

HUMIC ACID NITROGEN

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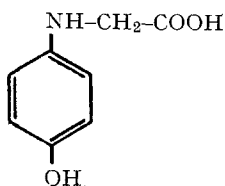
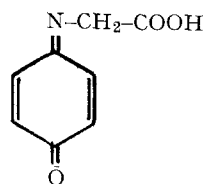
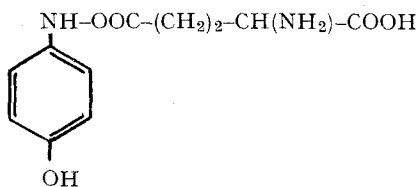
SUMMARY

An explanation has been advanced for the previously reported observation that alkaline hydrolysis of humic acids will release amino acids that are resistant to acid hydrolysis. It is thought that under the alkaline conditions oxidation of N-phenyl-amino acids to the more readily hydrolysed quinonimines occurs. The importance of oxidation in this step has been demonstrated by showing that acid hydrolysis in the presence of hydrogen peroxide will release amino acids that are stable to non-oxidative acid hydrolysis.

In some earlier work ¹ it was reported that after humic acid had been exhaustively hydrolysed by 6N hydrochloric acid, a further yield of amino acids could be obtained if the humic acid residue was hydrolysed by 2.5N sodium hydroxide. In the case of a low molecular weight fraction of humic acid, poor in nitrogen, these supplementary amino acids amounted to twice the yield obtained by acid hydrolysis, and in all cases the yield was significant. No explanation was advanced in the previous paper to account for these results but we now believe that the alkaline hydrolysis releases amino acids from N-phenoxy amino acid structures such as I. Such compounds can be readily formed by reaction of amino acids with phenols ² so their existence in humic acid is very probable. It has been shown that the amino acid will not be hydrolysed from structures such as I until they have been oxidised to the corresponding quinonimine II ³. Phenols are oxidised more readily under alkaline conditions ⁴ thus compounds such as I which are stable to acid hydrolysis could be oxidised and subsequently hydrolysed under alkaline hydrolysis conditions. As these alkaline hydrolyses ¹ were carried out in the presence of air the oxidation would proceed readily.

When proteins are allowed to react with phenols under oxidising

STRUCTURES

(I) N-(p-Hydroxyphenyl)-
glycine(II) N-(Methylcarboxy)-
quinonimine(III) N-(p-Hydroxyphenyl)-
glutamine

conditions, acid hydrolysis of the resulting polymer has been found to release all the amino acid except for the N-terminal amino acid and those amino acids that have a free amino group when incorporated into the protein *e.g.* lysine ⁵. Thus, if humic acid is formed by a complexing reaction between phenolic nuclei and peptides ^{3 6} acid hydrolysis would remove all the amino acids bound by peptide bonds and also those linked to quinone rings. The amino acids bonded to phenolic rings would not be released until the subsequent alkaline hydrolysis. If our concept of the alkaline hydrolysis mechanism is correct, then one would expect the alkaline hydrolysate to contain a preponderance of lysine and other amino acids with an extra functional group.

The results previously reported ¹ show that, compared to the

acid hydrolysates, the subsequent alkaline hydrolysates do consistently contain greater proportions of lysine and ornithine as well as serine, glutamic acid and aspartic acid. The increased proportions of glutamic and aspartic acids suggests that they were originally present as the corresponding amides, and the free amido group reacted with phenolic compounds to produce a structure such as III. This is not unreasonable in view of the substantial amount of amide nitrogen present in humic acid ⁷ and the greater stability of N-substituted amides ⁸.

In order to test the theory postulated and the role of oxidation in the hydrolysis, a sample of humic acid was exhaustively hydrolysed with 6*N* HCl for three 8 hour periods followed by a 24-hour-period. The residue was then hydrolysed by 6*N* HCl containing 3% H₂O₂. Total amino acid in each hydrolysate was measured by reaction with ninhydrin ⁹. From the table it can be seen that the yield of amino acid released by sequential acid hydrolysis quickly fell to very low levels, but subsequent hydrolysis under oxidising conditions produced a large increase in the yield. As the oxidising reagent is sufficiently strong to produce hydrolysable structures such as II from their non-hydrolysable counterparts I, this result is regarded as further evidence for the existence of N-phenoxy amino acids in humic acid.

Although the nitrogen and amino acid contents of humic acid fractions decrease with decreasing molecular weight ^{10 11} the amount

TABLE 1

Total amino acid produced by repeated hydrolysis of humic acid (100 mg)

Hydrolysis	Medium (75 mls)	Time of hydrolysis	Amino acid N released	N as % of total N	N as % of hydrolysable N
1	6 <i>N</i> HCl	8 hours	1.63 mg	35.4	79.0
2	6 <i>N</i> HCl	8 hours	0.093	2.02	4.5
3	6 <i>N</i> HCl	8 hours	0.044	0.96	2.14
4	6 <i>N</i> HCl	24 hours	0.050	1.08	2.41
5	3% H ₂ O ₂ in 6 <i>N</i> HCl	8 hours	0.24	5.30	11.8
			—	—	
			2.06	44.8	

The humic acid originally contained 4.6% N.

of nitrogen found as 'N-phenoxy amino acid' structures remains relatively constant¹. This supports the theory of Swift and Posner¹¹, who suggested that the high molecular weight units of humic acid are broken down by microbial attack and oxidation to form the smaller molecules, with a preferential loss of nitrogenous material. In this process the amino acids incorporated by peptide linkages would be attacked and removed by hydrolytic enzymes, while the amino acids bound to the phenolic nuclei would be stabilised and protected. Thus the proportion of humic acid nitrogen found as 'N-phenoxy amino acids' increases with decreasing molecular weight, finally amounting to most of the total in the smallest molecules. Amino acids bound into structures such as these may account for some of the non-hydrolysable nitrogen that has been reported in humic acid⁷.

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