorus or sulfur present. Nitrogen analysis gave inconsistent results. Traces of pyridine could be detected through sulfuric acid hydrolysis on some penicillin fractions, and under vigorous conditions both Kjeldahl and Dumas determinations yielded identical value of 4.15 per cent. These data suggested either the formula $C_{25}H_{32}O_9$ Ba or $C_{24}H_{32}N_2O_{11}$ Ba.

Penicillin is an extremely hygroscopic substance, freely soluble in water and in organic solvents. Acid, alkali and 100° centigrade cause rapid inactivation of aqueous solutions. Penicillin is a strong dibasic acid with the two dissociation constants nearly identical ($pK=2.4$ and 5.5). At relatively high pH a new acid group is formed with $pK=7.8$.

Basing their suggestion on their various analytical results, Mayer Chaffee, Cobb, Brosson, Schwend, and Fleischer (1842) proposed the empirical formula $C_{14}H_{17}NO_{6}$, possibly containing one mole of bound water. In 1843 more research revealed that after dilute sulfuric acid hydrolysis of penicillin, a deep blue-violet color with mihyacin is obtained (Abraham, Chain, Baker, Robinson).

These data relating to the chemical constitution of penicillin are far from a complete coverage of the field. Only those points which might be of interest here have been noted, so as to avoid possible confusion.

For the same reason only a few other antibiotics with their chemical nature will be discussed.

---

18 Ibid., pp. 581-586.
Penicillin is one of the antibiotics with a comparatively simple structure. This antibiotic was first described in 1928 by Fieser and Bliss as a metabolic product of Penicillium notatum. Its structure was established in 1938 (Birkenshaw, Oxford, and Reistick). In 1947 Oxford, Reistick, and Smith suggested that the keto-acid probably exists in tautomeric equilibrium with the unsaturated lactone:

\[
\begin{align*}
  &\text{CH}_3 \\
  &\text{CH}_2
\end{align*}
\]

Tautomeric forms of penicillin acid

Spinulosin and fumigatin are closely related structurally and are both derivatives of toluquinone. These antibiotics were isolated earlier but their structures were not established until 1938 (Anslow and Reistick).

Spinulosin

Fumigatin

The introduction of a second hydroxyl group into the fumigatin molecule results in a compound of decreased activity.

Clavacin has been termed clavatin, claviformin, and patulin. The structure of this antibiotic was summarized by Reistick, Birkenshaw, Michael, Bracken, and Hopkins (1943). Clavacin is a white crystalline compound obtained from Penicillium atatum, at 110 °C in a water solution. It is a strong reducing agent, optically inactive, and forms a 2:4 dinitrophenylhydrazone. It is a neutral substance, but is irreversibly inactivated by alkali. The established empirical formula is C₆H₈O₆.
The graphic formula is:

![Citrinin structure](image)

Citrinin is a yellow crystalline solid, melting at 170-171° centigrade, which gives an intense brown color with ferric chloride. Citrinin is precipitated upon acidification of the metabolite solution of Penicillium citrinum, which shows a relatively low antibacterial activity (Baist and Smith, 1941). There are two existing acid groups, pK 3.7 and 9.2. The empirical formula is \( C_{13}H_{14}O_5 \). The graphic formula is:

![Citrinin structure](image)

Citrinin has also been isolated from cultures of Aspergillus candidus (Thomson, 1942). Particular interest in this antibiotic lies in the fact that high yields are readily obtained and because purification is very simple. Its stability to autoclaving is also an important factor.

As was stated previously, only the chemical nature of these few antibiotics is to be discussed briefly in the hope that clarification of possible reactions may be achieved, rather than confusion through the presentation of an overpowering number of facts.
Chapter II

MOLD CULTURE AND MEDIA

The culture of mold derived originally from the mold growth on decaying Porto Rico sweet potatoes is more easily handled for purposes of experimentation when grown in liquid media. The medium found to be most adaptable to the surface culture of the mold is composed as follows:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown sugar</td>
<td>40.0 grams</td>
</tr>
<tr>
<td>Sodium Nitrate</td>
<td>8.0 grams</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.055 grams</td>
</tr>
<tr>
<td>Mono basic potassium phosphate</td>
<td>0.2 grams</td>
</tr>
<tr>
<td>Zinc sulphate</td>
<td>0.025 grams</td>
</tr>
<tr>
<td>Distilled water, added to make 1 liter of solution...</td>
<td>1000 milliliters</td>
</tr>
<tr>
<td>Certoos (commercial baby food)</td>
<td></td>
</tr>
<tr>
<td>Ortho phosphoric acid, added to bring pH to 5.5-6...</td>
<td></td>
</tr>
</tbody>
</table>

Seven hundred and fifty milliliters of the medium were placed in a small-necked gallon jug. The jug was stoppered with cotton, then autoclaved at fifteen pounds pressure for thirty minutes. After the sterilized medium was cooled, 5 milliliters of the mold spore suspension were added to the jug and shaken to disperse the mold spores throughout the medium. The seeded medium was stored in a room where the temperature was held at approximately 75° Fahrenheit.

