[CONTRIBUTION FROM THE LABORATORY OF SOIL FERTILITY INVESTIGATIONS.]

GUANINE FROM A HEATED SOIL.[1]

BY ELBERT C. LATHROP.

Received July 1, 1912.

The isolation from soils of the purine bases, xanthine,[2] hypoxanthine[3] and adenine,[4] has already been reported from these laboratories, and a study of the occurrence of these bases in many soils has been made.[5] During the course of an investigation on the chemical changes in soil organic matter, brought about by means of steam sterilization, guanine was isolated and identified.

The soil, Sassafras silt loam, which contained no guanine in the unheated state, was heated in an autoclave at 30 lbs. steam pressure for 3 hours. After cooling, 50 lbs. of the heated soil were extracted with 20 gals. of 3% sodium hydroxide solution for 7 hours, with constant stirring. After standing over night the clear solution was siphoned off, made acid with sulfuric acid, filtered through a filter press, carefully neutralized with sodium hydroxide, and concentrated in a vacuum pan to a volume of about 3 gals., at a temperature of about 52°. The concentrated solution was made strongly alkalin with sodium hydroxide, filtered from the precipitate of iron and aluminum hydroxides, and the purine bases precipitated by boiling with Fehling's solution and a little dextrose, after the method described by Balke,[6] and used for the isolation of the purine bases in this laboratory.[7] The precipitate was separated by filtration, washed and suspended in rather a large volume of boiling water and treated while hot with hydrogen sulfide. The filtrate from the copper sulfide was concentrated to a volume of about 100 cc. on the water bath, and when cool, was made slightly alkalin with ammonia and precipitated with an ammoniacal solution of silver nitrate. The gelatinous precipitate was washed, dissolved in boiling nitric acid, sp. gr. 1.10, and filtered hot. On standing for several hours a crystallin precipitate formed, which, after being filtered off and washed, was suspended in water and treated with hydrogen sulfide. After separating the silver sulfide the solution was again concentrated. A portion of this solution on evaporation with nitric acid left a yellow residue, which on treatment with a drop of sodium hydroxide turned a red color and on heating became purple, the xanthine test.

To the remaining solution ammonium hydroxide was added. A pre-

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[2] Schreiner and Shorey, *J. Biol. Chem.*, **8**, 391 (1910).

[3] *Ibid.*, **8**, 392 (1910).

[4] Shorey, *Bull.* **88**, Bur. Soils, U. S. Dept. Agr., 1912.

[5] Schreiner and Lathrop, *J. Frank. Inst.*, **172**, 145 (1911).

[6] *J. prakt. Chem.*, [2] **47**, 539 (1893).

[7] Shorey, Bur. Soils, *Bull.* **74**, 41 (1910).

cipitate immediately formed, which after standing a short time, was filtered off, washed with a little cold water and then dissolved in warm dilute hydrochloric acid. On evaporating a little of this solution to dryness with nitric acid and placing the dish containing the residue in an atmosphere of ammonia vapor, a pink color was developed. The solution therefore was divided into two portions. To the first portion, which had been previously warmed, a saturated solution of picric acid was added, when the picrate described by Capranica[1] separated out in crystallin form, which, under the microscope, appeared as pencil shaped, fern-like tufts of orange yellow needles. They were very insoluble in water and on drying took on a felt-like appearance with a silky luster, and became golden yellow in color. On heating they became orange red and began to decompose at 190°.

To the other portion a solution of potassium dichromate was added and on standing the dichromate described by Wulff[2] separated out in large, well formed, bright orange colored, elongated, four-sided prisms, with truncated ends. This salt was allowed to dry and was then heated to about 100°, when it lost water and turned a dark violet color.

By the method of obtaining this base, its solubility in water, ammonium, hydroxide and hydrochloric acid, the solubility of the silver nitrate salt in nitric acid, sp. gr. 1.10, the various color reactions and the two characteristic salts, the picrate and the dichromate, the identity of the compound is established as guanine.

This base, $C_5H_5N_5O$, which Fischer[3] has shown to be 2-amino-6-oxypurine, was first discovered in 1844 by Unger,[4] as a constituent of guano. It has since been met with in a very large number of tissues, both animal and vegetable, in the lungs, pancreas, liver, the excrement of birds and of the common garden spider.[5] In plants it has been found in the juice of turnips,[6] in the juice of ripe sugar cane,[7] in beer yeast,[8] in grass, in legumes, and in cucurbita.[9]

Kossel[10] has shown that together with xanthine, hypoxanthine and adenine, guanine is a decomposition product of nucleic acids and nucleoprotein. Bang[11] prepared from Hammerstein's pancreas-nucleoprotein a nucleic acid containing only the base guanine, and called it guanylic

[1] *Z. physiol. Chem.*, **4**, 233 (1880).
[2] *Ibid.*, **17**, 488 (1893).
[3] *Ber.*, **30**, 2241 (1897).
[4] *Ann.*, **59**, 58.
[5] Gorup, Will, *Ibid.*, **69**, 117.
[6] Lippmann, *Ber.*, **29**, 2653 (1896).
[7] Shorey, THIS JOURNAL, **21**, 609 (1899).
[8] Schindler, *Z. physiol. Chem.*, **13**, 442 (1889).
[9] Schulze, Bossard, *Ibid.*, **9**, 420 (1885).
[10] *Ber.*, **18**, 1928 (1885).
[11] *Z. physiol. Chem.*, **26**, 133 (1898).

acid. Schulze and Bossard[1] found in young vetch, clover, ergot, etc., a base, $C_{10}H_{13}N_5O_5$, which they called vernine, and which on hydrolysis with hydrochloric acid yielded guanine. Later Levene and Jacobs,[2] by partial hydrolysis of yeast nucleic acid, obtained a compound which they called guanosine, which on further hydrolysis with the hydrochloric acid yields guanine. This compound and vernine are identical.

