

pentachlorophenate). The results are given in Table I.

TABLE I
INHIBITING CONCENTRATIONS FOR THREE TEST ORGANISMS
(IN PER CENT. BY WEIGHT)

	<i>A. aerogenes</i> (increment 0.0001%)	<i>B. mycoides</i> (increment 0.0004%)	<i>A. niger</i> (increment 0.0004%)
5-Hydroxymercuri- vanillin	0.0004	0.0002	0.0010
Merfenel ^a	.0001	.00001	.0001
Lignasan ^b	.0010	.00016	.0006
Santobrite ^c	.0225	.0004	.0016

^a Technical phenylmercuric acetate. ^b Contains 6.25 ethylmercury phosphate. ^c Technical sodium pentachlorophenate.

TABLE II
TOXICITY TOWARD LAKE EMERALD SHINER
(*Notropis atherinoides*)

	Critical concn., p. p. m.	Survival period, min.
5-Hydroxymercurivanillin	5-7	600 (7.5 p. p. m.)
Merfenel ^a	0.02	15 (5.0 p. p. m.)
Lignasan ^b	0.8	128 (5.0 p. p. m.)
Santobrite ^c	0.4	16 (5.0 p. p. m.)

^a Technical phenylmercuric acetate. ^b Contains 6.25 ethylmercury phosphate. ^c Technical sodium pentachlorophenate.

Fish toxicity tests were run according to the method of Van Horn¹⁴ employing the lake emerald shiner (*Notropis atherinoides*) as the test fish. Van Horn has shown this fish to be one of the most sensitive for toxicity studies. The data are given in Table II.

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Summary

Reaction of vanillin with excess mercuric oxide and alkali in aqueous solution and acidification of the filtered reaction mixture with sulfur dioxide yields 60–70% vanillic acid. Similar reaction of vanillin with one or more moles of mercuric oxide and acidification with a non-reducing acid yields 5-hydroxymercurivanillin as the chief reaction product. 5-Hydroxymercurivanillin has demonstrated remarkable toxicity toward representative microorganisms, but was found to be much less toxic to fish than other well-known comparable antiseptics. Its use as a mill disinfectant or slime control agent is indicated.

(14) Van Horn, *Paper Trade J.*, 117, No. 24, 33 (1943).

APPLETON, WISCONSIN

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Bromination of Zein

BY W. W. BINKLEY¹

Investigations of the chemical nature of zein have been largely indirect. Extensive studies have been made of the hydrolytic products of this corn protein.² Few reagents are selective enough to reveal any part of the intimate structure of this molecule. Neuberger,³ using iodine and ammoniacal methanol, prepared an undegraded iodozein which indicated the combination of 0.064 g. atom of iodine with 100 g. of zein. The iodine was shown to be combined with the tyrosine residues in the protein. The halogenation of zein has been extended using chlorine and bromine.

The direct titration of zein with chlorine in acetic acid did not show the formation of a definite compound (Fig. 1). Bromine reacted with zein to form an undegraded or slightly degraded bromozein (Fig. 1). Iodine did not react with zein in the presence of acetic acid.

Dry ethyl ether and 0.25% aqueous sodium chloride were used as the precipitation media for the brominated zein. The product of the ether precipitation was acidic, reacted with sodium hy-

droxide, silver nitrate, and liberated iodine from potassium iodide; this compound was called bromozein hydrobromide. The other product was neutral, and did not react with sodium hydroxide, silver nitrate or potassium iodide; it was named bromozein.

A total of 0.24 g. atom of bromine was required to brominate 100 g. of zein (Fig. 1). Bromozein hydrobromide possessed 0.20 g. atom of this bromine (Table I).⁴ Sodium hydroxide or silver nitrate reacted readily with 0.13 g. atom of the bromine in bromozein hydrobromide, while 0.07 g. atom of the bromine was firmly bound. The amino acids which occur in zein were titrated with bromine in acetic acid. Two of these amino acids reacted under these conditions, *viz.*, tyrosine and histidine (Table II). Folin and Ciocalteu⁵ established the presence of 0.033 mole of tyrosine residues in 100 g. of zein. The bromination of these tyrosine residues would produce 0.066 g. atom of the alkali labile bromines and an equal number of the firmly bound bromines in bromozein hydrobromide. Osborne and Liddle⁶ found

(1) Research Associate of The Ohio State University Research Foundation.

(2) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 358.

(3) A. Neuberger, *Biochem. J.*, 28, 1892 (1934).

(4) Gram atoms of bromine are expressed per 100 g. of zein.

(5) O. Folin and V. Ciocalteu, *J. Biol. Chem.*, 78, 627 (1927).

(6) T. B. Osborne and L. M. Liddle, *Am. J. Physiol.*, 26, 295 (1910).