Three or four days after seeding the mold growth was visible on the surface of the medium and developed rapidly for twenty-one to twenty-five

Silvey, op. cit.
days. During the first ten to twelve days the coloration of the growth changed from pale to dark green while the growth itself became condensed into a mat completely covering the surface of the medium. As the culture aged, the mat grew thicker and darker in color. The surface of the mat was speckled with droplets of yellow liquid which became fluorescent under ultraviolet light. After twenty-five days of growth the yellow droplets were not noticeable, instead the mat was very dark and splotched or daubed with red. Upon further aging the mat began to shrink in size with the red coloration increasing in area and in depth of color.

In determination by titer, the antibiotic power of the material was found to be at its peak within twenty-one to twenty-five days of growth. The mat was filtered off about this time, leaving the filtrate containing the active secretion. Ten milliliters of the filtered medium were placed in 100 milliliters of sterile water to prepare the solution used in finding the antibacterial titer.

In order to determine the antibiotic properties of this particular whole secretion can be compared with those of other antibiotics, its titer was determined by its effect on a stock culture of *Staphylococcus aureus* B. For the antibacterial titer 0.5 milliliter of twenty-four hour *Staphylococcus aureus* B culture was placed in 100 milliliters of sterile water, thus making the stock culture solution. Of this stock solution 0.1 milliliter was seeded in a test tube containing 10 milliliters of sterile broth, and this was the concentration used in titrating. Similar test tubes are treated with varying amounts of the filtered medium solution described above. One test tube was left untreated to ascertain that the *Staphylococcus aureus* B was active, and one had no *Staphylococcus aureus* B in the tube but did have the whole medium to ascertain that the filtrate was not contaminated. The entire group of test tubes was then incubated at 38° centigrade for twenty-four hours and the results tabulated.
Two typical titer charts are shown in Tables 3 and 4.

**Table 3**

<table>
<thead>
<tr>
<th>Jug No.</th>
<th>pH of Medium Filtrate</th>
<th>Milliliters of Stock Medium Filtrate</th>
<th>Medium Control</th>
<th>Stap. Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.7</td>
<td>x x x x x</td>
<td>x</td>
<td>G</td>
</tr>
<tr>
<td>2</td>
<td>6.85</td>
<td>x x x x G</td>
<td>x</td>
<td>G</td>
</tr>
<tr>
<td>3</td>
<td>6.7</td>
<td>x x x x x</td>
<td>x</td>
<td>G</td>
</tr>
</tbody>
</table>

*a* Medium from 13 day cultures was used.

*b* No growth is signified by x.

*c* Growth is signified by G.

**Table 4**

<table>
<thead>
<tr>
<th>Jug No.</th>
<th>pH of Medium Filtrate</th>
<th>Milliliters of Stock Medium Filtrate</th>
<th>Medium Control</th>
<th>Stap. Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.4</td>
<td>x x x x G</td>
<td>x</td>
<td>G</td>
</tr>
<tr>
<td>2</td>
<td>7.45</td>
<td>x x x x G</td>
<td>x</td>
<td>G</td>
</tr>
<tr>
<td>3</td>
<td>7.45</td>
<td>x x x x x</td>
<td>x</td>
<td>G</td>
</tr>
</tbody>
</table>

*a* Medium from 21 day culture was used.

*b* No growth is signified by x.

*c* Growth is signified by G.
CHAPTER III

METHODS OF ANALYSIS

Substance for Analysis

Method of obtaining sample.—Medium from twenty-one day cultures of spores was taken from the culture jars and filtered through flannel to remove most of the mold spores. The filtered medium was placed in a freezing unit until cold, then removed. The pH of the medium was decreased to 1.5–2 by the addition of hydrochloric acid.

The acid medium was then extracted with ether by use of a circulation pump or a shaker. The ether was salted out with sodium chloride and recovered by decantation.

The ether extract was evaporated to dryness under reduced pressure, leaving a colored residue varying from light yellow to orange depending upon the concentration of components present.

Ether soluble extraction.—The dried sample was extracted with ether at 60° centigrade. An oily, brown liquid heavier than water settled out. The supernatant liquid was decanted and cooled. White needle-like crystals formed in the ether and were recovered by filtration.

Benzene soluble extraction.—The heavy, oily, brown liquid from the water extraction became a solid upon cooling to room temperature. The brown solid was extracted with benzene. A dark yellow to orange color was imparted to the benzene leaving a reddish-brown, gum-like residue, which was only slightly soluble in benzene. The benzene extract was
decanted, dried under reduced pressure, and so produced a reddish, brittle solid from the yellow-to-orange liquid.

