

it is remarkable that most of the amylopsin goes into the main liquid portion. Trypsin is found in the solid parts as well as in the liquid, while lipase is found mainly bound to the solids. This union is not so fixed, however, that when mixed with other substrates the ferments cannot readily separate and attack these.

The pancreas of the hog is distinguished from those of beef and sheep mainly in the power of starch digestion. It is suggested that in the last-named animals amylopsin may not be physiologically necessary because the starches can be digested by the ptyalin of the saliva through the aid of the prolonged cud-chewing process.

Lipase and esterase values do not vary in the same manner in the different fractions with the different animals. These variations are striking enough to indicate that the properties are associated with distinct enzymes.

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THE ISOLATION OF PARA-HYDROXYBENZOIC ACID FROM SOIL.

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In an investigation of a sandy soil from Florida an aromatic acid has been isolated and its identity with *p*-hydroxybenzoic acid established.

The compound was isolated in the following way: Twenty-three kilograms of soil were extracted with about 75 liters of an aqueous 2% solution of sodium hydroxide at room temperature for twenty-four hours. The extract was made slightly acid with sulfuric acid and filtered. The acid filtrate was then extracted with ether and the ether extract was concentrated to a volume of about 200 cc. and shaken up with a concentrated solution of sodium bisulfite to remove aldehydes or other substances which combine with this reagent. The bisulfite solution was drawn off and extracted several times with fresh ether. The combined ether extracts were then slowly evaporated on the surface of a small volume of warm water. The water solution was heated to boiling and filtered while hot to remove an insoluble oily residue. From the cold, concentrated aqueous solution a crystalline compound separated. This compound was purified by repeated crystallization from water. It persistently retained a slight tinge of color and only after many crystallizations and boiling with a small quantity of purified bone-black accompanied by much loss of material, could it be freed from color. The aqueous solution was then subjected to steam distillation to remove benzoic acid or any other volatile

substance which might be present. The solution remaining in the distilling flask was evaporated to dryness on a steam bath and the residue extracted with a small volume of chloroform to remove final traces of benzoic acid. The substance was finally recrystallized from water and dried between layers of hardened filter paper.

The properties of the compound thus obtained were found to be identical with those of *p*-hydroxybenzoic acid. The purified soil compound melts at 213–214°. *p*-Hydroxybenzoic acid melts at 213–214°. A mixture of chemically pure *p*-hydroxybenzoic acid (further purified by recrystallization from water) and the soil compound melted at 213–214°. ¹

The purified soil compound is soluble in ether and alcohol, slightly soluble in cold and more soluble in hot water, and insoluble in chloroform. It readily reduces potassium permanganate in cold, alkaline solution. With ferric chloride it gives a yellow flocculent precipitate which is soluble in excess of the reagent.

In cold, aqueous solution it decolorizes bromine instantaneously, a white precipitate ($C_6H_2Br_3OBr$) being formed with excess of bromine. This reaction is similar to the reaction between phenol and bromine. Tribromophenol was prepared from the soil compound and synthetic *p*-hydroxybenzoic acid in the following way: To an aqueous solution containing 0.05 g. of the substance was added an excess of a saturated aqueous solution of bromine. The bulky, curdy, white precipitate which was produced was filtered off and washed with cold water. It was then transferred to a beaker and treated gradually with an aqueous solution of acid sodium sulfite until a strong odor of sulfur dioxide persisted after stirring and warming to 40°. The compound $C_6H_2Br_4O$ first formed with bromine water loses one atom of bromine and is converted into the tribromo-derivative during the treatment with the bisulfite solution. The tribromo-derivative was filtered off and washed thoroughly with cold water and finally dissolved in a small volume of boiling 40% alcohol. Tribromophenol crystallized out in characteristic, long, hair-like needles from the cold solution. The crystals were collected, dried in a desiccator and the melting points determined. The product prepared from synthetic *p*-hydroxybenzoic acid melted at 92–93°. The product prepared from the soil compound melted at 92–93°. A mixture of these two compounds melted at 92–93°. The melting point of tribromophenol is 92.5–93.5°.

The compound obtained from soil gave on analysis:

¹ In all cases a slight sintering or softening above 205° was observed and the substance melted with evolution of gas. A strong odor of phenol was observed in the melting-point tubes after cooling. *p*-Hydroxybenzoic acid decomposes into phenol and carbon dioxide when heated above its melting point. The melting points were determined in sealed tubes.

