of a few drops of piperidine the color of the solution indicated the completion of the reaction in about one day. Upon acidification, the yellow crystals of the monomethylureide were precipitated. This product is soluble in alcohol or acetone; fairly soluble in chloroform, acetic ester or ligroin; insoluble in ether, benzene or carbon tetrachloride.

Calculated for $C_5H_7N_3O_4$: C, 34.64; H, 4.04
Found: C, 34.84; H, 3.88

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[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, SECTION OF AGRONOMY, IOWA AGR. EXPT. STA.]

THE CHEMICAL NATURE OF THE ORGANIC NITROGEN IN THE SOIL.

[SECOND PAPER.]

BY S. L. JODIDI.

Received October 26, 1911.

By applying the Hausmann-Osborne[1] method, as well as the formaldehyde[2] titration method perfected by Sörensen, the writer[3] has demonstrated that the acid-soluble organic nitrogen occurring in Iowa soils and in Michigan peat soils is made up chiefly of acid amides, monoamino acids and diamino acids. In the meantime, and in part before those publications, there appeared several papers dealing with the isolation of organic nitrogenous compounds from soils. Thus, Schreiner and Shorey succeeded in isolating from various soils the diamino acids arginine[4] and histidine, as well as the pyrimidine[5] derivative, cytosine, and the purine[6] bases, xanthine and hypoxanthine. Prior to that Shorey extracted from Hawaiian soils a compound which he identified as picolinecarboxylic acid.[7] Among the decomposition products of Michigan peat the monoamino acids leucine[8] and isoleucine were isolated and identified by Robinson, who also quantitatively determined the amino[9] nitrogen in the peat by Van Slyke's[10] nitrous acid method.

Adding, as the above researches do, to our knowledge concerning the nature of organic compounds in soils, yet it seems desirable, if not im-

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[1] *Z. physiol. Chem.*, 27, 95 (1899); 29, 47 (1900); 29, 136 (1900); 31, 215 (1900). THIS JOURNAL, 25, 323 (1903).

[2] *Biochem. Z.*, 7, 47(1907); 7, 407 (1908).

[3] THIS JOURNAL, 32, 396 (1910); 33, 1226 (1911). *Technical Bull.* 4, (1909) Mich. Agr. Exp. Sta.; *Research Bull.* 1, (1911) Iowa Agr. Exp. Sta.

[4] *J. Biol. Chem.*, 8, 381 (1910).

[5] *Ibid.*, 8, 385 (1910). *Bull.* 74, Bureau of Soils, U. S. Dept. Agr.

[6] *Ibid.*

[7] Ann. Rept. of Hawaii Agr. Exp. Sta., 1906, p. 55.

[8] THIS JOURNAL, 33, 564 (1911).

[9] Michigan Agr. Expt. Sta., *Techn. Bull.* 7, p. 11.

[10] *Ber.*, 43, 3170 (1910).

perative, to throw more light upon a question of such significance, bearing in mind that the question is of fundamental importance to agriculture since it is intimately connected with the momentous biological processes of ammonification, nitrification and denitrification. It is for this reason that it was decided to investigate an additional number of plots as to the nature of the organic nitrogen contained in them. The plots S, T, V, herein examined, form a part of the experiment station field which is situated on the Wisconsin drift. The plots, having the size of one-tenth of an acre each, were treated as follows: In 1906 they received a heavy application of manure, in 1907, 1908 and 1909 none. They grew corn in 1905 and 1906, oats and clover in 1907, corn in 1908 and 1909. The soil samples were taken in the fall of 1909 and represent composite samples of twenty-four borings taken from each plot to the depth of seven inches.

It was soon ascertained that the proportion of ammoniacal and nitric nitrogen in the plots under consideration was insignificant, from which it followed that the soil nitrogen consisted of organic nitrogenous compounds. Their separation into acid amides, diamino acids and monoamino acids was performed according to the Hausmann-Osborne method, namely by distilling the evaporated acid extract of the soil with mag-

TABLE I.—PERCENTAGE OF NITROGEN IN THE VARIOUS COMPOUNDS.