Chloroform soluble extraction.—The reddish-brown, gum-like residue remaining from the benzene extraction was extracted with chloroform. An orange color was imparted to the solvent, leaving a reddish-brown, gum-like residue which was only slightly soluble in chloroform. The chloroform soluble component was dried to produce a brittle, yellowish-red solid.

Alcohol soluble extraction.—The residue from the water, benzene and chloroform extraction dissolved in ninety-five per cent alcohol, giving an orange colored liquid which in dry form was a reddish-brown solid.

Separation of sample into components.—The separation of the sample into the four components (water extract, benzene extract, chloroform extract, alcohol extract) is summarized graphically by Table 5.

TABLE 5

| Ether extract of medium → soluble in water at 80°C → White needle-like crystals |
| Reozish-brown residue → soluble in benzene → Yellow to orange solution... Reddish, brittle solid upon drying |
| Reddish, gum-like residue → soluble in chloroform → Orange solution... Yellowish-red, brittle solid upon drying |
| Reddish-brown, gum-like residue → soluble in alcohol (25%) → Orange solution... Reddish-brown solid upon drying |
Analytical Tests

**Chromatograph.**—An adsorption column of calcium carbonate was prepared. The solvent used was passed through the column until the calcium carbonate was firm and uniformly packed. The solvent proper was followed by a small amount of the solvent containing the dissolved sample. After the sample had passed into the calcium carbonate, more solvent was added until the pattern developed as desired.

**Sodium Decomposition.**—A small piece of sodium was placed in a two inch test tube, suspended through a piece of asbestos board, and heated with a microburner until the sodium vapor stood about one-half inch in the test tube. Then the organic compound to be analyzed was dropped into the tube. After smoke ceased to come from the tube, the bottom of the test tube was placed in a beaker containing ten milliliters of distilled water. The tube was broken in the beaker of water. The resulting solution of water and compound was filtered and the filtrate was collected for analysis. This filtrate was called the stock solution.

**Sulfur.**—To one milliliter of stock solution (refer above), made slightly acid with acetic acid, a few drops of lead acetate solution were added. A black precipitate would indicate the presence of sulfur.

**Nitrogen.**—To three milliliters of stock solution (refer to sodium decomposition test) ten drops of potassium fluoride and five drops of ferrous sulphate solution were added and mixed well; after five minutes concentrated hydrochloric acid was added dropwise to acidify the solution. The immediate appearance of a brilliant Prussian blue color would indicate a positive test for nitrogen.

---

1 O liver, *Qualitative Organic Analysis*, p. 134.
Halogenes.—The milliliters of the stock solution (refer to sodium decomposition test) were acidified with dilute sulfuric acid and boiled well to expel any hydrogen sulfide or hydrogen cyanide. With the addition of dilute silver nitrate solution, a precipitate would denote the presence of halogenes.

Molecular weight determination by the freezing point depression method.—

Four to eight milligrams of the sample were weighed into a small, thin-walled, melting point tube. To this was added a known weight of camphor, weighing approximately fifteen times as much as the sample. The open end of the tube was sealed, taking care not to over heat the sample and camphor present in the tube. A similar tube was prepared containing only camphor. These two tubes were tied to a thermometer, calibrated to be read to a tenth of a degree, and the three were placed in a melting point bath and heated until the contents of both tubes were melted. After the sample and camphor were thoroughly mixed, the melting points of the camphor and sample end of the camphor were taken. The molecular weight was found by the following calculation:

\[
\text{Molecular weight} = \frac{S \times \Delta T}{c} \times 1000, \text{ where}
\]

- \( S \) = weight of sample
- \( c \) = weight of camphor
- \( \Delta T \) = depression of freezing point

\[
K = \text{molar depression constant} = 20.7^\circ C
\]

Molecular weight determination by boiling point rise method.—

A known weight of solvent, approximately 10 milliliters, was placed in a boiling point determination apparatus containing a thermometer calibrated to be read to a tenth of a degree. The solvent was brought to a boil, and after fifteen minutes, the temperature was read three times in three minutes. If the temperature readings were constant, a known weight of sample was added, approximately 0.1 grams, and the rise in temperature noted. The molecular weight was calculated as follows:
Molecular weight = $S \Delta T$

$K$—mole boiling constant

$G$—grams of sample

$S$—grams of solvent

$\Delta T$—elevation in boiling point

**Melting points.**—A small amount of the sample was placed in a melting point tube and packed down to form a compact unit at the bottom of the tube. The tube was then placed in a melting point bath, equipped with a thermometer calibrated to be read to a tenth of a degree. The exact melting point was noted.

**Optical activity.**—A polarimeter was used in the determination of the optical activity of a substance; the method given by Harrod was used.

**Ignition.**—A small amount of the sample was placed on a platinum foil and heated over a small flame. The melting was noted, along with the odor produced and the amount of smoke given off. After complete combustion, the foil was examined for the presence of an ash.

**Solubility.**—If as much as 0.1 gram of a compound dissolved in 10 milliliters of a solvent, it was stated that the compound was soluble in that particular solvent.

