colored compound. The choice of the meta linkage is, of course, purely arbitrary. The formula given accounts not only for the color of the substance, but explains the ease with which it passes into aniline nitrate by the addition of water, whereas, on the other hand, it also indicates that it should change readily into a nitraniline. Investigations regarding this last point are about to be made.

In the case of the *N*-substituted anilines where nitraniline derivatives are obtained with ease, we feel justified, at present, in regarding their formation as being due, primarily, to an addition of nitric acid to the nucleus. In cases where nitrosamines are the chief products, it is evident that the substituted amine group participates in the change. In all probability, in most cases, both reactions occur simultaneously, the experimental conditions, including the nitrating material, and the nature of the *N*-substituents determining which shall be the predominant reaction in any given instance. The question of the "protection" of the amino group has been already discussed in another connection in two of our earlier papers.[1]

The work is being continued at this university in the hope that it may throw further light on the very important and highly interesting questions referred to above. The present contribution can be regarded only as a preliminary survey of some of the ground which it will be necessary to explore before anything like positive conclusions can be reached.

The investigation described in the preceding pages was carried out at the Johns Hopkins University during 1906–07; it is being continued in this University by Mr. C. E. Burke under the direction of the senior author.

McMaster University, Toronto, Canada,
July, 1907.

THE ISOLATION OF DIHYDROXYSTEARIC ACID FROM SOILS.[2]

By Oswald Schreiner and Edmund C. Shorey.

Received August 8, 1908.

In the previous paper[3] dealing with the isolation of picoline carboxylic acid from soil it was pointed out that the productivity of a soil depends largely upon the condition of the organic matter in the soil and the processes which are at work in destroying plant remains. The great lack of knowledge, from a chemical point of view, concerning the organic matter of soils was also emphasized. It may not be out of place to remark that this chemical ignorance concerns an important soil ingredient,

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[1] *Am. Chem. J.*, **36**, 607 (1906); This Journal, **30**, 1395.

[2] Presented at the Chicago meeting, December, 1907, of the American Chemical Society by permission of the Secretary of Agriculture.

[3] This Journal, **30**, 1295.

the presence of which makes soil out of what would otherwise be rock powder or sand. There are in most soils tons of this organic matter to the acre-foot, matter which is being constantly changed through the activity of micro- and macro-organisms or growing crops, through cultivation and fertilization, matter which includes bodies toxic to vegetation, other bodies probably beneficial, and still other bodies wholly inert, but matter about which there is very little chemical knowledge.

As a result of the survey of the literature on this subject it might be stated that the most conspicuous feature is lack of specific knowledge concerning the organic matter of the soil. The humus bodies are a group of compounds of unknown composition and constitution, some of which seem to resemble the natural animal pigments known as melanins, themselves bodes of indefinite composition and unknown constitution, apparently of a protein nature. Of the humus bodies it might be said in illustration that their chemistry is as indefinite as was that of the essential oils in the middle ages and the term "humus bodies" as now used is fully as comprehensive as that of "essential oils," comprising in the latter case all volatile products obtainable from plant materials and in the former all dark-colored products obtainable from soils, vegetable minerable deposits, animal products, etc. It will be safe to say that the chemistry of these soil products will be as diverse and as interesting a study, both scientific and practical, as the chemistry of the volatile oils has proven itself to be. There is in the literature evidence that amino compounds are present in certain soils, but beyond this there is no knowledge what member or members of this large group of nitrogenous bodies may be present. The presence of waxes, resins, and other difficultly decomposable plant residues is indicated, and finally the isolation of a single definite and crystalline nitrogenous compound, picoline carboxylic acid, which doubtless constitutes but a very small portion of the organic matter.

The evidence that the toxic bodies which cause infertility are organic compounds, is both direct and indirect. As indirect evidence there is the absence of inorganic toxic material in the soils investigated; the ready changes in many cases from a toxic to a non-toxic form by oxidation, whether by the roots of growing plants or by cultivation; the similar changes effected by the addition of chemicals, both fertilizer salts and others; the change in toxic properties in many cases on heating and in other cases the carrying over of toxicity on distillation; and the removal of toxicity on burning. As final proof that there are in soils organic bodies harmful to plants there is, as shown in the paper referred to and in the present paper, the identification and isolation from the soil of organic bodies which are toxic to plants and behave in solution as does the extract from an infertile soil.