(a) 19.65 mg. of substance gave 43.7 mg. of CO₂ and 8.05 mg. of H₂O.

(b) 20.02 mg. of substance gave 44.8 mg. of CO₂ and 7.85 mg. of H₂O.

Synthetic *p*-hydroxybenzoic acid gave on analysis:

(a) 20.73 mg. of substance gave 46.02 mg. of CO₂ and 8.5 mg. of H₂O.

(b) 14.11 mg. of substance gave 31.42 mg. of CO₂ and 5.6 mg. of H₂O.¹

Sample.	% C.	% H.	% O.
(a) Soil compound.....	60.65	4.55	34.8
(b) Soil compound.....	61.02	4.36	34.62
(a) Synthetic <i>p</i> -hydroxy benzoic acid.....	60.54	4.55	34.91
(b) Synthetic <i>p</i> -hydroxy benzoic acid.....	60.73	4.41	34.86
Calculated for <i>p</i> -hydroxy benzoic acid.....	60.84	4.39	34.76

p-Hydroxybenzoic acid crystallizes with one molecule of water of crystallization which it loses when dried at 100°.

The compound obtained from soil gave on analysis:

(a) 0.0925 g. of substance crystallized from water gave 0.0107 g. of water of hydration.

(b) 0.1068 g. of substance crystallized from water gave 0.0120 g. of water of hydration.

Synthetic *p*-hydroxybenzoic acid gave on analysis:

(a) 0.1651 g. of substance crystallized from water gave 0.0192 g. of water of hydration.

(b) 0.1193 g. of substance crystallized from water gave 0.0136 g. of water of hydration.

	% H ₂ O.
(a) Soil compound.....	11.56
(b) Soil compound.....	11.24
(a) Synthetic <i>p</i> -hydroxybenzoic acid.....	11.62
(b) Synthetic <i>p</i> -hydroxybenzoic acid.....	11.40
Calculated for C ₇ H ₆ O ₃ .H ₂ O.....	11.54

Concentrated aqueous solutions of the neutral sodium salts of both the soil compound and synthetic *p*-hydroxybenzoic acid when treated with silver nitrate yielded heavy, white, micro-crystalline precipitates. These substances were found to be identical with the silver salt of *p*-hydroxybenzoic acid, having the formula AgC₇H₅O₃. The purified salt crystallizes from water in long needles or leaflets.

The silver salt prepared from the soil compound gave on analysis:

(a) 0.0338 g. yielded 0.0148 g. of silver.

(b) 0.0863 g. yielded 0.0381 g. of silver.

(c) 0.0449 g. yielded 0.0197 g. of silver.

The silver salt prepared from synthetic *p*-hydroxybenzoic acid gave on analysis:

(a) 0.0316 g. yielded 0.0140 g. of silver.

(b) 0.1575 g. yielded 0.0694 g. of silver.

¹ The writer wishes to express his thanks to Dr. Louis E. Wise who has very kindly made these combustions by his modification of Pregl's micro-combustion method.

	% Ag.
(a) Silver salt of soil compound.....	43.79
(b) Silver salt of soil compound.....	44.15
(c) Silver salt of soil compound.....	43.87
(a) Silver salt of synthetic <i>p</i> -hydroxybenzoic acid.....	44.30
(b) Silver salt of synthetic <i>p</i> -hydroxybenzoic acid.....	44.06
Calculated for $\frac{1}{2}\text{AgC}_7\text{H}_5\text{O}_3$	44.08

The reactions and analyses given above are sufficient to establish the identity of the compound obtained from soil as *p*-hydroxybenzoic acid, having the formula $\text{OH.C}_6\text{H}_4.\text{COOH}$.

Acids of this character cannot be accurately titrated with phenolphthalein as indicator since the phenolic hydroxy group begins to react with the alkali before the carboxyl group is completely neutralized. This prevents a sharp end reaction. The pink color develops slowly after the theoretical quantity of alkali necessary to neutralize the carboxyl group has been added.

p-Hydroxybenzoic acid has a dissociation constant of 2.8×10^{-5} and assuming that the acid dissociation constant of the phenolic hydroxyl group is of the same order of magnitude as that of phenol itself, which is 1×10^{-10} , an indicator which could theoretically yield good titration figures is bromocresol purple (dibromo-*o*-cresol sulfonphthalein). The change in color perceived when alkali is added is practically complete at a hydrogen-ion exponent of about 7.0.¹

In titrating, alkali was therefore added until a full purple color was developed. The following results were obtained.