**Beckman spectrophotometer.**—A very dilute solution of the sample was prepared, using a solvent which would absorb a minimum amount of light over the wave band covered by the absorption curve to be prepared. By use of a Beckman spectrophotometer, the optical density was obtained at various wave lengths.

**Carbon and hydrogen.**—Quantitative determination of carbon and hydrogen by the semi-micro combustion method was carried out, using a sample of approximately 20—milligrams in weight.

---


Oxidation test by potassium permanganate.—A small amount of the sample was dissolved in basic water, and a dilute solution of potassium permanganate was added dropwise. A change in color would yield a positive test.

Benedict's reagent.—A small amount of the sample was dissolved in basic water, and added dropwise to boiling Benedict's reagent. Decolorization of the Benedict's reagent was noted as a positive test for an aldehyde or ketone.

Tollen's reagent.—A small amount of sample was added to a test tube containing 1 milliliters of Tollens's reagent. The tube was then heated in a water bath. Formation of a silver mirror was taken as positive test for an aldehyde or ketone.

Bromide oxidation.—Bromine water was added dropwise to a basic solution of the sample. A change in color or a precipitate would indicate a positive test.

Phenylhydrazine.—A small amount of sample was dissolved in alcohol and water added dropwise until the sample just dissolved. Then a quantity of phenylhydrazine, equal in weight to that of the sample being tested, was added. The appearance of crystals would show a reaction, thus indicating the presence of a carbonyl group or of a group easily oxidized to a carboxyl group.

Reduction by metals in basic solutions.—A small piece of aluminum was placed in a basic solution of the sample. A change in color would indicate reduction.

Aniline.—A small amount of aniline was added to a warm alcoholic solution of the sample, made slightly basic with sodium hydroxide. A red color was taken as a positive test for quinones.
Sodium hydrosulfite.—Solid hydrosulfite was added to a basic solution of the sample. A positive test for quinones could be indicated by the disappearance of color from the solution.

Sodium salt.—Sodium salts of acid were prepared by placing the acid in a warm, ten per cent solution of sodium hydroxide.

Fuming sulfuric acid.—A small amount of dry sample was placed in 3 milliliters of fuming sulfuric acid. If the substance went into solution without excessive charring, an aromatic was indicated.
Chapter IV

LATE AND RASOLO

Chromatographic Description

The ether extract obtained as described in the preceding chapter (p. 12), was dissolved in 10 milliliters of chloroform. This solution was chromatographed (p. 16), using chloroform as the solvent. Upon development of the column three, distinct, yellow-colored rings formed. Two of these rings were near the top, but separated by a non-colored area approximately 2 centimeters wide. The third ring was near the bottom of the column, approximately 10 centimeters below the bottom of the second or middle ring. For convenience in future discussion the top ring will be referred to as compound I, the middle ring as compound II, and the lower ring as compound III.

The calcium carbonate was removed from the adsorption tube intact. Each colored section was separated from the column, placed in individual containers, and the colored compound or pigment removed by extraction with 95 per cent alcohol. Each pigment solution was diluted with 57 per cent alcohol and the optical density of the dilute solutions determined at various wave lengths, from 250 to 660 millimicrons, using a Beckman spectrophotometer (p. 16). In the graphs on pages 20 and 21 wave lengths have been plotted against optical density. In order to remove concentration as a variable factor, the optical density at 660 millimicron wave length was taken as unity and the data plotted with respect to this value.

Due to the resemblance of the antibiotic properties of a mixture of
Fig. 1

COMPARISON OF ABSORTION CURVES OF COMPOUNDS:

Compound I

Compound II

Compound III

Penicillin
Fig. 2

Comparison of Absorption Curves of Compounds

Compound I

Compound II

Compound III

Penicillin
these compounds to the antibiotic properties of penicillin, the absorption curve on pages 20 and 21 represents the optical density of an alcoholic (95 per cent) solution of penicillin at the wavelengths indicated, from 220 to 460 millimicrons. The optical density at 360 millimicrons wave length was taken as unity.

Separation by Use of Solvents

The ether extract (p. 12) was further separated by the solvent method (pp. 12 and 13) as illustrated by the separation chart in the preceding chapter (Table 5, p. 13).

A portion of each of the extracts was dried under reduced pressure and dissolved in 95 per cent alcohol. The optical density of each of the alcoholic solutions of the extracts was determined at the wavelengths indicated, from 220 to 460 millimicrons, using a Beckman spectrophotometer. In all cases the optical density at 360 millimicrons wavelength was taken as unity and all data plotted accordingly.

The absorption curve on pages 23 and 24, obtained by plotting the optical density against wavelengths of the alcoholic extract, is identical to the curve obtained in the same manner on compound I (pp. 20 and 21). Likewise, the absorption curve (pp. 25 and 26) produced by the benzene extract is identical to the absorption curve produced by compound II (pp. 20 and 21).