The extension of these investigations involves among others, three problems, each complex in itself. These are the identity and nature of the toxic bodies in soils; their origin or the way in which they arise or get into the soils; and finally the relation of toxic bodies to each other, to non-toxic or even beneficial bodies and to added bodies such as fertilizers both organic and inorganic. These questions are very complex and it would seem most improbable that the practice of agriculture could ever become scientific except through the complete clearing up of the questions involved. Every effort should therefore be made to shed light on the identity, source, and general properties, including destruction or alteration, of toxic soil compounds. It is only through a full understanding of the scientific principles involved that the practice of agriculture can be placed on the same plane with other great industries which have profited so much by chemical research in the past, a development in which agriculture has not shared to its fullest extent.

With the first of the problems stated, the isolation, identification and general properties of a toxic soil compound the present paper is chiefly concerned. The comparatively slight toxicity of picoline carboxylic acid isolated from the Takoma soil described in the earlier paper referred to, coupled with the apparently very small amount of this body present in this soil led to the conclusion that there must be some bodies other than this present, bodies of much more toxic character and perhaps not in any way connected with the one obtained or others indicated. With the object of obtaining, if possible, such other bodies from this soil a number of other methods or soil treatments were tried.

One which finally gave definite results was as follows: The soil was treated with 2 per cent. solution of sodium hydroxide as in the method first used. This alkaline extract was made acid with a slight excess of acetic acid and filtered from the humus precipitate. There was thus obtained a dark colored extract containing organic matter, sodium acetate, acetic acid, and some mineral matter from the soil. This acid extract was shaken out with ether and the ether allowed to evaporate on the surface of a small quantity of water in a warm place. The ether on evaporating left on the surface of the water some colored resinous material together with nodules or concretions of a crystalline body. On heating the water to boiling these crystals went into solution while the resinous matter remained undissolved and on pouring the solution on a filter previously wet with hot water the resinous material remained on the filter. On cooling the solution so obtained crystals separated, which generally appeared as plates or leaflets arranged in radiating clusters. These, if still colored, could be purified by solution in hot water and filtering as before. This body, which is white when pure, melts at 98–

99°, is little soluble in cold water, somewhat readily in hot, and quite soluble in alcohol or ether. A water solution is acid to litmus and decomposes barium and calcium carbonate, forming the corresponding salts, which are difficultly soluble in cold water. This body was found to be quite toxic to wheat seedlings.

With the object of extending the research to other soils, six more or less infertile soils, which had been selected for this purpose, were treated as follows: Eight hundred grams of each were treated with 2000 cc. of a 2 per cent. sodium hydroxide solution for twenty-four hours, with frequent shaking. After standing, 1000 cc. of the supernatant clear extract were treated with a slight excess of acetic acid and filtered. The filtrate was shaken out twice with ether and the ether allowed to evaporate on the surface of a small quantity of water, the water boiled and filtered and the filtrate cooled. The character of any crystals which formed and the approximate amount was observed. From four of the soils examined there was obtained crystalline material having the same general appearance and properties as that obtained by the same method from the Takoma soil. The amount of this material obtained from 1000 cc. of the original extract was then dissolved in water and made up to 1000 cc. with distilled water and used as a culture medium for wheat seedlings. Dilutions of this solution with one and nine parts of pure distilled water were also used. The results obtained were in direct relation to the amount of crystalline material, the solution containing the greatest portion being the most toxic. The soil from which, by this treatment, the most material was obtained and which furnished the most toxic medium was from Tennessee, a soil which had been in cultivation for more than fifteen years, for the most part in cotton. The soil is classified as Clarksville silt loam, and although light colored, contains 3.26 per cent. organic matter and 0.16 per cent. nitrogen. The surface soil, which was collected, is a gray silt loam 6 inches deep. The subsoil to a depth of three feet is a yellow silt loam, having a close compact structure. The field is in a large area of this type, the Clarksville silt loam occupying the broad, nearly level top of a ridge. There were a few apple trees in the field but the sample was taken at some distance from them. The yields of cotton have been light. On an area of about fifty feet square, from which the sample was taken, the plants were only about one-half to one-fourth as large as in other parts of the field and matured very few bolls. Where vacancies had occurred in the cotton rows, corn had been planted. The same difference existed in the corn as in the cotton. Near where the sample was taken the stalks were small, with a yellow color, and had failed to form good ears. In other parts of the field the corn had a good color and average sized ears had been produced. The east half of the field contains twenty apple trees set five years ago. One tree stands about thirty feet from where the sample was taken and is smaller than the trees further away, thus agreeing with the growth of the cotton and the corn in different parts of the field.