Plot.		Gram.	% of oven-dried soil.	% of total soil nitrogen.	% of nitrogen in solution.
S.	Total nitrogen in solution (obtained by boiling with hydrochloric acid)	0.16069	0.1844	87.37	100.00
	Ammoniacal nitrogen	0.00081	0.0009	0.44	0.50
	Nitrogen of acid amides	0.05397	0.0619	29.35	33.59
	Nitrogen of diamino acids	0.02773	0.0318	15.08	17.26
	Nitrogen of monoamino acids (difference from 100)	0.07818	0.0897	42.51	48.65
T.	Total nitrogen in solution (obtained by boiling with hydrochloric acid)	0.20247	0.2199	92.41	100.00
	Ammoniacal nitrogen	0.00099	0.0011	0.45	0.49
	Nitrogen of acid amides	0.06789	0.0737	30.99	33.53
	Nitrogen of diamino acids	0.04029	0.0438	18.39	19.90
	Nitrogen of monoamino acids (difference from 100)	0.09330	0.1013	42.58	46.08
V.	Total nitrogen in solution (obtained by boiling with hydrochloric acid)	0.12675	0.2216	88.98	100.00
	Ammoniacal nitrogen	0.00062	0.0011	0.43	0.49
	Nitrogen of acid amides	0.04207	0.0735	29.53	33.19
	Nitrogen of diamino acids	0.01797	0.0314	12.61	14.18
	Nitrogen of monoamino acids (difference from 100)	0.06609	0.1155	46.40	52.14

nesia, which gave, in the form of ammonia, all the nitrogen corresponding to the amides present in the soil. The residue which remained on distillation of the soil extract with magnesia was thoroughly extracted with water and concentrated to 100 cc., to which sulfuric acid and enough phosphotungstic acid were added to throw down the diamino acids present in the extract. The determination of the nitrogen in the washed phosphotungstic acid precipitate gave the amount of the diamino nitrogen. The percentage of the monoamino nitrogen was obtained by subtracting the sum of ammoniacal, amido, and diamino nitrogen from 100. The results secured are recorded in Table I.

While the phosphotungstic acid precipitate and the filtrate from that precipitate, in the case of protein decomposition products, virtually represent diamino acids and monoamino acids respectively, it may not hold good for soil extracts. However, the facts presented in Table II go to show that the substances designated in Table I as diamino and monoamino acids actually display reactions and possess properties which are characteristic of those compounds.

As was pointed out elsewhere,[1] not all of the nitrogen obtained by the Hausmann-Osborne method as such, of diamino and monoamino acids actually represent, diamino and monoamino nitrogen, but that a portion of that nitrogen belongs to classes other than amino acids. To ascertain just what proportion of that nitrogen is virtually made up of diamino and monoamino acids, the soil extracts from the plots S, T, V were subjected to formaldehyde titration. Before presenting the results, however, a few notes may not be amiss in connection with the formaldehyde titration method. In order to avoid mistakes, it is necessary to use for titration not too small amounts of the amino acids; the greater the quantity employed for the formaldehyde titration the more reliable are the results. Further, it is of advantage to start the formaldehyde titration, not in the alkaline but in the acid state, *i. e.*, the soil extract to be titrated is slightly acidified with hydrochloric acid so as to require, say, from one to three cc. of 0.2 N barium hydroxide. The operations in question are conveniently carried out as follows: The soil extract containing amino acids, upon examination of the optical activity, is made up to a definit volume, say, to 60 cc., for which the nitrogen in 15 cc. is determined by the Kjeldahl method to ascertain the total nitrogen present in the sample, 15 cc. are titrated in the ordinary way to determin the acidity of the substance, and two portions of 15 cc. each are titrated with formaldehyde to find the increase of acidity due to the reaction with formaldehyde. These three operations furnish all the data which are necessary for the calculation of the percentage of diamino or mono-

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[1] THIS JOURNAL, 33, 1239 (1911).

TABLE II.

