

SUMMARY.

In the foregoing pages results have been presented which indicate the following conclusions:

(1) Neutral salts such as the chlorides in the presence of carbonates can be comparatively readily and completely leached from the soil.

(2) With continued leaching of soils containing "black alkali" there is an increase in the rate at which percolation takes place, due probably to the reduction of the amount of alkali present and its effect on the physical structure of the soil.

(3) With continued leaching there is a comparatively rapid reduction of normal carbonates in the soil water, due in large measure to conversion into bicarbonates.

(4) Bicarbonates are rapidly removed at first and then continue to be slowly removed in the soil water in very small amounts, diminishing so slowly as to be practically constant for an indefinite period.

(5) Soils containing "black alkali" can be reclaimed by leaching, but the time and the amount of water required are probably much greater than in the case of "white alkali."

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THE CRYSTALLINE SUBSTANCES OF PRICKLY ASH BARK.¹

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THE discovery of a crystalline substance in northern prickly ash (*Xanthoxylum fraxineum*, s. *X. Americanum*) is due to E. Staples.² The same crystals were later isolated by J. U. Lloyd³ and more fully examined by G. Eberhardt.⁴ To this substance the name of xanthoxylin (xanthoxyline) was given by Staples.

Another crystalline substance was isolated by G. Colton⁵ from southern prickly ash (*Xanthoxylum Carolinianum*, s. *X. Clava-Herculis*) and was shown by Eberhardt⁶ to be different from the

¹ A preliminary report of this work was read before the A.Ph.A. in 1903.

² Am. J. Pharm. 1829, 163.

³ Ibid. 1890, 229.

⁴ Ibid. 1890, 231.

⁵ Ibid. 1890, 191.

⁶ Loc. cit.

one contained in the northern variety of the drug. This substance, too, was named xanthoxylin.

Still another substance, also named xanthoxylin by its discoverer, was isolated by Stenhouse¹ from Japanese pepper (*Xanthoxylum piperitum* DC.).

The xanthoxylin of Stenhouse is isomeric with cantharidin and seems to be entirely different from either of the crystalline principles contained in the prickly ash barks.

In order to avoid confusion I propose to retain the name xanthoxylin for the substance isolated by Stenhouse from Japanese pepper, and to designate with the name xanthoxylin N the crystalline principle first obtained by Staples from northern prickly ash, and with the name xanthoxylin S the crystalline principle of southern prickly ash.

XANTHOXYLIN N.

Among the many substances which accompany xanthoxylin N in the northern prickly ash bark are a considerable amount of fixed oil, in which the xanthoxylin N seems to be very easily soluble, and an amorphous wax-like substance also easily soluble in the oil and in hot alcohol, but difficultly soluble in cold alcohol.

As xanthoxylin N is also easily soluble in hot, but difficultly soluble in cold alcohol, it is quite difficult to isolate xanthoxylin N in perfect purity by simple recrystallization from this solvent even if recrystallization be repeated several times. As the solubilities of xanthoxylin N and of the waxy substance in other solvents also seem to be very much alike, recrystallization from other solvents, too, is very unsatisfactory and connected with great waste of material. This accounts for the small yield obtained by previous investigators.

A preliminary examination of some pure xanthoxylin N obtained from a previous batch by Staple's method showed that, while xanthoxylin N is almost insoluble in cold water and only difficultly soluble in cold alcohol, it is extremely easily soluble in cold alcohol containing a few per cent. potassium hydroxide. Such a solution of xanthoxylin N in alcoholic potassium hydroxide can be diluted with water to any extent without causing the slightest turbidity even after standing for several months. If excess of mineral acid or acetic acid be added to the alkaline

¹ Ann. 89, 257; 104, 236.

solution, the xanthoxylin N, particularly when not quite pure, separates out as an oily liquid, but if the alkaline liquid be saturated with carbon dioxide the xanthoxylin N separates out unchanged and crystalline provided it had not been boiled for some time with the alcoholic solution of the alkali.

Making use of this behavior of xanthoxylin N, the following method seems to be the best for its isolation.

The powdered northern prickly ash bark is exhausted with cold benzene and, after the solvent is distilled off completely, the oily residue is mixed with an equal volume of 5½ per cent. solution of potassium hydroxide in alcohol. After standing for three or four hours the liquid is diluted with an amount of water equal to double the amount of alcohol taken and, after thorough shaking, transferred to a separating funnel. On standing over night a dark layer containing most of the xanthoxylin N dissolved in the alkaline liquid settles at the lower part of the separating funnel while a yellow oil floats on the surface. The alkaline liquid is drawn off and saturated with carbon dioxide. At first the liquid becomes turbid but after a while beautiful shining crystals commence to appear which look very pretty in the dark liquid. The crystals were collected after a few days' standing of the liquid and, after thorough washing with water, dried and weighed. The crude xanthoxylin N obtained by this method amounted to 3.5 per cent.