0.005 g. mole of histidine residues in 100 g. of zein. The bromine uniting with the histidine residues would account for all the firmly combined bromine in bromozein hydrobromide. An amount of 0.037 g. atom of the labile bromines of bromozein hydrobromide liberated iodine from potassium iodide.

TABLE I
DISTRIBUTION OF THE ELEMENTS IN ZEIN, BROMOZEIN AND ITS HYDROBROMIDE*

Elements	Zein,	Bromo-	Bromo-	Bromozein
	g. atoms 100 g. zein	zein hydro- bromide, g. atoms 100 g. zein residue	zein, g. atoms 100 g. zein residue	from bromozein hydro- bromide, g. atoms 100 g. zein residue
Carbon	4.60		4.32	4.31
Nitrogen	Total	1.15	1.13	1.06
	Amide	0.33	0.33	0.31
Bromine		0.20	0.07	0.07

* Calcd. as follows

$$\frac{\% \text{ of element present}}{100 - \% \text{ Br present}} \times \frac{1}{\text{at. wt. of element}} \times 100 = \frac{\text{g. atoms element}}{100 \text{ g. zein residue}}$$

TABLE II
TITRATION OF THE AMINO ACID RESIDUES WHICH OCCUR IN ZEIN WITH BROMINE IN ACETIC ACID

Amino acid ^a	Bromine consumed,	Bromide ion	Bromine
	g. atoms mole of amino acid	formed, g. ions mole of amino acid	combined with amino acid, g. atoms mole of amino acid
Tyrosine	4.1	2.1	2.0
Histidine	2.3	1.2	1.1

^a The other amino acids which occur in zein had no significant bromine titration.

Bromozein hydrobromide was converted readily into bromozein with silver nitrate or sodium hydroxide. A total of 0.07 g. atom of bromine was found in bromozein (Table I).

The solubility characteristics of the bromozein and its hydrobromide were similar to those of zein itself but, unlike the latter substance, they showed only a slight tendency toward gelation.

Experimental

Materials.—The zein was obtained from the Corn Products Refining Co., Argo, Ill.

Anal. Moisture, 6.6; ash, 0.5; fat, 1.0; N, 14.70 (16.00, cor. for moisture, ash and fat); amide N, 4.58.

Titration of Zein with Chlorine, Bromine and Iodine.—Chlorine was passed into 1 liter of glacial acetic acid in an ambered-colored glass bottle surrounded by an ice-and-water-bath. After the addition of 4–5 g. of chlorine, 25-ml. aliquots of the resulting solution were titrated with 0.1 *N* sodium thiosulfate in the presence of an excess of potassium iodide using starch as the indicator. Four samples, each containing 5 g. of zein in 100 ml. of 90% acetic acid (10% water), were treated with 50, 100, 200, 300 ml., respectively, of the standardized chlorine solution and allowed to stand one hour at room temperature in diffuse daylight. After the addition of an excess of potassium iodide, the solutions were titrated with 0.1 *N* sodium thiosulfate using starch as the indicator.

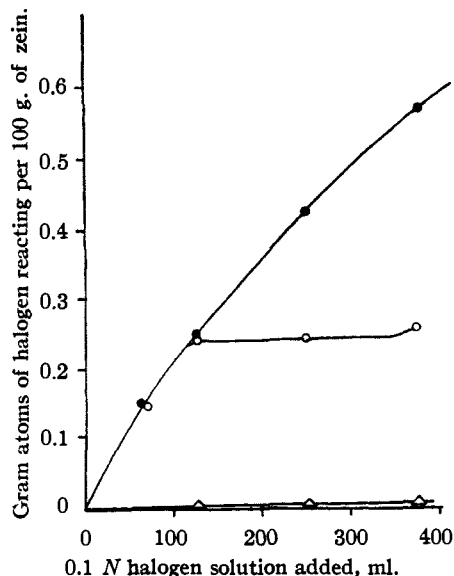


Fig. 1.—Titration of zein with chlorine, bromine and iodine in acetic acid: ●, chlorine; ○, bromine; △, iodine.

An amount of 10 g. of bromine was made up to 1 liter with glacial acetic acid and titrated with 0.1 *N* sodium thiosulfate as previously indicated. Zein was allowed to react with bromine under the conditions already described for chlorine.

An amount of 15.9 g. of iodine was made up to 1 liter with glacial acetic acid and titrated with 0.1 *N* sodium thiosulfate as previously indicated. Zein was treated with iodine under the conditions already described for chlorine. The analytical results are shown graphically in Fig. 1.

Titration of Amino Acids with Bromine.—An amount of 100 mg. of amino acid⁷ in 20 ml. of 90% acetic acid was allowed to react with 40 ml. of 0.125 *N* bromine in glacial acetic acid for one hour at room temperature. The excess bromine was titrated with 0.1 *N* sodium thiosulfate as previously indicated.

