short, corpuscular protein, zein. The results are in very satisfactory agreement with sedimentation, diffusion and viscosity measurements on this protein when compared on the basis of a prolate ellipsoidal molecule roughly 300–400 Å. in length.

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[CONTRIBUTION FROM THE DEPARTMENT OF FUEL TECHNOLOGY OF THE PENNSYLVANIA STATE COLLEGE]

The Chemistry of Nitrogen in Humic Acids from Nitric Acid Treated Coal[1]

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The partial oxidation of coals yields acidic substances which resemble the naturally occurring humic acids of peat and brown coals in many respects. A particularly effective oxidizing reagent for this purpose is concentrated nitric acid because of the rapidity[2] with which it attacks coal and yet does not carry the oxidation appreciably beyond the humic acid stage. Similar results have been obtained with 1 *N* nitric acid,[3] but a longer period of heating is required. Nitrogen, as well as oxygen, is introduced into the coal and while the character of the oxygen-containing groups has been fairly well established[2,3,4] nothing appears to be known about the nitrogen groups. In an attempt to find a method for determining the nature of these groups, a study has been made of the action of alkaline permanganate under a variety of conditions on these humic acid-like compounds as well as on selected nitrogen-containing substances and the original coal for comparison.

The coal from which the humic acids were made was of the high volatile A bituminous rank and was taken from the Upper Freeport bed in central Pennsylvania. An analysis appears in Table II. The humic acids were prepared by heating the coal with concentrated nitric acid followed by extraction with alkali and precipitation with dilute hydrochloric acid. Four variations in the method of treating the acids were used. Procedure 1 consisted of determining (a) the amount of ammonia which was liberated from the humic acids by vigorous oxidation with alkaline permanganate, (b) the amount of nitrogen converted to the nitrate ion, and (c) the amount of carbon oxidized to carbon dioxide and volatile acids. Procedure 2 consisted of determining the amount of nitrogen in the humic acids which could be distilled as ammonia by alkaline hydrolysis before oxidizing them with permanganate and continuing as in Procedure 1. Procedure 3 consisted of alkaline hydrolysis, followed by alkaline reduction with zinc, and continuing as in Procedure 1. Procedure 4 consisted of alkaline hydrolysis and reduction in one operation, and then by following Procedure 1.

The results of the experiments on the humic acids and the known compounds are presented in Table I. Those on the humic acids following Procedure 1 indicate that there are at least two forms of nitrogen present. The first of these was liberated as ammonia by alkaline permanganate and amounted to 21.9%. The second was converted to the nitrate ion and amounted to 68.4%. The first form is undoubtedly in a more reduced state because the ammonia was removed in the presence of a strongly oxidizing reagent. The second form appears to be in a higher state of oxidation because it was liberated as the nitrate ion.

Confirmation that a reduced form of nitrogen may be liberated as ammonia by alkaline permanganate oxidation and that an oxidized form of nitrogen may be converted to the nitrate ion was obtained with the known nitrogen compounds appearing in Table I. The amino group in the glycine was freed as ammonia by permanganate oxidation to the extent of 88.5%, while in the case of the more resistant *p*-aminobenzoic acid, only 66.7% of the amino nitrogen was released as ammonia. At the same time 1.0% of the nitrogen in glycine and 8.4% in *p*-aminobenzoic acid was converted to nitrate ion. Under similar conditions only 0.2% of the nitrogen in *p*-nitrobenzoic acid was liberated as ammonia while 39.8% was converted to the nitrate ion. These results indicate that the amount of nitrogen converted to ammonia and nitrate ion by permanganate oxidation depends to a very large degree upon the nature of the nitrogen-containing groups. The low yield of nitrate from *p*-nitrobenzoic acid was undoubtedly due to the resistance of the compound to oxidation because only 21.1% of the carbon was liberated as carbon dioxide and volatile acids. With the humic acids, on the other hand, extensive oxidation occurred and it was possible to account for 93.8% of the carbon and 90.3% of the nitrogen.

Alkaline hydrolysis also liberated ammonia from the humic acids,[5] Procedure 2, Table I, but it was not expected that the quantity would be the same, within the experimental error, as that released by boiling alkaline permanganate. At the same time over half of the remaining nitrogen which appears to be in a higher state of oxidation was reduced to the point where it was evolved as ammonia upon subsequent oxidation with per-

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[1] Presented before the Division of Gas and Fuel Chemistry, American Chemical Society, New York, N. Y., September 14, 1944.

[2] Fuchs and Sandhoff, *Fuel*, **19**, 45, 69 (1940).