For purification the xanthoxylin N had to be recrystallized eight times, using animal charcoal in the last recrystallizations, before the melting-point remained constant.

Xanthoxylin N forms dazzling white needle-shaped crystals. It is extremely easily soluble in benzene, chloroform and hot alcohol. It is a little less soluble in cold acetone and less yet in ether. It is soluble in 49 parts of cold alcohol and about 25,000 parts of cold water. It melts at 132.5° to a clear yellowish liquid which does not re-solidify on cooling for about two hours. At 110° it assumes a yellowish tint but becomes white again on cooling.

A solution of xanthoxylin N in ethyl or methyl alcohol is colorless. Upon the addition of potassium hydroxide the alcoholic solution becomes yellow but the color disappears upon neutralizing the alkali with an acid. A saturated solution of xanthoxylin N in water has no color if kept protected from the air. Exposed

to the air or upon addition of alkali, the aqueous solution soon assumes a bluish fluorescence. The fluorescence is intensified by addition of potassium hydroxide. Addition of about 10 per cent. alcohol or a few drops of dilute acid destroys the fluorescence.

In presence of potassium hydroxide, xanthoxylin N is very easily soluble in cold alcohol but solution is not effected immediately.

On covering 1 gram xanthoxylin N with 10 cc. of a 5 per cent. solution of potassium hydroxide in alcohol it takes about an hour to dissolve all of the substance. On evaporating the alcohol from such a solution after diluting it with four times the amount of water, the liquid assumes a dark yellow color but no xanthoxylin N separates out on standing if the solution be protected from the carbon dioxide of the air.

Xanthoxylin N is tasteless in aqueous solution and has a very slight pricking taste in alcoholic solution.

It is optically inactive.

Cold aqueous potassium hydroxide does not affect xanthoxylin N. When boiled for a long time with a 10 per cent. solution of the alkali it very slowly goes into solution, forming a dark yellow liquid from which it is reprecipitated unchanged by a current of carbon dioxide.

Sulphuric acid colors xanthoxylin N red. On digesting xanthoxylin N in cold sulphuric acid till it is dissolved and then throwing the red solution into much water the xanthoxylin N separates out unchanged as shown by melting-point and general behavior. If xanthoxylin N be digested over night with sulphuric acid at a temperature of 80° and the dark red solution then thrown into water a resinous precipitate separates out which is easily soluble in alkalies and alkaline carbonates but difficultly soluble in barium hydroxide.

The formula of xanthoxylin N given in the preliminary report was corroborated by new analysis.

The analysis gave C, 69.65, 69.64, 69.89 and 69.93; H, 5.33, 5.53, 5.39 and 5.70. Calculated for $C_{15}H_{14}O_4$: C, 69.73; H, 5.47.

A molecular weight estimation by the lowering of the freezing-point, using benzene as solvent, gave 225, 254, 273 and 289. Calculated for $C_{15}H_{14}O_4$, 258.14.

Tested by Zeisel's method xanthoxylin N was found to contain

one methoxyl group. In carrying out the method it was found that when hydriodic acid alone is used the substance becomes one solid lump which does not break up during the whole operation, hence the results obtained are about 1 per cent. too low; but if the substance is first dissolved in 8 cc. of warm glacial acetic acid and the solution then mixed with 10 cc. hydriodic acid (sp. gr. 1.7) the results are very fair.

Analysis gave 11.47 and 11.46 CH_3O . Calculated for $\text{C}_{14}\text{H}_{11}(\text{CH}_3\text{O})\text{O}_8$, 12.03 CH_3O .

Hydrated Xanthoxylin N Dibromide, $\text{C}_{18}\text{H}_{14}\text{Br}_2\text{O}_4\cdot\text{H}_2\text{O}$.—The presence of a double binding in xanthoxylin N is shown by the absorption of one molecule of bromine by a molecule of xanthoxylin N. Five grams of xanthoxylin N were dissolved in 30 cc. glacial acetic acid and a 20 per cent. solution of bromine in the same solvent then added drop by drop till the slightly yellowish tint, which appears after the addition of the first drop of the bromine solution, changes to a decided red. About 3.2 grams of bromine were consumed and no evolution of hydrobromic acid could be noticed. The reddish liquid was then thrown into about 700 cc. of very cold water with constant stirring. The white precipitate which separated was thoroughly washed with water and after drying on porous plates recrystallized from a mixture of acetone and alcohol. The brominated xanthoxylin N crystallizes in white warts, melting at 171° with effervescence. Analysis of the compound showed that in the treatment with bromine under the above conditions xanthoxylin N takes up besides two atoms of bromine one molecule of water. This water cannot be removed either by keeping the brominated compound in vacuum over sulphuric or by heating it to 110° for three hours.