The purine bases are susceptible to the action of microörganisms and enzymes, and are thereby transformed one into the other. Schindler[3] observed the transformation of adenine and guanine into hypoxanthine and xanthine, respectively, during the process of putrefaction. That putrefactive bacteria, especially the colon bacillus, are able to effect this change was also shown by Schittenhelm and Schröter.[4] Burian[5] showed that xanthine and hypoxanthine in the animal organisms are oxidized to uric acid by an oxid zing enzyme. Adenine and guanine, however, are not susceptible to this change, and must first be deaminized by enzymes of another character, being converted thereby to hypoxanthine and xanthine.[6] On the other hand, Kutscher[7] found that in the steril auto-digestion of yeast, xanthine and hypoxanthine soon disappeared, leaving only guanine and adenine.

It would seem then that the sources of guanine in the soil are various, although the largest amount, no doubt, is formed from nucleoprotein material. In the heated soil there is a possibility of three sources. It may either be formed from the hydrolysis of guanosin, guanylic acid, or other nucleic acids. As to the presence of the first two in soil there is doubt, but nucleic acids were isolated as such from the unheated soil. Since the guanine was not found in the unheated soil, it must have arisen in the heated soil from the breaking down of higher forms of nitrogenous material, probably nucleic acids.

It is interesting to note that guanine, as yet, has not been found in unheated soil. Xanthine and hypoxanthine have been found in quite a number of soils; adenine in two soils. That guanine is formed in the soil in the same manner as the other purine bases, there can be no doubt, but it is apparent that it does not remain there long. What becomes of it cannot be stated, but it would seem very probable that through the action of microörganisms and enzymes it is changed into xanthine, and the process does not necessarily stop even there. In this respect of rapid change guanine resembles arginine and adenine, which, although found in

[1] *Z. physiol. Chem.*, **10**, 80 (1886).
[2] *Ber.*, **42**, 2469, 2474 (1909).
[3] *Z. physiol. Chem.*, **8**, 432 (1884).
[4] *Ibid.*, **32**, 203 (1901).
[5] *Ibid.*, **43**, 497 (1905).
[6] Jones and Austrian, *Ibid.*, **43**, 110 (1904); *J. Biol. Chem.*, **3**, 227 (1907).
[7] *Z. physiol. Chem.*, **32**, 66 (1901).

soils, are of rather infrequent occurrence, and do not seem to retain their chemical identity long.

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ON THE COLORING MATTERS IN ALFALFA, ALFALFA INVESTIGATION, III.[1]

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Received July 1, 1912.

That chlorophyll plays one of the most important parts in the physiology of the plant cannot be gainsaid, but whether or not its function is directly connected with the fixation of atmospheric nitrogen by leguminous plants is not known. At any rate a knowledge of the character and quantity of the chlorophyll in alfalfa is indispensable to a study of its metabolic functions.

There is now no longer a question that chlorophyll, the green coloring matter in leaves, is a mixture of two distinct chemical substances. These components of chlorophyll are both green in color, but in solution one is of a darker green than the other.

A difference of opinion has existed among chlorophyll chemists regarding the ratio in which the two components are present,[2] but after an exhaustive study of the chlorophyll of some twelve varieties of leaves, Jacobson and Marchlewski[3] arrived at the conclusion that the components do not occur in a definit ratio in different species of plants, and that the ratio may not be the same in the same species. In a later communication the same authors[4] have presented two methods for determining the two components, neo- and allo-chlorophyll, in the presence of one another.[5]

One of these methods, namely the photographic, was employed for investigating the alfalfa chlorophyll. This method is based upon the fact that the absorption bands of neo- and allochlorophyllan in the ultraviolet part of the spectrum are different and that these bands, which are not influenced to an appreciable extent by impurities, are photographed and the photograph compared with a standard series.

[1] In recent years the chemistry of chlorophyll has been greatly advanced by Prof. Marchlewski's optical methods and so I decided to apply these methods to my chlorophyll products obtained from alfalfa. This investigation was carried out in Prof. Marchlewski's laboratory at Cracow, Austria; and it is with a deep sense of gratitude that I acknowledge his many valuable suggestions and the use of his laboratory appliances.

[2] *Proc. Roy. Soc.*, **21**, 442 (1873); *Ber.*, **41**, 1352 (1908); *Trans. Chem. Soc.*, **77**, 1080 (1900); *Biochem. Z.*, **35**, 413 (1911).

[3] *Am. Chem. J.*, **47**, 221 (1912).

[4] *Ibid.*, **47**, Aug. (1912).

[5] This paper appeared first in the *Bull. Acad. Sci., Cracovie*, Feb., 1912.