[3] Juettner, Smith and Howard, THIS JOURNAL, **57**, 2322 (1935).

[4] Chowdhury and Biswas, *J. Indian Chem. Soc.*, **19**, 289 (1942).

[5] Fuchs and Horn, *Brennstoff-Chem.*, **12**, 60 (1931), first observed this property.

TABLE I

YIELDS OF PRODUCTS

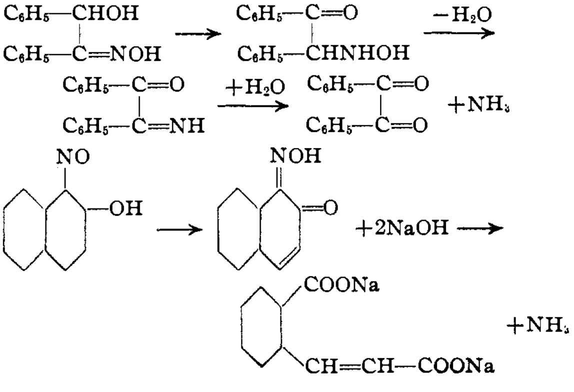
Substance	Procedure	Per cent. total nitrogen converted to: NH_3 by alkaline hydrolysis	NH_3 by alkaline reduction	NH_3 by alkaline oxidation	NO_3^- by alkaline oxidation	Total nitrogen recovered	Per cent. total carbon oxidized to: CO_2	Volatile acids	Total carbon recovered
Humic acids[a]	1			21.9	68.4	90.3	89.8	4.0	93.8
	2	21.3		36.3	31.1	88.7	89.7	..	89.7
	3	22.3	23.7	45.6	4.2	95.8	74.0	12.9	86.9
	4	[b]	43.9	42.1	5.5	91.5	47.2	7.5	54.7
Glycine	2	0.6		88.5	1.0	90.1	95.4	9.9	105.3
p-Aminobenzoic acid	1			66.7	8.4	75.1	86.2	15.2	101.4
	2	0.0		55.8	1.9	57.7	40.4	3.2	43.6
p-Nitrobenzoic acid	1			0.2	39.8	40.0	19.7	1.4	21.1
	2	0.3		0.3	37.8	38.4	8.5	2.9	11.4
α-Benzoin oxime	1			68.1	16.0	84.1	68.9	0.5	69.4
	2	87.2		1.5	2.2	90.9	46.3	3.3	49.6
α-Nitroso-β-naphthol	2	80.7		3.5	0.8	85.0	59.0	6.0	65.0
Upper Freeport bed coal[c]	1			63.8	33.0	96.8	29.9	1.9	31.8
	2	0.3		..	..	..	..	..	...

[a] Total nitrogen on the as-used basis obtained by Beet's improved Kjeldahl method (*Fuel*, **13,** 343 (1934)) was 4.34%; by the improved Dumas method (Fieldner and Taylor, *Ind. Eng. Chem.*, **7,** 106 (1915)), 5.03%. The per cent. of total nitrogen removed from the regenerated humic acids is based on the Dumas analysis. [b] Evolved and included during alkaline reduction. [c] The per cent. of nitrogen was 1.37% by both the Kjeldahl and Dumas methods on the as-used basis.

manganate. This nitrogen amounted to 36.3% while that converted to nitrate ion was 31.1%. These results suggest that the 21.3% nitrogen evolved by the boiling alkali was eliminated by means of an internal oxidation–reduction reaction in which the oxidized nitrogen played the same role as the permanganate in Procedure 1. This was shown to be untenable, however, by boiling the humic acids with an alkaline reducing mixture, Procedures 3 and 4. In Procedure 4, 43.9% of the nitrogen was evolved by boiling alkali and zinc dust. In Procedure 3, 23.7% was evolved after the humic acids were first boiled with alkali. The difference, 20.2%, again checks the amount liberated by the alkaline oxidizing reagent and the alkali alone. Consequently, it appears to be quite certain that this quantity of nitrogen in the humic acids is present in a form which can be released readily by alkaline hydrolysis and that the oxidized form of the nitrogen is not responsible for its release.