To check the purity of the alcoholic, benzene, and chloroform extracts, each extract was chromatographed, using benzene as the solvent. In each case a single yellow ring appeared and stayed together upon development of the column. The colored area was divided arbitrarily into three sections: the lower section containing the greatest concentration of pigment, the

1 Penicillin for use as standard (#252861, 775 U/mg) was obtained from The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana.
Fig. 3

ABSORPTION CURVE OF ALCOHOLIC EXTRACTED COMPOUND

Alcoholic Extracted Compound
Fig. 4

ABSORPTION CURVE OF ALCOHOLIC EXTRACTED COMPOUND

Alcoholic Extracted Compound

WAVE LENGTH
Fig. 5

ABSORPTION CURVE OF BENZENE

EXTRACTED COMPOUND

Benzene Extracted
Compound

\[ \text{WAVELENGTH} \]

320 340 360 380 400 420 440 460
Fig. 6

Absorption Curve of Benzene Extracted Compound

Benzene Extracted Compound

Wavelength
Fig. 7

ABSORPTION CURVE OF CHLOROFORM

EXTRACTED COMPOUND

Chloroform Extracted Compound
Fig. 8

ABSORPTION CURVE OF CHLOROFORM EXTRACTED COMPOUND

Chloroform Extracted Compound
Fig. 9

PURITY OF CHLOROFORM

EXTRACTED COMPOUND

Top Division

Middle Division

Lower Division
Fig. 11

PURITY OF ALCOHOLIC EXTRACTED COMPOUND

Top Division

Middle Division

Lower Division

WAVE LENGTH

320 340 360 380 400 420 440 460
middle and upper sections containing a very small amount of pigment. The lower, middle and upper sections of the colored region of the calcium carbonate column of each extract were recovered and dissolved in 25 per cent alcohol. The optical density of each fraction was determined at the wave lengths indicated on the graphs on pages 26, 30, and 31 by the use of a Beckman spectrophotometer. It will be noted that the absorption curve (p. 31) of the lower fraction of the alcoholic extract is identical with the absorption curve obtained from compound I (pp. 26 and 31). The absorption curve of the lower fraction of the benzene extract (p. 30) is identical with the absorption curve of compound II (pp. 26 and 31). The absorption curve of the lower fraction of the chloroform extract (p. 26) is identical with the absorption curve of compound III (p. 26 and 31).

The water extract, obtained as described in the preceding chapter and as charted on page 1, was recrystallized from water, giving long needle-like crystals. These crystals closely resembled those of benzoic acid and had a sharp melting point, so they were set aside and not used in the absorption analysis.

ANALYSIS OF COMPOUNDS

Solubility. The following results were obtained by tests (p. 16).

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>WATER</th>
<th>DIL. HCL</th>
<th>DIL. KOH</th>
<th>5% NaHCO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Extract</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Soluble</td>
<td>Soluble</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Soluble</td>
<td>Soluble</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Soluble</td>
<td>Soluble</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Soluble</td>
<td>Soluble</td>
</tr>
</tbody>
</table>

Kanam, op. cit., Solubility table attached to Fly i.e.f.
**Sodium decapsulation and element analysis.**—Using the procedures described on pages 14 and 15, the following data was obtained.

**Table 7**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>SULPHUR</th>
<th>NITROGEN</th>
<th>HALOGEN</th>
<th>PHOSPHATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Extract</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Ignition test (p. 16).**—The following data was obtained.

**Table 8**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>RESULTS</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Extract</td>
<td>Negative</td>
<td>Burns with a smoky flame</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>Negative</td>
<td>Burns with a very heavy sooty smoke</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>Negative</td>
<td>Burns with a smoky flame</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>Negative</td>
<td>Burns with a smoky flame</td>
</tr>
</tbody>
</table>

**Melting point determination (p. 16).**—The following data was obtained.

**Table 9**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Melting Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Extract</td>
<td>121° - 121.5° C.</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>Approximately 82° - 84° C.</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>Approximately 86° - 88° C.</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>Approximately 80° - 94° C.</td>
</tr>
</tbody>
</table>
Optical activity (p. 16).—The following data was obtained.

### Table 10

**Optical Activity**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract</td>
<td>No optical activity</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>No optical activity</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>No optical activity</td>
</tr>
<tr>
<td>Benzene extract</td>
<td>No optical activity</td>
</tr>
</tbody>
</table>

Classification reactions.—Using the methods described on pages 16–18, the following data was obtained.

