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Chlorinated Tyrosine in Municipal Waste Treatment Plant Products after Superchlorination

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For years, investigators have been concerned about the possible toxicity of new chlorinated organic compounds formed during the disinfection of drinking water and wastewater with chlorine (BELLAR AND LICHTENBERG 1974, JOLLEY 1974, GLAZE et al.1976). Many organochlorine compounds such as carbon tetrachloride are known to be toxic, and evidence is growing that most organochlorine compounds can be expected to be detrimental to health. A particular candidate for metabolic interference would be a chlorine-substituted naturally occurring amino acid in which the chlorine has replaced the carbon-bonded hydrogen. Since free amino acids are known to occur in sewage (HUNTER 1971) the disinfection step, particularly where "superchlorination" (use of high chlorine doses-typically 2000 - 4000 mg/l) is used, is a likely point for production and discharge of these potentially toxic compounds into the environment.

Early workers (LANGHELD 1909, DAKIN 1917, and WRIGHT 1936), studying the effects of chlorine and other chlorinating agents on amino acids in aqueous solution, found that an N-chloroamine initially is formed, which then undergoes oxidative deamination and/or decarboxylation to give the corresponding aldehyde or nitrile, their proportions depending on pH and chlorine:amino acid ratio:

$$\underset{\substack{|\\ NH_2}}{RCH}_2CO_2H \xrightarrow{[Cl]} RCHO \quad + \quad RCN$$

Later, KANTOUCH and ABDEL-FATTAH (1971) obtained similar results, and stated that with tyrosine, hypochlorite gave the 3-chloro and 3,5-dichloro derivatives which were said to be detected by paper chromatography. No details of the structure elucidation were given, however. Previously, ALEKSLEV et al. (1969) had stated that treatment of L-leucyl-L-tyrosine or oxytocin in 85% formic acid with chlorine-saturated carbon tetrachloride produced 3,5-dichlorotyrosine as shown by paper chromatography and electrophoresis. PEREIRA and coworkers (1973) showed by gas chromatography/mass spectrometry (GC/MS) that

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3-chloro and 3,5-dichlorophenylacetonitrile and the corresponding phenylacetaldehydes were produced by the chlorination of tyrosine, but again no unequivocal evidence for the formation of carbon-bound chlorine in an amino acid was given. In this same study, it was shown that phenylalanine did not appear to ring-chlorinate, and that simple dipeptides underwent only N-chlorination at the terminal amino group.

FIRNAU and FRITZ (1973), after labeling desalted blood serum with ^{82}Br, inferred the existence of 3-bromotyrosine on the basis of the position of bromine activity in the elution pattern of a calibrated cation-exchange column. Recently, SOARES FORTUNATO (1976) reported that oral administration of 3,5-dibromotyrosine to rats inhibited the de novo synthesis of thyroid hormones from inorganic iodine, affecting hormone concentrations in both the thyroid and vascular spaces. This antithyroid action was apparently not closely related to the goitrogenic effect of this compound. In view of the intense toxicity of organochlorine compounds and the above pharmacological data, we would like to report the first detection of 3-chloro and 3,5-dichlorotyrosine in chlorinated municipal waste treatment plant products.

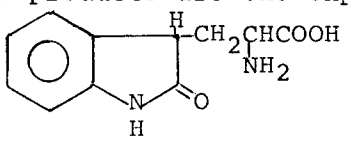
MATERIALS AND METHODS

Laboratory chlorination of standard solutions of amino acids was performed by adding 1 ml of 2000 mg/l (28 µm Cl_2/ml) aqueous chlorine solution to 1 ml of 20 µm/ml amino acid solution adjusted to pH 1-2 with hydrochloric acid. The reaction was allowed to proceed for 30 minutes, then the mixture extracted with 1 ml of ether. The ether solution was analyzed using a Finnigan 3200 GC/MS system. A portion of the aqueous reaction mixture was also analyzed by GC/MS, and the balance of the solution taken to dryness, then derivatized as the N-heptafluorobutyryl n-propyl ester (JÖNSSON 1973). The product was analyzed by GC/MS using a 2 mm I.D. X 1.5 m glass column containing either 5% or 10% SP-2100 (Supelco, Inc.) on Supelcoport 100/120. Programming from 50° to 280°C at 4° min^{-1} was sufficient to separate the amino acid derivatives, using a carrier gas flow rate of 20 ml min^{-1} helium. When it was necessary to separate the amino acids from complex sample matrices, the ion-exchange method of GARDNER and LEE (1973) was employed, followed by ligand exchange as used by SIEGAL and DEGENS (1966), prior to drying and derivatization. When analyzing actual samples, the residual chlorine was quenched at the time of sampling, using sodium sulfite or thiosulfate. Centrifugation and filtration were used to remove the particulate matter.