The liberation of ammonia by alkaline hydrolysis indicates that some such group as cyanide, amide, or ammonium salt must be present, but such groups are highly unlikely considering how the humic acids were made. The quantity of ammonia evolved corresponds closely with the amount of nitrogen present in the raw coal, but alkaline hydrolysis of the raw coal does not yield appreciable amounts of ammonia (Procedure 2, Table I) and as a source of the ammonia from the humic acids seems improbable. It also seems unlikely that amino nitrogen would yield appreciable quantities of ammonia on alkaline hydrolysis and this was substantiated by the negative results obtained with glycine and *p*-aminobenzoic acid (Table I). Consequently, other nitrogen derivatives were studied to determine whether a reductive hydrolysis releasing ammonia from a group in a higher state of oxidation were not possible. The compounds which were found to behave in this way were α-benzoin oxime which gave up 87.2% of its nitrogen as ammonia in boiling alkali (Procedure 2) and α-nitroso-β-naphthol which gave up 80.7%. In both cases only a small percentage of additional nitrogen was accounted for by conversion to nitrate upon subsequent oxidation. These results are very similar to those observed with the humic acids and suggest a close similarity of the nitrogen-containing groups.

It might be expected that α-benzoin oxime and α-nitroso-β-naphthol would be hydrolyzed by boiling alkali to hydroxylamine. However, since hydroxylamine will not distill from an alkaline solution and is rapidly oxidized by permanganate, the liberation of ammonia indicates that this substance is the primary product of the reaction rather than hydroxylamine. The following reactions would account for the direct elimination of ammonia.



$$\begin{array}{l} C_6H_5\text{—}CHOH \\ \quad | \\ C_6H_5\text{—}C{=}NOH \end{array} \longrightarrow \begin{array}{l} C_6H_5\text{—}C{=}O \\ \quad | \\ C_6H_5\text{—}CHNHOH \end{array} \xrightarrow{-H_2O} \begin{array}{l} C_6H_5\text{—}C{=}O \\ \quad | \\ C_6H_5\text{—}C{=}NH \end{array} \xrightarrow{+H_2O} \begin{array}{l} C_6H_5\text{—}C{=}O \\ \quad | \\ C_6H_5\text{—}C{=}O \end{array} + NH_3$$

The introduction of nitroso or isonitroso groups into the coal substance is quite possible because the nitric acid treatment liberates large amounts of nitrogen oxides. Furthermore, Fuchs[6] has shown that natural humic acids extracted from German brown coal react with nitrogen oxides. These facts, together with the similarity in behavior of the humic acids and the known nitroso and isonitroso compounds, indicate that the humic acids contain these groups to the extent of about 25%.

Since nitro or possibly nitrate ester groups are the only other likely radicals to be introduced by the nitric acid treatment at a higher oxidation level than the nitroso or isonitroso level, it appears probable that the 68.4% of the nitrogen converted to nitrate by permanganate oxidation is in the form of these groups. Considering the method of treating the coal it seems very improbable that appreciable amounts of nitric acid esters were formed and that for the most part nitro groups were introduced. The presence of nitro groups is further suggested by the mildly explosive properties exhibited by these humic acids when heated to about 250° as well as the difficulties experienced with the Kjeldahl nitrogen analysis.[7]

It appears that a part of the nitro groups behave differently on reduction because 23.7% was reduced and evolved as ammonia in Procedure 3, while 45.6% remained in the humic acids and was released as ammonia by permanganate oxidation. The elimination of only a part of the nitrogen during the alkaline reduction indicates that the nitro groups either occupy different positions in the humic acid molecule or that their reduction takes a different course at some intermediate stage. Considering that complete reduction of a nitro group would give an amino group which ordinarily would not undergo hydrolysis to ammonia, it seems more likely that certain structural arrangements in the humic acids favor the elimination of ammonia from some intermediate reduction product. In Procedure 3 the total nitrogen reduced by the zinc and alkali and evolved as ammonia together with that evolved on permanganate oxidation amounted to 69.3%. This checks the quantity oxidized to nitrate ion in Procedure 1, 68.4%, and the amount liberated in Procedure 2 by the permanganate as ammonia and nitrate ion, 67.4% very closely and indicates the fairly quantitative nature of these reactions. In Procedures 3 and 4, small amounts of nitrogen were also oxidized to nitrate ion by the permanganate, 4.2 and 5.5%, respectively, making totals of 95.8 and 91.5%.

In Table I it will be noticed that the values for total nitrogen do not equal 100%. It is possible that the boiling alkaline permanganate is not capable of liberating all of the nitrogen in certain cases or that a part is liberated as free nitrogen or nitrous oxide, but later experiments indicate that the Winkler boric acid technique[8,9,10] used was inadequate to retain all of the ammonia.[11] By using 0.1 *N* sulfuric acid instead of boric acid the amount of nitrogen recovered from glycine as ammonia was raised to 91.5% and by replacing the beaker-receiver with an Erlenmeyer flask the recovery was raised to 98.9%. By using a sintered glass bubbler and a beaker the value was 99.0%. Consequently, it seems likely that with more care taken to retain the ammonia it would be possible to account for the nitrogen of organic compounds which are oxidized by alkaline permanganate in a very nearly quantitative manner, the yield of ammonia and nitrate ion depending upon the character of the substance oxidized.