A water extract of this soil was a poor medium for the growth of wheat seedlings and was improved by treatment with carbon black.

Having then a soil unproductive in the field, toxic in the laboratory, and yielding by simple treatment a crystalline body also toxic, the foundation was laid for the isolation of a toxic soil compound in sufficient quantity to determine its identity and study its properties. Large quantities of this soil were treated by the method already mentioned, extracting with 2 per cent. sodium hydroxide solution, acidifying with acetic acid, filtering from the humus precipitate, shaking out the acid filtrate with ether and crystallizing the water-soluble portion of the ether extract from water. By this treatment, extracting each portion of the soil twice, the body described was obtained in a fairly pure condition in amounts of 0.05 gram per kilo of soil, which is 50 parts per million or 200 pounds per acre to the depth of one foot. The quantity actually present in the soil must, of course, be much larger, for when all is said the methods of extraction are very crude and far from quantitative and the amount in a crystalline form does not even represent the amount extracted. It is, of course, impossible to say what relation exists between the amount extracted and the amount present, except that the amount present is much the greater, perhaps even several times as great.

The properties of this compound, some of which have already been noted, are: Slight solubility in cold, more ready solubility in hot water, ready solubility in alcohol and ether. It crystallizes in radiating clusters or concretions, and when pure in very small plates or leaflets, which are generally arranged in star-like groups as shown in the microphotograph in Fig. 1. The melting point is 98–99°. Calcium and barium salts are readily formed by decomposition of the corresponding carbonates. These are little soluble in cold water, but quite readily in hot water. The barium salt crystallizes in somewhat characteristic circular concretions of radiating structure. The silver salt is amorphous and quite insoluble in water.

All the properties of this compound agree with those of dihydroxystearic acid prepared from elaïdic acid.

Analysis:

Calculated for $C_{18}H_{36}O_4$, C, 68.3; H, 11.4

Found, C, 67.5, 68.0; H, 12.2, 11.7.

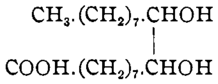
Analysis of the silver salt gave 25.77 per cent. Ag. Calculated, 25.53.

The melting point of the soil compound remained unchanged when this was mixed with dihydroxystearic acid prepared from elaïdic acid.

The figures together with the correspondence noted between the properties of the body obtained from the soil and those of dihydroxystearic acid from elaïdic acid are sufficient to establish the identity of the soil compound as dihydroxystearic acid.

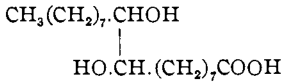
There are four isomeric dihydroxystearic acids known, three being laboratory products. The one found in the soil is that obtained in the laboratory by the oxidation of elaïdic acid and described by Saytzeff.[1] This acid can easily be prepared, starting with oleic acid. If oleic acid be treated for a few minutes at a low temperature with nitrous anhydride, generated in any convenient way, say by the action of nitric acid on arsenic trioxide, it is changed to the isomeric elaïdic acid. This, after washing with water, and recrystallizing from ether or alcohol, melts at 45–47°. If this elaïdic acid be dissolved in a dilute solution of potassium hydroxide and treated with potassium permanganate it is oxidized, dihydroxystearic acid being one of the products. The yield obtained in this way is low, the dihydroxystearic acid first formed being easily subject to further oxidation, pelargonic and azelaic, and finally oxalic acids being formed. Edmed[2] in studying the oxidation products of oleic and elaïdic acids obtained from the latter by this treatment with alkaline permanganate, dihydroxystearic acid, 33 per cent.; pelargonic acid, 13 to 14 per cent.; azelaic acid, 26 per cent.; and oxalic acid, 15 to 20 per cent. The dihydroxystearic acid formed from elaïdic acid is much more easily oxidized than the isomeric acid obtained by oxidation of oleic acid and this has been explained by the juxtaposition of the hydroxyl groups in the former as shown in the structural formulas.