Diamino acids.		Monoamino acids.	
Reactions.	Rotation.	Reaction with formaldehyde.	Rotation.
The diamino acids extracted from the various plots (S, T, V) displayed the following reactions: 1. Phosphotungstic acid gave a heavy, white precipitate. 2. Phosphomolybdic acid gave a yellow precipitate. 3. Mercuric chloride gave a grayish white flocculent precipitate. 4. Silver nitrate gave a grayish or yellowish white precipitate, soluble in excess of ammonia. 5. Picric acid either failed to give a precipitate, or the precipitate obtained after some time was quite insignificant. 6 The aqueous solution of the diamino acids was strongly alkaline. 7. Addition of neutralized formaldehyde to the alkaline solution caused it to turn acid, pointing to the presence of carboxyl and amino groups.	S. The diamino acid solution containing 0.006 g. nitrogen, on acidulating with hydrochloric acid, rotated in the 2 dm. tube +0.09° (Ventzke). T. The rotation of the acidulated diamino acid solution, with 0.008 g. nitrogen, was +0.22° (Ventzke). V. The diamino acid solution, with 0.007 g. nitrogen, having been strongly acidulated with hydrochloric acid, showed the rotation of +0.20° (Ventzke).	The monoamino acids extracted from the soils examined, on being mixed with neutralized formaldehyde, immediately turned acid, thus indicating the presence of carboxyl and amino groups.	S. The monoamino acid solution, with 0.042 g. nitrogen, having been acidulated with hydrochloric acid, showed in the 2 dm. tube the rotation +0.61° (Ventzke). T. The monoamino acid solution containing 0.040 g. nitrogen, rotated —0.75°. When acidulated with 5 cc. concentrated hydrochloric acid, the same solution showed the rotation +0.1° (Ventzke). V. The aqueous solution of the monoamino acids, extracted from about 150 grams soil, showed the rotation —0.73 (Ventzke). Acidulated with 3 cc. concentrated hydrochloric acid, the same solution showed the rotation +0.5° (Ventzke).

amino acids present in the substance under examination. The results are given in Table III.

While the data before us to a certain degree modify the results reported in previous publications, especially as far as the percentage of diamino and monoamino acids is concerned, yet this modification does not go far enough to shift the ratio of the acid amides to the diamino and monoamino acids. Here, as in the earlier publications, the percentage of monoamino acids is predominant, next in proportion follow the acid amides, and finally the diamino acids. The percentage of the ammoniacal nitro-

TABLE III.

Diamino acids.

Plot S.—The soil extract containing diamino acids was made up to 60 cc., of which 15 cc. were titrated in the usual way and required 0.2 cc. 0.2 *N* barium hydroxide, and 2 portions of 15 cc. each were titrated with formaldehyde and required on the average 0.6 cc. 0.2 *N* barium hydroxide. This means that the increase of the acidity caused by the presence of diamino acids corresponds to 0.4 cc. 0.2 *N* barium hydroxide. Unfortunately, 15 cc. of the soil extract oxidized according to Kjeldahl were lost, so that the percentage of the diamino acids could not be calculated.

Plot T.—The soil extract supposed to contain diamino acids was made up to 60 cc. The nitrogen determination in 15 cc. showed that they contained 0.006 g. nitrogen. 15 cc. titrated in the ordinary way required 1.47 cc. 0.2 *N* barium hydroxide; two portions of 15 cc. each titrated with formaldehyde required 2.04 cc. 0.2 *N* barium hydroxide. This means that the 60 cc. substance, through formaldehyde, increased in acidity equivalent to 2.28 cc. 0.2 *N* barium hydroxide or 0.01280 g. nitrogen (53.3 per cent.), if lysine alone were present; or 0.01921 g. nitrogen (80.0 per cent.), if histidine alone were present; or 0.02561 g. nitrogen (106.7 per cent.), if arginine only were present. While these figures do not give the actual percentage of each one of the diamino acids, they do show that a large portion of what is given in Table I as nitrogen of diamino acids actually represents diamino nitrogen.

