6. The total sulfur trioxide precipitated at Madison, Wis., with the rain amounted in the five months of June to October, 1910, inclusive, to 11.7 pounds per acre. The annual amount may tentatively be placed at 15–20 pounds.

7. The losses of sulfur trioxide by drainage, based on the analysis of the drainage waters at Rothamsted, England, and on a yearly drainage of 10 inches, would amount to about 50 pounds per acre yearly.

8. Even with much less loss by drainage it does not appear that the atmosphere can serve as a complete compensating factor for losses of sulfur trioxide which soils sustain through both cropping and drainage. The partial depletion of the sulfur of the soil by continued cropping without fertilization is evidence in support of this view.

9. From the data here presented it appears that for permanent and increased production of farm crops such systems of fertilization must be inaugurated as will supply to the soil from time to time, in addition to the elements now recognized as necessary, a sufficient quantity of sulfur to meet the losses sustained by cropping and drainage.

10. Such sources of sulfur are farm manures; the trade fertilizers, such as superphosphate, ammonium sulfate and sulfate of potassium; and the so-called soil stimulant, gypsum or calcium sulfate.

No attention, so far as we are aware, has been directed to this problem in America. It is hoped that the thesis here presented may be made the subject for further research by chemists and agronomists and the relative importance and necessity for sulfur in systems of fertilization finally established.

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TWO COMPOUNDS ISOLATED FROM PEAT SOILS.

BY CHAS. S. ROBINSON.

Received February 11, 1911.

Although the organic nitrogenous compounds in peat have been under investigation for over a hundred years the actual isolation of such compounds in the crystallin form had been attained only very recently. Suzuki,[1] by means of Fischer's esterification method, succeeded in isolating several of the mono- and di-aminoacids from a natural humic acid. Previous to this, Shorey[2] had isolated picolinecarboxylic acid from a soil containing 12.47 per cent. humus and Schreiner and Shorey[3] have since isolated this compound from several other soils. They have also isolated several diamino acids, purine bases and pyrimidine derivatives.[4] In all cases they extracted the soil with dilute alkali.

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[1] *Bull. Coll. Agric. Tokyo*, 7, 513; abst. in *Chem. Zentr.*, 1907, II, 1.

[2] *Rept. Agr. Exp. Sta. Hawaii*, 1906, 37.

[3] U. S. Dept. of Agr., Bur. of Soils, *Bull.* 53.

[4] *Ibid.*, *Bull.* 74.

Isolation of Leucine and Isoleucine from Peat.

The peat used for this purpose was a brown peat of a type common to this country. After drying at 110° it contained 2.53 per cent. nitrogen of which 9.18 per cent. was in the amido form. This was determined by heating with sulphuric acid, filtering and distilling off the ammonia after making the liquid alkaline with magnesium oxide. In the air-dried state it contained 11.18 per cent. moisture and 16.00 per cent. ash. It was ground in a mill so that practically all of it would pass through an 80-mesh sieve.

The nitrogenous material was extracted by heating it for forty-eight hours on the sand bath with 25 parts by weight of 25 per cent. sulphuric acid in a flask fitted with a reflux condenser. While still hot, the dark brown liquid was filtered, with suction, through a Buchner funnel having a solid unglazed, porcelain bottom. The insoluble material was returned to the flask, heated with a fresh supply of water and again filtered. This was repeated several times until the wash water came through colorless. The filtrate and washings were made alkaline with slaked lime and the precipitate of calcium sulfate allowed to settle. The supernatant liquid was then poured through a filter and the precipitate washed several times by decantation with hot water. The liquid thus obtained was concentrated to about one-third its volume and filtered from a small precipitate of calcium sulfate and humus. Barium hydroxide was added to the filtrate in slight excess and the precipitate of barium sulfate filtered off and thoroughly washed.[1] The hydroxides of barium and calcium remaining in solution were then removed by passing carbon dioxide into the hot solution and filtering off the precipitated carbonates by suction. By this process all of the sulfuric acid can be removed together with the reagents used for its removal, leaving only the products of the extraction in solution. Most of the brown, amorphous, humus material is held back so that at the end one has, at most, only a light brown solution. The amount of mineral matter was very small and was precipitated as a light-colored, sandy powder, during the concentration, before the organic matter came down. After removing this small precipitate, the solution was made slightly acid and a solution of phosphotungstic acid slowly added until it failed to cause a further precipitation. The precipitate thus formed was light gray and flocculent. It was filtered off and washed with hot water. Both the filtrate and precipitate were then boiled with an excess of barium hydroxide and filtered, after which the excess of barium hydroxide was removed with carbon dioxide and the