Dihydroxystearic acid m. p. 99°
from elaïdic acid.



$$\begin{array}{c} CH_3.(CH_2)_7.CHOH \\ | \\ COOH.(CH_2)_7.CHOH \end{array}$$

Dihydroxystearic acid m. p. 134°
from oleic acid.



$$\begin{array}{c} CH_3(CH_2)_7.CHOH \\ | \\ HO.CH.(CH_2)_7COOH \end{array}$$

The known dihydroxystearic acids, other than that obtained by the oxidation of elaïdic acid and found in soils, are that obtained by the oxidation of oleic acid already mentioned, melting at 134°; that obtained from isoleic acid, melting at 79°; and a natural product found in castor oil melting at 140–141° and described by Juillard[3] and Meyer.[4]

Besides the differences in melting points noted, these acids show quite important differences in their behavior towards solvents, but all agree in

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[1] *J. prakt. Chem.*, **33**, 300 (1886); **50**, 76 (1894).

[2] *J. Chem. Soc.*, **73**, 627 (1898).

[3] *Bull. soc. chim.*, **13**, 238 (1895).

[4] *Arch. Pharm.*, **235**, 184 (1897).

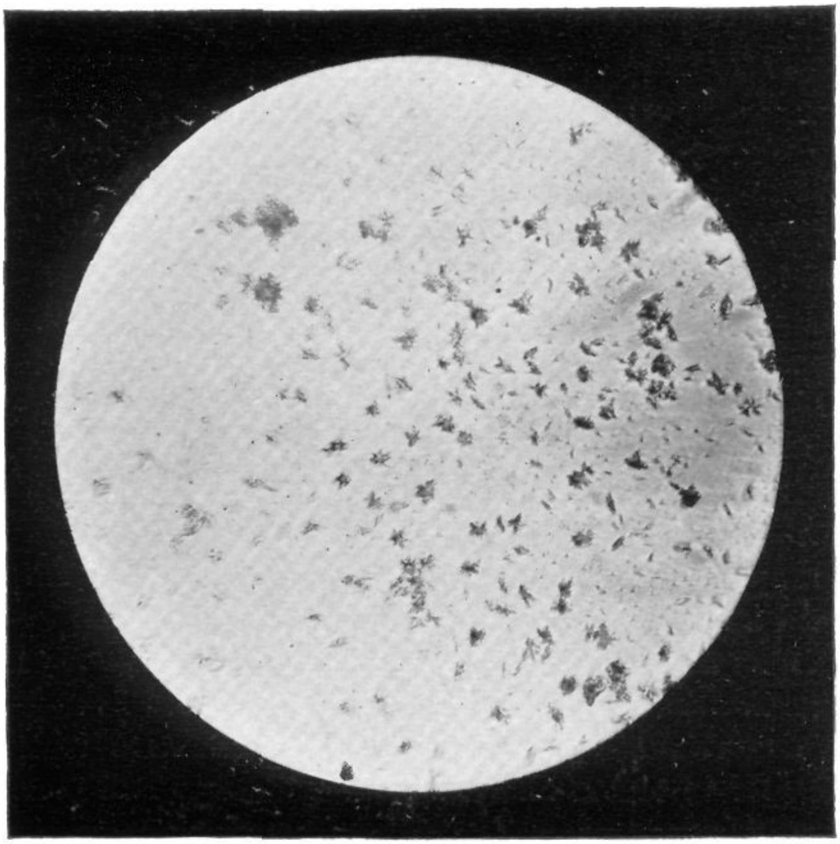

Fig. 1.