0.0526 g. of synthetic *p*-hydroxybenzoic acid required 0.0152 g. of sodium hydroxide for neutralization.

0.0524 g. of soil compound required 0.0152 g. of sodium hydroxide for neutralization.

	Neutralization equivalent (molecular weight).
Found for synthetic <i>p</i> -hydroxybenzoic acid.....	138.1
Found for soil compound.....	137.9
Calculated for $\text{C}_7\text{H}_5\text{O}_3$	138.06

Benzoic acid was also isolated from this Florida soil. The isolation of benzoic acid from a Florida subsoil has been reported by Shorey² in a previous publication from this laboratory. The benzoic acid crystallized out with the *p*-hydroxybenzoic acid and was separated from the latter by distilling with steam as already mentioned. The distillate was collected in a flask containing a measured quantity of standard sodium hydroxide solution. The distillate was made acid with sulfuric acid and extracted several times with ether. The ether extract was evaporated

¹ Clark, W. M., and Lubs, H. A., "The Colorimetric Determination of Hydrogen-Ion Concentration and Its Application to Bacteriology," *J. Bacter.*, **2**, 109-135 (1917).

² Shorey, E. C., "The Presence of Some Benzene Derivatives in Soils," U. S. Dept. of Agr., *J. Agr. Res.*, **1**, 357-363 (1914).

on the surface of a small volume of warm water. The benzoic acid which separated from the cold, aqueous solution was finally purified by sublimation when a pure white product was obtained.

The properties of this compound were compared with those of pure benzoic acid¹ and found to be identical.

The compound crystallized in leaflets which were readily soluble in ether, alcohol, and chloroform, sparingly soluble in cold and more soluble in hot water. It melted at 121°. Benzoic acid melts at 121°. A mixture of the soil compound and pure benzoic acid melted at 121°.

A determination of the neutralization equivalent (molecular weight) gave the following results: 11.8 mg. of the soil compound required 1.95 cc. 0.05 N NaOH for complete neutralization with phenolphthalein as indicator. The neutralization equivalent was found to be 121. The neutralization equivalent calculated for benzoic acid (C₇H₆O₂) is 122. When an aqueous solution of the neutral sodium salt is treated with a dilute solution of ferric chloride, a light red precipitate is formed which is insoluble in acetic acid. When a solution of copper sulfate is added to an aqueous solution of the neutral sodium salt a light blue micro-crystalline precipitate is formed. This substance crystallized from water is light blue needles or plates united in spherical masses and was found to be similar to the copper salt prepared in the same way from synthetic benzoic acid having the formula Cu(C₇H₅O₂)₂·2H₂O. The acid also gave Mohler's reaction.²

p-Hydroxybenzoic acid and its derivatives have not been found as widely distributed in nature as those of its isomer, salicylic acid. Piutti and Comanducci³ have obtained *p*-hydroxybenzoic acid and protocatechuic acid from an acid extract of the unripe fruit of *Bignonia catalpa*. They believed that these acids exist in the fruit in the form of glucosides. They also showed that the *p*-hydroxybenzoic acid which they isolated was the same as the catalpic acid previously isolated by Sardo.⁴

p-Hydroxybenzoic acid has been found to occur in the urine of horses.⁵

This acid is also obtained from certain plant resins by fusing with alkali.⁶

¹ A sample of pure benzoic acid was obtained from the U. S. Bureau of Standards.

² This reaction is carried out by heating the substance to be tested with sulfuric acid until charring takes place, sulfobenzoic acid being formed if benzoic acid is present in the original material. On heating with potassium nitrate this will be transformed into *m*-dinitro-benzoic acid. On adding excess of ammonia to this acid and then a few drops of a colorless solution of ammonium sulfide a red color is produced.

³ Piutti, A., and Comanducci, E., "Sur les acides du '*Bignonia catalpa*,'" *Bull. soc. chim.*, [3] 27, 615-620 (1902).

⁴ Sardo, M. S., "Prime ricerche sulla *Bignonia Catalpa*. Acido catalpico," *Gaz. chim. ital.*, 14, 134-139 (1884).

⁵ Neuberger, C., "Der Harn," Berlin, 1, 501 (1911).