The application of Procedure 1 to the raw coal resulted in the liberation of 63.8% of its nitrogen as ammonia and 33.0% as nitrate ion. These results tend to indicate that about two-thirds of the nitrogen is in a reduced combination and one-third in a more oxidized state. However, the coal is relatively resistant to permanganate oxidation and appears, even, to follow a different course as compared with that of the humic acids prepared by nitric acid treatment of the coal. Thus it will be noticed in Table I that only 31.8% of the carbon in the raw coal was oxidized to carbon dioxide and volatile acids during the liberation of nitrogen as compared with 93.8% of the carbon of the humic acids. It is true that the raw coal contains some fusain which is particularly resistant to oxidation and which has been removed in the preparation of the humic acids, but the amount is quite insufficient to account for the difference. It is also true that the coal is undispersed at the beginning of the oxidation, but oxidation produces carboxyl groups and dispersion is soon affected. In addition the coal was heated for seventy-seven hours as compared with thirty-five for the humic acids. Similar results were observed by Juettner[12] in his study of the production of mellitic acid from coals and coke. In view of the greater resistance of the coal to oxidation and the observation that more resistant amino compounds yield more nitrate on oxidation (compare *p*-aminobenzoic acid and glycine) it seems probable that a very considerable part of the nitrogen of the coal appearing as nitrate ion is in a reduced state in the coal but became oxidized during the prolonged period of oxidation.

Experimental

Preparation of Humic Acids.—To a 100-g. sample of a high volatile A bituminous coal from the Upper Freeport bed (see analysis in Table II) in a 1-liter flask under a reflux condenser, 20-ml. portions of concentrated nitric acid were added at twenty-minute intervals until a total of 280 ml. (400 g.) had been added. The mixture was then heated on a steam-bath for eighteen hours, filtered, washed

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(6) Fuchs, *Brennstoff-Chem.*, **9**, 178 (1928).

(7) Footnote *a* Table I.

(8) Winkler, *Z. angew. Chem.*, **26**, 231 (1913).

(9) Wagner, *Ind. Eng. Chem., Anal. Ed.*, **12**, 771 (1940).

(10) Taylor and Smith, *ibid.*, **14**, 437 (1942).

(11) Miller, *ibid.*, **8**, 50 (1936).

(12) Juettner, THIS JOURNAL, **59**, 208–213 (1937).

until neutral to "alka-acid" paper and dried on a steam-bath.

Nineteen such batches were combined, thoroughly mixed, and successive batches of 90 g. were extracted with 600 ml. of 0.5 *N* sodium hydroxide, stirred well, centrifuged, filtered and washed with distilled water, the washings being added to the filtrate. The humic acids were precipitated by the addition of 310 ml. of 1 *N* hydrochloric acid, centrifuged, dried on a steam-bath, further dried for seven hours at 70° at reduced pressure and then washed free from chlorides. Drying the humic acids prevented peptization during washing. After again drying as before, the batches were thoroughly mixed and stored in lots of 100 g. under nitrogen in sealed bottles. Their analysis is given in Table II.

TABLE II

PERCENTAGE YIELDS AND ANALYSES OF PRODUCTS OBTAINED IN THE PREPARATION OF THE HUMIC ACIDS

		Raw coal	HNO_3 treated coal	Humic acids	Alkali insol. treated coal
Yield, moisture-free			107.88	89.24	14.95
Moisture		0.64	8.27	3.21	6.52
Ash		7.62	3.86	1.54	26.52
Moisture- and ash-free	C	86.31	62.01	62.40	68.83
	H	5.42	3.65	3.21	4.15
	N[a]	1.50[b]	4.77	4.56[c]	3.60
	S	1.95	0.57	0.55	0.48
	O	4.82	29.00	29.28	22.94

[a] Beet's improved Kjeldahl method was used, *Fuel*, **13**, 343 (1934). [b] 1.50% was also obtained by the Dumas method, Fieldner and Taylor, *Ind. Eng. Chem.*, **7**, 106 (1915). [c] 5.28% was obtained by the Dumas method.