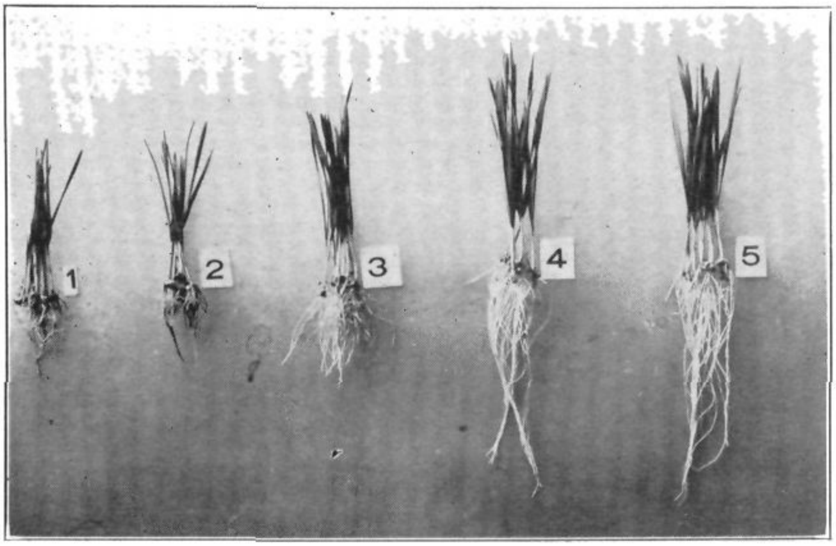

Fig. 2.

being little soluble in cold water. They are all monobasic acids, forming well-defined salts.

In describing the method used in isolating dihydroxystearic acid from the soil, mention was made of a resinous body which was present in the ether extract and which was separated from the dihydroxystearic acid after evaporation of the ether by filtration of the hot solution. This resinous material, which at the temperature of boiling water is a brown oil, becomes at room temperature a thick viscous mass. It is not soluble in water or dilute acids. In dilute alkalies it forms a slimy emulsion and dissolves readily in alcohol and ether. So far no crystalline body has been obtained from this material, nor has it been possible to make any crystalline derivatives. No characteristic color, or other reactions, have been noted and it is not known whether it is a simple body or a mixture. Elementary analysis gave: C, 63.84; H, 9.65. It is very insoluble in water and when wholly free from dihydroxystearic acid it does not impart any toxic properties to distilled water on boiling with it.

Concerning the possible origin of dihydroxystearic acid in the soil, it might not be amiss to state briefly some of the results obtained, although no definite conclusion can as yet be drawn from them. The Takoma soil, from which picoline carboxylic acid had been isolated, was, as stated above, also found to contain dihydroxystearic acid in almost as large amounts as that from Tennessee. This Takoma soil seems to be a good medium for the growth of fungi and it was noted that the rootlets of the oak trees growing on this ground, were infested with mold. This growth of mold was at times so abundant that in digging up the small roots and shaking free of soil the attached mold mycelia furnished as much material as the roots themselves. A quantity of these roots with mold attached was washed free of adhering soil and treated just as the soil had been in the isolation of dihydroxystearic acid. There was obtained in this way a small quantity of crystalline material having the appearance and properties of the dihydroxystearic acid isolated from the soil. The crystalline form, melting point, solubility, formation and appearance of barium salt all correspond with those of the body isolated from the soil and there is little or no doubt of their identity. Similar oak roots from another locality where they were not infested with mold were treated in the same way, but no indication whatever was obtained of the presence of dihydroxystearic acid or any other crystalline body, by this method. It would seem to be a fair conclusion from this that mold can form dihydroxystearic acid from material associated with oak roots and that in this case at least the dihydroxystearic acid found in the soil may have been formed in this way. As to what material furnishes the foundation for this formation of dihydroxystearic acid by fungi, whether it be root excretions or root tissue or cells, it is possible at present to theorize only, and such discussion does not come within the scope of this paper. It may, however, not be out of place to mention that the conditions existing in nature in this case are similar to those shown in the laboratory to lead to the formation of dihydroxystearic acid. Oleic acid, as part of the complex molecules of fats and readily decomposed lecithins, is present in all living parts of plants; nitrous acid, by which oleic acid is changed to elaïdic acid, is constantly being formed in soils, either as a product of denitrification or as one of the stages of nitrification, and finally there are oxidizing agents—the air, enzymes in living roots, and microörganisms.