### Table 11

**Classification Reactions**

<table>
<thead>
<tr>
<th>REAGENTS</th>
<th>WATER EXTRACT</th>
<th>CHLOROFORM EXTRACT</th>
<th>ALCOHOLIC EXTRACT</th>
<th>BENZENE EXTRACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Permanganate</td>
<td>Negative</td>
<td>Reduced to a green color</td>
<td>Reduced to a green color</td>
<td>Reduced to a green color</td>
</tr>
<tr>
<td>Benedict's Reagent</td>
<td>Negative</td>
<td>Decolorizes</td>
<td>Decolorizes</td>
<td>Decolorizes</td>
</tr>
<tr>
<td>Tollens Reagent</td>
<td>Negative</td>
<td>Silver Mirror</td>
<td>Silver Mirror</td>
<td>Silver Mirror</td>
</tr>
<tr>
<td>Fuming Sulfuric Acid</td>
<td>Tends to go into solution in each case without excess charring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylene Chloride</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Ferric Chloride</td>
<td>No color</td>
<td>No color</td>
<td>No color</td>
<td>No color</td>
</tr>
<tr>
<td>Iodine Oxidation</td>
<td>Negative</td>
<td>Decolorizes</td>
<td>Decolorizes</td>
<td>Decolorizes</td>
</tr>
<tr>
<td>Aluminum Reduction</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Phenyl-hydrazine</td>
<td>Negative</td>
<td>Slight crystal formation—action slow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>REAGENTS</td>
<td>WATER EXTRACT</td>
<td>CHLOROFORM EXTRACT</td>
<td>ALCOHOLIC EXTRACT</td>
<td>BENZENE EXTRACT</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------</td>
<td>--------------------</td>
<td>--------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Aniline</td>
<td>Solid obtained</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
| Sodium Hydro-
| sulfite           | Negative       | Slight decolorization in each remaining case |                  |                 |
| Sodium Hydride    | Sodium Salt formed in each case |                   |                    |                 |

**Molecular weight determination by freezing point depression.**

Using the method described on page 13, the following data was obtained.

**Table 12**

**Molecular Weight Determination by freezing point depression**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt. of Sample</th>
<th>Wt. Camphor</th>
<th>Melting Point of Camphor</th>
<th>Melting Point of Sample and Camphor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform Extract</td>
<td>3.80 mg</td>
<td>67.4 mg</td>
<td>176.6° C.</td>
<td>168.9° C.</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>3.85 mg</td>
<td>47.5 mg</td>
<td>176.6° C.</td>
<td>168.2° C.</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>4.20 mg</td>
<td>60.3 mg</td>
<td>177.3° C.</td>
<td>166° C.</td>
</tr>
</tbody>
</table>

**Molecular weight determination by boiling point rise using the method described on page 13.**

Data was obtained on the compounds derived from the Porto Rico sweet potato mole. This data is contained in Table 15 on the following page.
TABLE 13
MOLECULAR WEIGHT DETERMINATION
BY BOILING POINT RISE

<table>
<thead>
<tr>
<th>Sample</th>
<th>SOLVENT</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>Absolute Alcohol</td>
<td></td>
</tr>
<tr>
<td>Extract</td>
<td>Chloroform</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td></td>
</tr>
<tr>
<td>Alcoholic</td>
<td>Absolute Alcohol</td>
<td>Decomposition with decreased solubility occurred in each instance</td>
</tr>
<tr>
<td>Extract</td>
<td>Chloroform</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>Absolute Alcohol</td>
<td></td>
</tr>
<tr>
<td>Extract</td>
<td>Chloroform</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td></td>
</tr>
</tbody>
</table>

Carbon and hydrogen analysis.—Using the method referred to on page 16, the following results were obtained.

TABLE 14
CARBON AND HYDROGEN ANALYSIS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt. of Sample</th>
<th>Wt. of Carbon Dioxide</th>
<th>Wt. of Water</th>
<th>%C</th>
<th>%H</th>
<th>%O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>16.371 mg</td>
<td>44.457 mg</td>
<td>15.055 mg</td>
<td>7.55%</td>
<td>65.34%</td>
<td>26.11%</td>
</tr>
<tr>
<td>Extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcoholic</td>
<td>20.769 mg</td>
<td>41.167 mg</td>
<td>12.466 mg</td>
<td>6.72%</td>
<td>64.81%</td>
<td>30.64%</td>
</tr>
<tr>
<td>Extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>26.597 mg</td>
<td>51.934 mg</td>
<td>15.416 mg</td>
<td>6.43%</td>
<td>52.75%</td>
<td>40.78%</td>
</tr>
<tr>
<td>Extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER V

CONCLUSION

Four compounds were isolated from the filtrate produced by the strain of mold resembling *Penicillium notatum* growing. The isolation of the four compounds has been discussed in Chapter IV, and their degree of purity has been shown by the graphs on pages 36, 38 and 39. Therefore the purpose of this chapter will be to sum up the chemical nature of each individual compound. The summation will be given in brief form for clarity.

Enter Extracted Compound

I. Physical examination

1. By recrystallization long, white, needle-like crystals were obtained.

2. By the ignition test the melting point appeared sharply. The resulting liquid burned with a smoky flame and gave a very sharp odor.