Analysis gave C, 41.19 and 41.05; H, 3.59 and 3.55; Br, 36.33 and 36.94. Calculated for $\text{C}_{15}\text{H}_{14}\text{Br}_2\text{O}_4\cdot\text{H}_2\text{O}$: C, 41.28; H, 3.71; Br, 36.67.

On prolonged exposure to the light the dibromide assumes a yellowish tint. The dibromide is easily soluble in cold alcohol and insoluble in water. It is very easily soluble in cold alcohol containing potassium hydroxide and the alkaline alcoholic solution can be diluted with water to any extent.

Dihydroxanthoxylin N, $\text{C}_{18}\text{H}_{16}\text{O}_4$.—On passing a current of hydriodic acid into a solution of xanthoxylin N in eight times its amount of glacial acetic acid the substance takes up two hydrogen

atoms and separates out as a hard resinous mass upon dilution of the acid liquid with water. After removal of the iodine by the usual methods and recrystallizing repeatedly from hot alcohol the dihydro compound was obtained in white needles melting at 142–143°. The dihydro compound is easily soluble in ether, chloroform, benzene and hot alcohol. It is difficultly soluble in cold alcohol and almost insoluble in water. Towards aqueous and alcoholic potassium hydroxide it behaves like xanthoxilin N itself.

Analysis gave C, 69.31 and 69.05; H, 6.50 and 6.25. Calculated for $C_{15}H_{16}O_4$: C, 69.19; H, 6.21.

The dihydroxanthoxilin N, like xanthoxilin N, contains one methoxyl group.

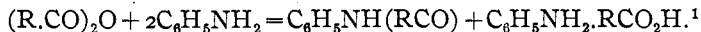
Analysis gave 11.56 and 11.50 CH_3O . Calculated for $C_{14}H_{18}(CH_3O)_3$, 11.93 CH_3O .

All attempts to prove the presence of hydroxyl or ketone groups in xanthoxilin N by the usual methods were unsuccessful. In every case the substance was recovered unchanged. The behavior of the substance towards alcoholic potassium hydroxide and subsequent dilution with water would seem to indicate that xanthoxilin N is a lactone. That this is not so is shown by the fact that it does not form a benzoyl compound in alkaline solution.

The experiment was carried out as follows: The xanthoxilin N was dissolved in about six times its amount of pyridine and the solution shaken three hours with a 20 per cent. solution of sodium hydroxide, using an amount of the alkaline solution equal to the amount of pyridine taken. The liquid which became miscible with water was then shaken with benzoyl chloride, after adding some more dilute sodium hydroxide.

The solution was then treated with an excess of carbon dioxide and the precipitate which separated out recrystallized from hot alcohol. It was found to be unchanged xanthoxilin N as shown by its melting-point and general behavior.

Xanthoxilin N does not behave like an anhydride of a mono-basic acid as it does not form an anilide when treated with aniline.



The experiment was carried out by dissolving 0.5 gram xanthoxilin and 0.5 gram aniline in 10 cc. of hot alcohol and setting

¹ Menshutkin and Wassiliew: J. russ. chem. Ges. 1889, 192.

the liquid aside over night. Most of the xanthoxylin N crystallized out unchanged as was shown by its melting-point and the absence of nitrogen by Lassaigne's test. On the other hand, towards other reagents xanthoxylin N acts like a monobasic acid.

When xanthoxylin N is dissolved in an excess of standardized solution of potassium hydroxide in methyl alcohol and, after dilution with water, the excess of alkali titrated with standard acid, no acid properties are indicated by methyl orange, but if phenolphthalein be used as indicator the xanthoxylin N behaves like a monobasic acid. 1.0127 grams xanthoxylin N neutralized 7.9 cc. N/2 potassium hydroxide. Calculated for $C_{16}H_{14}O_4$ as a monobasic acid, 7.7 cc. If the neutralized liquid is set aside in a stoppered flask it turns red again in about an hour. On now adding more standard acid till the red color disappears and setting the liquid aside, it again assumes a red color on standing. This phenomenon is repeated till an amount of acid is added which is equivalent to the amount of alkali used. The whole operation occupied about three days. Hence xanthoxylin N shows the phenomenon of slow (abnormal) neutralization like some pseudo-acids studied by Hantzsch.

Towards resorcinol xanthoxylin N behaves like an anhydride of a dibasic acid, giving the phthalein reaction with great brilliancy. On heating xanthoxylin N with four times the amount of resorcinol and a small amount of fused zinc chloride to 150° till a drop of the melt becomes soluble in cold dilute potassium hydroxide (about two hours), dissolving the melt in dilute alkali and then adding an excess of acetic acid, a yellow precipitate is formed which is very little soluble in water or dilute acids but dissolves in dilute alkali with intense blue fluorescence. A blank experiment with resorcinol alone showed that the phthalein reaction was due solely to the xanthoxylin N, as the resorcinol melt gives with alkali only a green fluorescence but no yellow precipitate upon addition of acids.