The dihydroxystearic acid isolated from the soil was obtained without much difficulty in quantity sufficient for cultural experiments and as its method of preparation was such that it could easily be obtained free from any reagents which might have a toxic effect, the body obtained from the soil was used in testing the toxicity of this compound. As a preliminary test a saturated solution of dihydroxystearic acid, which was found to contain approximately 200 parts per million at room temperature, was used as a culture solution for the growth of wheat seedlings. Ten days' growth of the seedlings in this solution gave a green weight of plants 52.5 as compared with 100 for the same number of seedlings in distilled water for the same time. The water transpired by the plants growing in dihydroxystearic acid solution was 12.3 against 100 transpired by the plants growing in distilled water.

The toxicity of this compound in not very high concentration being thus established, culture sets comprising several concentrations were then grown, the result of the ten days' growth being as follows:

EFFECT OF DIHYDROXYSTEARIC ACID ON WHEAT SEEDLINGS.

	Solution.	Relative transpiration.	Relative green weight.
5	Control in distilled water	100	100
4	Dihydroxystearic acid 20 parts per million	75	87
3	" " 50 " " "	56	78
2	" " 100 " " "	24	53
1	" " 200 " " "	20	54

The plants are shown in Fig. 2, the numbers in the figure corresponding with the numbers in the table.

The toxicity of this body, it will be noted, is as great in a concentration of 100 parts per million as in a concentration of 200 parts per million, which is approximately a saturated solution, and the toxic effect is marked even when the concentration is as low as 20 parts per million.

Artificial dihydroxystearic acid prepared as described from elaïdic acid as well as the isomeric dihydroxystearic acid formed on the oxidation of oleic acid gave in concentration of 200 parts per million practically the same results, very little transpiration, green weight about half of that in the control solutions, and death of the plants in 12 or 15 days.

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THE COLORIMETRIC ESTIMATION OF BENZALDEHYDE IN ALMOND EXTRACTS.

BY A. G. WOODMAN AND E. F. LYFORD.

Received August 10, 1908.

The recent successful application of the reaction with fuchsine-sulphurous acid to the colorimetric determination of acetaldehyde[1] and of citral[2] suggested its similar use for the estimation of small amounts of benzaldehyde. Such a method would find immediate use in the assay of commercial almond extracts, for which the methods at present given in text-books are entirely inadequate, yielding results often 40 to 50 per cent. too low. A procedure based on the colorimetric estimation has been worked out and is described in this paper.

Preparation of Reagents.

Fuchsine-Sulphurous Acid.—0.5 gram of pure fuchsine is dissolved in 100 cc. of water and a freshly prepared solution of sulphurous acid containing twenty grams of sulphur dioxide is added. When the solution is decolorized, sufficient water is added to make a liter. The amount of sulphur dioxide should be determined by titration with iodine, or, if freshly prepared, its strength can be determined with sufficient accuracy from its specific gravity as found with a Westphal balance. A convenient method of preparing the solution is to balance the fuchsine solution on a scale pan and then to pass in a current of sulphur dioxide until the weight has increased by twenty grams.

In order to get a colorless solution, pure fuchsine should be used, and the solution can be relied upon for about ten days, the sulphuric acid formed by the oxidation of the sulphur dioxide decreasing its sensitiveness so much in that time that it is better to use a fresh solution. This strength of sulphur dioxide was chosen after various experiments as giving less trouble in the use of alcohol containing traces of aldehyde, and being at the same time sufficiently sensitive. The difference in color produced by 0.05 milligram of benzaldehyde is readily detected.

It has been stated by Prud'homme[3] that benzaldehyde reacts but slightly with the ordinary fuchsine-aldehyde reagent and he has suggested

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[1] Tolman, THIS JOURNAL, 28, 1625.

[2] *Ibid.*, 1472.

[3] *Bull. soc. ind. Mülhouse*, 74, 169.