Procedure 1.—The oxidations were carried out in a 5-liter flask fitted with a Kjeldahl trap and condenser, dropping funnel, and a stopcock opening to the air to equalize changes in pressure within the flask. Forty grams of potassium hydroxide and 60 g. of potassium permanganate were dissolved in 300 ml. of water and heated to boiling. To this was added dropwise a 5.0000-g. sample of the humic acids dissolved in 100 ml. of 10% potassium hydroxide. The distillate was collected in lots of about 300 ml. in 400-ml. high-form beakers containing 10 ml. of saturated boric acid diluted to 75 ml. using a four-opening bubbler to aid in the absorption of the ammonia. The ammonia was titrated with 0.1 *N* sulfuric acid using methyl red indicator. The solution was distilled during the daytime only and until the titration of 300 ml. of distillate was 0.5 ml. of acid or less. Water was added through the dropping funnel as needed. A total of thirty-five hours was required to complete the oxidation of the humic acids.

The extent of the oxidation of the carbon was determined by adding 50 ml. of concentrated sulfuric acid diluted with an equal volume of water to the flask and again distilling. The volatile acids were collected in a liter flask attached to the condenser. The carbon dioxide passing through was dried with anhydrone and then absorbed in a weighed Nesbitt bottle packed with ascarite and anhydrone. A second Nesbitt bottle was attached to protect the weighed bottle from the atmosphere. All of the carbon dioxide was carried into the weighed Nesbitt bottle at the end of the run by removing the water from the condenser and steaming out the condenser and receiver. Suction was also applied at the far end to aid the process. The volatile acids were titrated with 0.1 *N* sodium hydroxide and calculated as acetic. The amount of carbon dioxide was obtained from the increase in weight of the Nesbitt bottle.

The quantity of nitrate ion produced during the oxidation was determined on the residue in the 5-liter flask as follows. The manganese dioxide was filtered and washed well. The filtrate and washings were returned to the flask and reduced by adding 40 g. of 20-mesh zinc and an additional 25 ml. of concentrated sulfuric acid. When the zinc had all dissolved 200 g. of potassium hydroxide was added and the ammonia distilled into boric acid and titrated as before.

Procedure 2.—A 5.0000-g. sample of the humic acids was dissolved in 50 g. of potassium hydroxide in 300 ml. of water placed in the 5-liter flask arranged as described in Procedure 1. The mixture was distilled intermittently until the titration of 300 ml. of distillate took 0.5 ml. of 0.1 *N* acid or less. Thirty-five hours were required.

The humic acids were then oxidized by adding 10-g. portions of permanganate dissolved in water until the color persisted in the boiling solution and no more ammonia distilled out. A total of 40 g. of permanganate was required and twenty hours boiling. The quantities of ammonia, carbon dioxide, volatile acids, and nitrate were determined as described in Procedure 1.

Procedure 3.—A 5.0000-g. sample of the humic acids was boiled with 50 g. of potassium hydroxide as described in Procedure 2 until no more ammonia distilled over. Then a total of 44 g. of zinc dust was added and the mixture again distilled as long as ammonia was evolved. This took a total of forty hours.

The residue was next oxidized by adding 10-g. lots of permanganate as described in Procedure 2. A total of 80 g. of permanganate and thirty-five hours of heating were required. The quantities of products were again determined as described in Procedure 1.

Procedure 4.—A 5.0000-g. sample was boiled with 100 g. of potassium hydroxide dissolved in 300 ml. of water and 44 g. of zinc dust. The ammonia was distilled out as before, a total of one hundred five hours being required.

The residue was then oxidized by adding permanganate and the amount of oxidation products determined as in Procedures 2 and 3. A total of 50 g. of potassium permanganate and thirty-two hours were required.

Summary

The nitrogen in the regenerated humic acids prepared from nitric acid treated bituminous coal is present in two distinctly different forms. The majority is in a highly oxidized state and is liberated as nitrate ion upon permanganate oxidation. A smaller amount is in a less oxidized state and is liberated as ammonia not only by alkaline permanganate but also by boiling alkali.

A comparison of the humic acids with known nitrogen compounds under a variety of oxidizing and reducing conditions indicates that the highly oxidized nitrogen must be in the form of nitro groups while the less oxidized nitrogen is at the nitroso or isonitroso level.

Similar experiments on the raw coal indicate that at least two-thirds of its nitrogen is in a reduced condition, probably at the amine level. The remainder of the nitrogen appears as nitrate ion on oxidation. Very likely this is due to the resistance of the coal to oxidation, although it is possible that coal contains a more or less oxidized form of nitrogen.

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