⁶ Dunstan, W. R., and Henry, T. A., "A Chemical Investigation of the Constituents of Indian and American *Podophyllum*," *J. Chem. Soc.*, 73, 209-226 (1898).

p-Hydroxybenzoic acid is obtained from certain pigments or anthocyanins by hydrolysis with acids. The recent investigations of Willstätter¹ have shown that these pigments which occur in the blossoms in many fruits and in some leaves of plants are glucosides and that they are derivatives of the complex benzopyrylium nucleus. Many of these pigments have been investigated. On hydrolysis with acids, delphinin obtained from larkspur breaks up into glucose, *p*-hydroxybenzoic acid and delphinidin.²

Pelargonium,³ a hydrolysis product of pelargonin which is the pigment obtained from *Pelargonium*, yields *p*-hydroxybenzoic acid and phloroglucinol when heated with alkali.

These observations suggest the possibility of the formation of *p*-hydroxybenzoic acid or its derivatives in soil during the decomposition of the complex plant pigments.

Benzoic acid occurs in gum benzoin and other plant resins in the form of esters. In gum benzoin it is present chiefly as the ester, benzyl benzoate. It is also present in some fruits such as cranberries and plums. It results from the oxidation of aromatic compounds possessing a side chain and in this way it may be formed from a great variety of vegetable products.

These two compounds are known to possess strong antiseptic properties and when present in soils in appreciable quantities they may have a great effect on the soil microorganisms or the growth of higher plants. These acids probably exist in the soil in the form of salts or esters.

The soil from which these two compounds were isolated is composed chiefly of quartz sand containing a considerable portion of organic matter which gives the soil a dark brown color. The organic matter is largely deposited in layers on the sand particles and when the soil is treated with dilute alkali the organic matter is dissolved or loosened and almost pure quartz sand remains. The soil from which this sample was taken is at present devoted to orange culture and is in a section which is badly affected with the citrus disease commonly known as "dieback."

p-Hydroxybenzoic acid was present in much larger quantities than benzoic acid. Approximately 0.5 g. of pure *p*-hydroxybenzoic acid was isolated from 23 kg. of soils. This amount corresponds to 21.6 parts per million or approximately 86.6 pounds per acre foot, assuming the weight of an acre foot to be four million pounds. In the case of benzoic acid only 40 mg. were isolated from 23 kg. of soil. This amount corresponds to 1.7 parts per million or about 6.9 pounds per acre. In the

¹ Willstätter, R., and Stoll, A., "Untersuchungen über Chlorophyll," Berlin, 1913.

² Willstätter, R., and Mieg, W. V., "Über ein Anthocyan des Rittersporns," *Ann.*, 408, 61-62 (1915).

³ Willstätter, R., and Bolten, E. K., "IV. Über den Farbstoff der Scharlachpelargonie," *Ann.*, 408, 42-61 (1915).

isolation of these compounds there is necessarily much loss of material and these figures are therefore given as minimal values.

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CORRECTIONS.

Page 1421, line 21, read 28.39 instead of 28.29.

Page 1422, line 7, read 12.69 instead of 12.09.

Page 1423, line 3, read 3.81 instead of 3.51.

Page 1424, line 3, read 42.26 instead of 42.46.

NEW BOOKS.

The Life of Robert Hare. By EDGAR FAHS SMITH. J. B. Lippincott & Co.

This is an important contribution to the history of American Science. In the absorption in present-day problems and achievements we are apt to underestimate and ignore the pioneers who laid the foundation for our building. It is a tardy recognition, but due these men, that we take account of their work.

Robert Hare belongs to the group of men which includes Franklin, Bache, Silliman, Joseph Henry and, later, Wolcott Gibbs. He was born in 1781 and died in 1858 and throughout his long life devoted his untiring energy and great ability to the development of the two kindred sciences of physics and chemistry. As Dr. Smith observes, he would in these days have been classed as a physical chemist.

At twenty years of age he was made a member of the Chemical Society of Pennsylvania, the first chemical society in the world, and presented a paper on his new invention, the oxy-hydrogen blowpipe, showing by means of it the fusion of platinum. This proved the beginning of the platinum industry and the introduction of this valuable instrument into the industries in general.

His early years were spent at work in his father's brewery and through his mechanical ingenuity many improvements were introduced into the business. In 1818 he served for a few months as professor of natural philosophy and chemistry at the College of William and Mary and the same year was made professor of chemistry in the medical department of the University of Pennsylvania, holding this position until his retirement after twenty-nine years of service.

His eagerness for research was most noteworthy in an age when teachers were generally content to limit their activities to lecture work, and when the providing of apparatus, commonly self-made, formed a heavy burden both in cost and labor. His own expression was that he "longed to return again to rove unshackled in the path of experiment."

It is impracticable in a brief review to mention all of his inventions and