II. Physical constants

1. The melting point was 101° to 101.5° centigrade.

2. There was no apparent optical activity.

III. Elementary analysis

1. Nitrogen, sulfur, halogens, and phosphate were absent.

IV. Solubility tests indicated the compound was an acid or a phenol.

1. The compound was insoluble in water and dilute acid.

2. The compound was soluble in dilute alkali and ether.

37
V. Classification reactions indicated an aromatic acid.

1. With ferric chloride the compound produced no color, so indicated it was not a phenol.

2. With sodium hydroxide the compound formed a sodium salt, so indicated it was an acid.

3. With fuming sulfuric acid, the compound went into solution without excessive charring. This indicated that it was an aromatic compound.

4. With sodium bicarbonate the compound went into solution forming a sodium salt, indicating that it was an acid.

VI. Consultation of literature indicated the compound was benzoic acid or maleic acid.

VII. Preparation of a derivative indicated the water extracted compound to be benzoic acid.

1. Aniline derivative of the compound melted at 155° - 160° centigrade.

2. Aniline derivative of benzoic acid melted at 160° centigrade.

VIII. Conclusion: The water extracted compound was benzoic acid.

Benzene Extracted Compound

I. Physical examination

1. All attempts at crystallization failed. A yellowish-red gum-like solid formed in each case.

2. The compound has a characteristic odor.

3. The compound melted rather sharply. The resulting liquid decomposed and burned with a smoky flame, yielding a very sharp pungent odor.

1 Referred to arbitrarily as compound II in previous chapter.
II. Physical constants indicated the chromophoric grouping to be similar to that of penicillin.

1. The melting point was difficult to determine due to the color of the melted substance, but was approximately 90° to 94° centigrade.

2. There was no apparent optical activity.

3. The molecular weight as determined by the freezing point depression method was found to be 278.50 ± 5.

4. Absorption curves are on pages 20 and 21.

III. Elementary analysis

1. Nitrogen, sulfur, chlorine and phosphates were absent.

2. Carbon present equaled 53.46 per cent.

3. Hydrogen present equaled 3.48 per cent.

4. Oxygen present equaled 43.56 per cent.

IV. Solubility tests indicated the compound was an acid or phenol, or possibly a negatively substituted quinone.

1. The compound was insoluble in water and dilute acid.

2. The compound was soluble in dilute alkali and ether.

V. Classification reactions indicated an aromatic compound possessing an acid group and possibly an aldehyde group, probably in the form of a negatively substituted quinone.

1. With ferric chloride there was no color, so the compound was not a phenol.

2. The compound reduced potassium permanganate, so indicated a possible aldehyde or ketone group.

3. Tollen's reagent yielded positive reaction, indicating an aldehyde or ketone group.
4. The compound decolorized Benedict's reagent, so indicated an aldehyde.

5. With acetyl chloride negative results indicated no alcohol.

6. Reaction of the compound by aluminum in potassium hydroxide was negative, indicating a rather stable compound.

7. With phenylhydrazine a few crystals formed, but the action was slow, indicating a negatively substituted carbonyl group.

8. The compound dissolved in fuming sulfuric acid without excess charring, indicating an aromatic.

9. Oxidation by bromine decolorized the solution, showing oxidation by a strong oxidizing agent.

10. The aniline test was negative, not giving a positive test for a quinone.

11. With sodium hydrosulfite the solution decolorized slightly, indicating negatively substituted quinone.

12. Sodium salt formed with sodium hydroxide, indicating an acid group.

VI. Consultation of literature yielded no compound having properties corresponding to the properties of this compound.

VII. No derivatives were obtained.

VIII. Conclusion: These tests indicated an aromatic compound, whose molecular weight is approximately 276.50 - 54, composed of 53.25 per cent carbon, 6.45 per cent hydrogen, and 40.36 per cent oxygen. The compound possesses an acid group and possibly an aldehyde group, probably in the form of a negatively substituted quinone. The indications were that the chromophoric grouping is similar to that of penicillin. A possible molecular formula is C$_{12}$H$_{17}$O$_{7}$. 
Chloroform Extracted Compound

I. Physical examination

1. A crystalline compound was not obtained.

2. The compound was light orange in color, very brittle and hard when dry.

3. The melting point appeared rather sharply and the resulting liquid decomposed and burned with a smoky flame.

II. Physical constants indicated the compound to be closely related to the benzene extracted compound and the chromophoric grouping to be very similar to that of penicillin.

1. The melting point was approximately 82° to 84° centigrade.

2. There was no apparent optical activity.

3. The molecular weight as determined by the freezing point depression method, was 265.46 ± 0.51.

4. Absorption curves are on pages 20 and 21.

III. Elemental analysis.

1. Nitrogen, sulfur, halogens, and phosphates were absent.

2. Carbon present equaled 65.34 per cent.

3. Hydrogen present equaled 7.56 per cent.

4. Oxygen present equaled 26.11 per cent.

IV. Solubility tests placed the compound in the class of phenols, acids, and negatively substituted quinones.

1. The compound was insoluble in water and dilute acid.

2. The compound was soluble in dilute alkali and ether.

V. Classification reactions indicated an aromatic compound possessing an acid group and possibly an aldehyde group, probably in the form of a negatively substituted quinone.