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[1] Where only small quantities of peat and sulphuric acid are used, it is, of course, better to precipitate all of the acid with barium hydroxide. When large quantities of acid are being used, however, it is more economical to precipitate the bulk of it with lime and use the barium only for that which remains in solution as calcium sulfate.

solutions concentrated. The one resulting from the decomposition of the phosphotungstic acid precipitate yielded only a brown, viscous mass, which failed to crystallize.

A light yellow scale formed on the surface of the other solution and as the concentration was continued, waxy knobs and clusters were formed on the sides of the dish. This material was separated from the rather oily mother liquor, dissolved in water and, after boiling with animal charcoal, recrystallized. After several recrystallizations it was obtained colorless, separating in waxy knobs and clusters when allowed to crystallize slowly or as a white crust when the mother liquor was more concentrated. It was further purified by precipitation from a concentrated aqueous solution with absolute alcohol. After being purified in this way it crystallized in white flakes. It was readily soluble in water but almost insoluble in absolute alcohol and most organic solvents. The aqueous solution was neutral to litmus. When the solid substance was heated in a capillary tube it softened at 230° and decomposed at 265-269°, giving a white crystallin sublimate in the upper part of the tube. The mother substance analyzed as follows:

0.0696 g. subst. gave 6.7 cc. N at 25° and 748 mm.

Calculated for $C_6H_{13}O_2N$: N, 10.68. Found, 10.56 per cent.

Copper Salt.—Several hundred milligrams were dissolved in boiling water and the solution saturated with copper carbonate. Carbon dioxide was evolved and the solution turned dark blue. A light blue scum formed on the surface. The solution was filtered hot and concentrated, upon which a dark blue crystallin copper compound separated out. It gave 19.63 per cent. copper; calculated for $(C_6H_{12}NO_2)_2Cu$, 19.64 per cent.

These figures indicated that it might be one of the aminocaproic acids of which leucine is the most prominent. The light blue scum which had been filtered off was dissolved in water, in which it was difficultly soluble. From this it was evident that the original material contained two compounds, one forming a copper salt easily soluble in water, the other forming a copper salt difficultly soluble in this solvent. As was shown by Ehrlich, such is the case with leucine and isoleucine. His method for separating these two substances was based on the fact that the isoleucine copper salt is readily soluble in cold concentrated methyl alcohol while the corresponding leucine compound is practically insoluble. Unfortunately this ideal condition of affairs does not hold absolutely when both substances are present. In a mixture of these compounds some of the leucine copper salt dissolves in the alcoholic solution of the isoleucine salt while some of the latter is held back in the crystals of the former. For complete purification of the leucine, Ehrlich[1] suggested that, after extracting as thoroughly as possible, the leucine

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[1] *Ber.*, 37, 1809 (1904).

copper salt be decomposed with hydrogen sulfide, the copper sulfide be filtered off and the copper salt again formed and extracted as before. Several repetitions of this procedure should remove all of the isoleucine. Recrystallization from water removes the leucine completely from the isoleucine.

Leucine.—The solution of the mixed copper salts as originally obtained was taken to dryness on the steam bath. The resulting light blue, crystallin mass was extracted with small portions of cold concentrated methyl alcohol until the solvent was no longer colored after having been shaken some time with the salt. The solid residue was then dissolved in water and decomposed with hydrogen sulfide. After filtering off the copper sulfide the solution was saturated with copper carbonate, the excess filtered off and the solution taken to dryness as before. This copper salt was again extracted with methyl alcohol and the process repeated twice. The last extraction removed only a trace of copper from the solid residue. This was then dissolved in water, and decomposed with hydrogen sulfide. The copper sulfide was filtered off and the solution concentrated. It was levorotatory. The white crystallin substance which separated out gave the following rotation in 20 per cent. hydrochloric acid.