RESULTS AND DISCUSSION

Laboratory chlorination of alanine, valine, leucine, and phenylalanine gave results in agreement with previous work. Serine and threonine failed to yield any detectable products other than starting material, which was seen to decrease in quantity. Analysis of the ether extract from the reaction with tyrosine showed approximately equal proportions of mono and dichlorinated phenylacetonitrile and phenylacetaldehyde. The chlorine atoms were shown by MS to be on the ring or the methylene group, presumably on the 3 and 5 ring positions; however, the exact position cannot be specified at this time because of the ring expansion process which takes place during the fragmentation. Analysis of the HFBA-n-Pr derivatized portion revealed the presence of chloro and dichlorotyrosine with the chlorine atoms in the same locations, removing any doubt as to the presence of carbon-bound chlorine in these products.

Preliminary results with tryptophane have shown that two of the products are the expected oxindole,



and a chlorooxindole, where the chlorine atom is known to be located in the fused ring system.

Results from the analysis of actual samples are shown in Table 1. Sample 1 is raw sewage from the influent of a municipal waste treatment plant. Sample 2 is superchlorinated anaerobic digestor supernatant from a different municipality, while samples 3 and 4 are superchlorinated combined primary and secondary sludges from still another location. Sample 3 was dechlorinated two hours after sampling while sample 4 was allowed to stand in the laboratory for four days before quenching the remaining chlorine.

The minimum detectable limit for the amino acids except histidine and tyrptophane is between one and eight nanograms by the analytical procedure used. Histidine and tryptophane suffer in the derivatization step, and their MDL's vary, typical values being 182 and 20 ng, respectively. Sample sizes were 0.1,25, 2, and 2 liters, respectively.

SUMMARY

Previous work has shown that 3,5-dibromotyrosine acts as a thyroxine production/secretion inhibitor which may imply a similar response to chlorotyrosine derivatives. Chloro- and dichlorotyrosine have been

TABLE I

AMINO ACIDS IN RAW SEWAGE AND SUPERCHLORINATED EFFLUENTS (1)

	#1 Raw Sewage	#2 Anaerobic Digestor Supernatant	#3 Combined Secondary 2hr quench	#4 Primary & Effluents 4 day quench
Alanine	250	3.9	3.1	3.2
Glycine	40	24.0	8.9	5.1
Valine	200	9.0	11.0	3.2
Threonine	120	3.0	0.9	tr
Serine	40	3.7	2.4	1.2
Leucine	380	-	7.9	tr
Isoleucine	130	9.9	7.1	5.8
Proline	40	-	-	-
Hydroxyproline	50	-	-	-
Methionine	20	-	-	-
Phenylalanine	90	5.0	11.0	3.6
Aspartic Acid	80	-	-	-
Lysine	110	5.0	3.5	3.6
Glutamic Acid	50	-	15.0	4.5
Histidine	130	-	-	-
Arginine	-	-	-	-
Tyrosine	150	3.0	1.4	1.1
Chlorotyrosine	-	3.0	1.0	1.3
Dichlorotyrosine	-	-	-	0.5

(1) Concentrations are in μg/l; - means none found; tr means trace

produced in the laboratory by the action of aqueous chlorine upon tyrosine at low pH, and have been found in the effluent from superchlorination facilities in use at three municipal waste treatment plants. The extent to which these and other possible chlorinated amino acids occur in superchlorinated waste products, and their fate in receiving streams is not known, and is under investigation by this laboratory.

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