It follows from these experiments that of the four oxygen atoms of xanthoxylin N the function of only one is established with certainty as belonging to a methoxyl group. As to the other three oxygen atoms it is as yet impossible to say whether they are

present in the form of a $(R.CO)_2O$ group, or a $\begin{array}{c} R-CO \\ | \\ R-CO \end{array} \rangle O$ group

or in some other form. Further work which I have in hand will, I believe, clear up the subject.

XANTHOXYLIN S.

The xanthoxylin S existing in southern prickly ash seems to be nearly related to the xanthoxylin N of the northern bark. Analysis seems to indicate that it is the next lower homologue of xanthoxylin N, *i. e.*, it contains CH_2 less than xanthoxylin N.

Xanthoxylin S does not behave towards potassium hydroxide and carbon dioxide like xanthoxylin N, and the method used for the separation of the latter from the oil cannot be used for the separation or purification of xanthoxylin S. As the xanthoxylin S does not seem to be affected by boiling alcoholic potassium hydroxide, it might be possible to separate it from the oil by saponifying the oil and shaking out with benzene or ether. I intend to try this method later on. For the present, I have obtained a small amount of the xanthoxylin S in the following way:

The powdered southern prickly ash was exhausted with benzene, the latter distilled off completely, and the oily residue mixed with twice its volume of petroleum ether. On standing over night a considerable amount of a crystalline sediment separated out. The crystals were taken up with cold ether, the ethereal solution filtered, the ether distilled off, and the residue recrystallized repeatedly from hot alcohol.

Thus obtained the crystals were snow-white, and melted at $119-120^\circ$.

Analysis gave the following results:

(1) 0.28585 gram substance gave 0.71587 gram CO_2 and 0.1429 gram H_2O .

(2) 0.1150 gram substance gave 0.2901 gram CO_2 and 0.0575 gram H_2O .

Calculated for $\text{C}_{14}\text{H}_{12}\text{O}_4$: C, 68.81; H, 4.96. Found: C, 68.54; H, 5.62.

No methoxyl group could be found by Zeisel's method. The xanthoxylin S is possibly an alcohol or a phenol of which xanthoxylin N is the methyl ether. The formula of xanthoxylin S could then be written $\text{C}_{14}\text{H}_{11}(\text{OH})\text{O}_3$, and that of xanthoxylin N, $\text{C}_{14}\text{H}_{11}(\text{O}\cdot\text{CH}_3)\text{O}_3$. The figures obtained by the elementary analysis of xanthoxylin S would also correspond to the formula

$C_{21}H_{18}O_6$. An estimation of the molecular weight by the freezing-point method with benzene as solvent, gave as an average 326. The formula $C_{14}H_{12}O_4$ gives 244.12; the formula $C_{21}H_{18}O_6$ gives 366 for the molecular weight. A study of other derivatives will be required for the establishing of the exact formula of xanthoxylin S. This work is to be continued.

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[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE.]

INVESTIGATIONS ON THE PROPERTIES OF WHEAT PROTEINS.

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I. THE SEPARATION OF THE PROTEINS.

THE proteins of wheat according to the work of Osborne and Voorhees,¹ are five in number, *viz.*, gliadin, glutenin, an albumin, a globulin and proteose. The first of these, gliadin, is soluble in 70 per cent. alcohol. Glutenin is insoluble in alcohol, and together with gliadin constitutes about 80 to 85 per cent. of the total proteins of the wheat. The other three proteins, *viz.*, the albumin, the globulin and the proteose are soluble in dilute salt solutions and together constitute about 15 to 20 per cent. of the total proteins present.

This separation of the proteid substance of wheat into these five individual proteins is not accepted by all who have worked upon the problem, *e. g.*, F. Kutscher² claims, from a study of the cleavage products, that there are but three proteins in wheat, *viz.*, gluten casein, corresponding to Osborne and Voorhees' glutenin, gliadin, readily soluble in 60 per cent. alcohol, and gluten fibrin, slightly soluble in 60 per cent. alcohol. Ritthausen³ had previously distinguished four proteins, one insoluble in alcohol, gluten casein, and three soluble in alcohol, *viz.*, gliadin, gluten fibrin and mucedin. Kutscher⁴ claims, from a study of the products of hydrolysis, that mucedin and gliadin are the same.

¹ Am. Ch. J. 15, 392 (1893).

² Z. physiol. Chem. 38, 111 (1903).

³ "Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Oelsamen" (Bonn, 1872, Dissert.).

⁴ Loc. cit.