0.0455 gram substance in 11.1373 gram solution (0.4085 per cent.; 1.086 sp. gr.) gave +0.40° (Ventzke) rot. in a 2 dm. tube at 20°, sodium light.

$$[\alpha]_D^{20} \text{ found} = +15.62.$$
$$[\alpha]_D^{20}\ l\text{-leucine} = +17.3.$$

When heated quickly in a capillary tube it melted at 287°. The copper salt gave the following figures for nitrogen and copper:

0.0585 gram substance gave 5.0 cc. moist nitrogen (28°, 738 mm.).

0.0291 gram substance gave 0.0072 gram CuO.

Calculated for $(C_6H_{12}O_2N)_2Cu$: N, 8.65; Cu, 19.64. Found: N, 9.10; Cu, 19.76 per cent.

Isoleucine.—The combined methyl alcohol extracts from the leucine were concentrated. After drying at 110°, the dark blue copper compound analyzed as follows: 0.0539 gram substance gave 0.0133 gram CuO.

Calculated for $(C_6H_{13}O_2N)_2Cu$: Cu, 19.64. Found, 19.72 per cent.

After recrystallizing once from water, the copper was precipitated by hydrogen sulfide and the solution concentrated. The pure, white crystallin compound was filtered from the mother liquor, washed with alcohol and ether and dried at 110°. It melted at 272° when heated quickly. It had the following specific rotation:

In water, 0.3227 gram substance in 14.6053 grams solution (2.21 per cent.; 1.0053 sp. gr.) gave +1.1° (Ventzke) rotation in a 2 dm. tube at 20°, sodium light.

$[\alpha]_D^{20}$ found = +8.58.
$[\alpha]_D^{20}$ for isoleucine = +9.74.

In 20 per cent. hydrochloric acid, 0.1965 gram substance in 13.4543 grams solution (1.46 per cent.; 1.0871 sp. gr.) gave +3.25° (Ventzke) rotation in a 2 dcm. tube at 20°, sodium light.

$[\alpha]_D^{20°}$ found = +35.47.
$[\alpha]_D^{20°}$ isoleucine = +36.80.

It analyzed as follows for nitrogen:

Calculated for $C_6H_{13}O_2N$: N, 10.68. Found: N, 10.89 per cent.

From these results there can be no doubt as to the identity of the two compounds in question. Both of them are well known decomposition products of protein and one of them, leucine, was among the compounds isolated by Suzuki from humic acid.

Compounds from Black Peat.

Compounds from Black Peat.—A sample of black peat described by Jodidi[1] was extracted with 25 per cent. sulfuric acid and the extract treated as above. Upon concentrating the sulfate-free liquid, the characteristic yellow scale formed. It was separated from the mother liquor and purified as in the previous case. The quantity was too small to permit of a separation of the isomers. The material was converted into the copper salt which analyzed as follows:

0.0720 gram substance gave 0.1169 gram CO_2 and 0.0485 gram H_2O.
0.0302 gram substance gave 0.0075 gram CuO.

Calculated for $(C_6H_{12}NO_2)_2Cu$: C, 44.48; H, 7.47; Cu, 19.64.
Found: C, 44.29; H, 7.53; Cu, 19.85.

The substance was evidently a mixture of the two compounds described above.

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FASTING STUDIES. III. NITROGEN PARTITION OF TWO MEN THROUGH SEVEN-DAY FASTS FOLLOWING THE PROLONGED INGESTION OF A LOW-PROTEIN DIET; SUPPLEMENTED BY COMPARATIVE DATA FROM THE SUBSEQUENT FEEDING PERIOD.

BY PAUL E. HOWE, H. A. MATTILL, AND P. B. HAWK.

Received February 13, 1911.

Introduction.

The metabolism of fasting men has been studied by a number of investigators but accurate studies of the nitrogen partition during fasting, in

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[1] Mich. Exp. Sta., *Technical Bull.* 4, 25.