Plot V.—60 cc. of the soil extract were analyzed as in S. It was found that the increase of acidity through formaldehyde was equal to 0.67 cc. 0.2 *N* barium hydroxide. The Kjeldahl determination in 15 cc. of this solution was unfortunately lost by accident, which rendered the calculation of the percentage of diamino nitrogen impossible.

Monoamino acids.

Plot S.—Sixty cc. of the soil extract containing monoamino acids were treated as follows: the nitrogen determination in 15 cc. gave 0.01407 g. nitrogen; 15 cc. were titrated and required 2.2 cc. 0.2 *N* barium hydroxide; two portions of 15 cc. each were titrated after adding formaldehyde and required on the average 5.61 cc. 0.2 *N* barium hydroxide. This means that 3.41 cc. 0.2 *N* barium hydroxide equivalent to 0.00957 g. nitrogen, or 68.02 per cent. of what is given in Table I as nitrogen of monoamino acids, actually represent monoamino nitrogen. The other 31.98 per cent. belongs to classes other than monoamino acids.

Plot T.—The titration with formaldehyde of 60 cc. substance, with 0.0552 g. nitrogen, showed that 82.03 per cent. of what is given in Table I as nitrogen of monoamino acids actually represent monoamino nitrogen, the rest consisting of nitrogenous compounds other than monoamino acids.

Plot V.—The substance containing monoamino acids was analyzed with the following result: 15 cc. were found to contain 0.0097 g. nitrogen. The increase of acidity in 15 cc. substance, due to the reaction with formaldehyde, was equivalent to 2.97 cc. 0.2 *N* barium hydroxide, which is equal to 0.00834 g. nitrogen. Or 85.98 per cent. of what is recorded in Table I as nitrogen of monoamino acids actually represent monoamino nitrogen.

gen is insignificant. In comparing the results, the differences of which may in part be due to some difference between the soils S, T, V, on the one hand, and Θ,[1] U, on the other, it is to be borne in mind that whereas in laboratory or pot experiments one can easily get good average samples, it is quite different with field or plot experiments. In the first place it is not an easy task to obtain one or several tons of manure, hay or straw as a uniform and homogeneous mass. Nor is it possible absolutely evenly to distribute such organic materials throughout the plots. Likewise, it is very difficult, if at all possible, to get a truly average soil sample from a plot of the size of one-tenth of an acre. It is for these reasons that samples from the same plot, and still more so from different plots, though treated with the same organic materials, may in some measure differ from each other. This may account for some differences in the results reported, yet, on the whole, the data secured in the above experiments confirm the data presented in the former[1] publications.

Conclusions.

1. The principal portion of the acid-soluble organic nitrogen, contained in the soils investigated, is made up of acid amides, monoamino acids and diamino acids.

2. The larger part of the phosphotungstic acid precipitate, recorded in Table I as nitrogen of diamino acids, actually represents diamino nitrogen, the smaller part belonging to classes other than diamino acids.

3. In the case of the filtrate from the phosphotungstic acid precipitate, presented in Table I as nitrogen of monoamino acids, it was found that from 68.02 to 85.98 per cent. of that filtrate represent in fact monoamino nitrogen, the rest (from 31.98 to 14.02 per cent.) consisting of nitrogenous compounds other than monoamino acids.

[FROM THE LABORATORY OF FERTILITY INVESTIGATIONS.]

THE ISOLATION OF CREATININE FROM SOILS.[2]

BY EDMUND C. SHOREY.

Received November 2, 1911.

In the course of investigations into the nature of the organic constituents of soils a crystallin organic compound was isolated and identified as creatinine.

Reactions indicating the presence of this compound were first observed in a solution of a portion of the soil organic matter obtained in the following manner: The soil was treated with 2% sodium hydroxide solution for half an hour, and then without separation of the alkaline extract from the soil, acetic acid in slight excess was added and the acid solution filtered from the soil and precipitate formed. The acid filtrate was neutralized with sodium hydroxide and, without filtering, a solution of

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[1] *Loc. cit.*

[2] Published by permission of the Secretary of Agriculture.