Referred to arbitrarily as compound III in previous chapter.
1. With ferric chloride there was no color, so the compound was not a phenol.

2. Potassium permanganate was reduced, as the test indicated a possible aldehyde or ketone group.

3. Tollen's reagent yielded a positive reaction, indicating an aldehyde or ketone group.

4. The compound decolorized Benedict's reagent, so indicated an aldehyde or ketone.

5. With acetyl chloride negative results indicated no alcohol.

6. Reduction of the compound by alumina in potassium hydroxide was negative, indicating a rather stable compound.

7. With phenylhydrazine a few crystals formed, but the action was slow, indicating a negatively substituted carbonyl group.

8. The compound dissolved in fuming sulfuric acid without excess charring, indicating an aromatic.

9. Oxidation by bromine decolorized the solution, showing oxidation by a strong oxidizing agent.

10. The ninhydrin test was negative, not giving a positive test for a quinone.

11. With sodium hyposulfite the solution decolorized slightly, indicating negatively substituted quinone.

12. Sodium salt formed with sodium hydroxide, indicating an acid group.

VI. Consultation of literature yielded no compound having properties corresponding to the properties of this compound.

VII. No derivatives were obtained.

VIII. Conclusion: These tests indicate an aromatic compound, whose molecular weight is approximately 166.46 ± 5%, composed of
65.34 per cent carbon, 7.35 per cent hydrogen, and 26.11 per cent oxygen. The compound possesses an acid group and possibly an aldehyde group, probably in the form of a negatively substituted quinone. The indications were that this compound is closely related to the benzene extracted compound and that the chromophoric grouping is similar to that of penicillin.

A possible molecular formula is \( C_{16} H_{22} O_4 \).

Alcoholic Extracted Compound

I. Physical examination

1. All attempts at recrystallization failed. A reddish-brown, brittle solid formed in each case.

2. The compound has a characteristic odor.

3. The compound melted rather sharply. The resulting liquid decomposed and burned with a smoky flame.

II. Physical constants indicated this compound to have somewhat different chromophoric grouping from that of the benzene and the chloroform extracted compound and less like the chromophoric grouping of penicillin.

1. The melting point was approximately 86° to 88° centigrade.

2. There was no apparent optical activity.

3. The molecular weight, as determined by the freezing point depression method, was 257.80 \( \pm 0.6 \).

4. Absorption curves are on pages 29 and 31.

III. Elementary analysis

1. Nitrogen, sulfur, halogens, and phosphates were absent.

2. Carbon present equaled 62.61 per cent.

3. Hydrogen present equaled 6.73 per cent.

4. Oxygen present equaled 40.6 per cent.

Referred to arbitrarily as compound I in previous chapter.
IV. Solubility tests indicated the compound to be an acid, phenol or a negatively substituted quinone.

1. The compound was insoluble in water and dilute acid.
2. The compound was soluble in dilute alkali and ether.

V. Classification reactions indicated an aromatic compound possessing an acid group and an aldehyde group.

1. With ferric chloride there was color, so the compound was not a phenol.
2. Potassium permanganate was reduced so the test indicated a possible aldehyde or ketone group.
3. Tollen's reagent yielded a positive reaction, indicating an aldehyde or ketone group.
4. The compound decolorized Benedict's reagent, so indicated an aldehyde or ketone.
5. With acetyl chloride negative results indicated no alcohol.
6. Reduction of the compound by aluminum in potassium hydroxide was negative, indicating a rather stable compound.
7. With phenylhydrazine a few crystals formed, but the action was slow, indicating a negatively substituted carbonyl group.
8. The compound dissolved in fuming sulfuric acid without excess charring, indicating an aromatic.
9. Oxidation by bromine decolorized the solution, showing oxidation by a strong oxidizing agent.
10. The aniline test was negative, not giving a positive test for a quinone.
11. With sodium hydrosulfite the solution decolorized slightly, indicating negatively substituted quinone.
12. Sodium salt formed with sodium hydroxide, indicating an acid group.
VI. Consultation of literature yielded no compound having properties corresponding to the properties of this compound.

VII. No derivatives were obtained.

VIII. Conclusion: These tests indicated an aromatic compound, whose molecular weight is approximately 237.80 ± 5%, composed of 87.8% per cent carbon, 6.73 per cent hydrogen, and 5.64 per cent oxygen. The compound possesses an acidic group and possibly an aldehyde group. The indications were that this compound has a somewhat different chromophoric grouping from that of the benzene and the chloroform extracted compounds. A possible molecular formula is

\[ C_{12}H_7O_5 \]
BIBLIOGRAPHY