An amount of 100 mg. of amino acid was allowed to react with bromine as indicated above. The excess bromine in the reaction was reduced to bromide ion with 0.1 *N* sodium bisulfite. This solution was diluted with 100 ml. of water and titrated with 0.1 *N* silver nitrate using eosin as the indicator. Analytical data are collected in Table II.

Preparation of Dibromotyrosine Hydrobromide.—An amount of 100 mg. of tyrosine was allowed to react with bromine as described above. The solvents and excess bromine were removed under reduced pressure with a bath temperature of 50°. The crystalline residue was washed with cold dry ethyl ether; yield, 232 mg. These crystals were recrystallized from ethanol–ethyl ether solution; m. p. 213–215° (dec.). They were soluble in water, ethanol and acetic acid; they were not soluble in ethyl ether. They were acid to methyl red and reacted with aqueous silver nitrate.

Anal. Calcd. for C₉H₉O₂NBr₂·HBr: total Br, 57.11; Br as HBr, 2.38 ml. of 0.1 *N* AgNO₃ per 100 mg. Found: total Br, 57.02; Br as HBr 2.40 ml. AgNO₃ per 100 mg. These data are summarized in Table II.

Bromination of Histidine.—Histidine dihydrobromide (150 mg.) was allowed to react with bromine as indicated previously. The solvents and excess bromine were removed almost completely under reduced pressure in the presence of carbon dioxide with a bath temperature of 30°.

(7) The amino acids were obtained from the Eastman Kodak Co. Rochester, N. Y.

The residue was poured into 20 ml. of cold, dry ethyl ether with vigorous stirring. The pink precipitate which formed, was collected on a filter, washed with 20 ml. cold, dry ethyl ether and dried in the absence of air at 25°; yield, 150 mg. The brominated histidine decomposed rapidly on exposure to air. It was soluble in water, ethanol and acetic acid, insoluble in ethyl ether. It was acid to methyl red and reacted with aqueous silver nitrate.

Anal. Calcd. for $C_8H_{10}O_2N_3Br \cdot 2HBr$: total Br, 60.57; Br as HBr, 5.95 ml. 0.1 *N* $AgNO_3$ per 100 mg. Found: total Br, 57.8; Br as HBr, 5.40 ml. 0.1 *N* $AgNO_3$ per 100 mg. These data are summarized in Table II.

Preparation of Bromozein Hydrobromide.—Ten grams of bromine in 500 ml. of glacial acetic acid was added slowly to a solution of 50 g. of zein in 450 ml. of 90% acetic acid. The reaction mixture was allowed to stand twenty-four hours at 25° in diffuse daylight and then poured slowly into 7 liters of dry ethyl ether with vigorous stirring. The white precipitate which formed, was collected on a filter, washed with 3 to 4 liters of dry ethyl ether and dried forty-eight hours at 50°; yield, 55.00 g. This solid was soluble in 90% acetic acid and 70% ethanol. It reacted with sodium hydroxide and silver nitrate in ethanol and liberated iodine from potassium iodide in acetic acid. An accelerated gelation test of bromozein hydrobromide in 70% ethanol showed only a slight tendency toward gel formation.

Anal. Moisture, 5.2; N, 12.90; amide N, 3.96; Br, 13.10; three samples, each of 500 mg., required 5.25 ml. 0.1 *N* NaOH, 5.25 ml. 0.1 *N* $AgNO_3$, and 1.50 ml. 0.1 *N* $Na_2S_2O_3$, respectively.

Preparation of Bromozein.—Zein was allowed to react with bromine as described in the preparation of bromozein hydrobromide. The reaction mixture was poured into 20 liters of 0.25% aqueous sodium chloride with vigorous stirring. The bromozein coagulated readily and the supernatant liquid was removed with a siphon. The product was resuspended in 20 liters of distilled water and allowed to settle. This washing process was repeated twice. The bromozein was collected on a filter and dried forty-eight hours at 50° and two hours at 105°; yield, 47.63 g. It was soluble in acetic acid and 70% ethanol but did not react with sodium hydroxide, silver nitrate, or potassium iodide. An accelerated gelation test of bromozein in 70% ethanol showed only a slight tendency toward gel formation.

Anal. N, 14.10; amide N, 4.06; Br, 5.34; C, 49.10.

The first supernatant liquid from the precipitation of bromozein was concentrated to 200 ml. and filtered. The addition of sodium hydroxide to this concentrate

produced ammonia before and after digestion with sulfuric acid.

Conversion of Bromozein Hydrobromide into Bromozein.—An amount of 5 g. of bromozein hydrobromide was dissolved in 500 ml. of 0.05 *N* sodium hydroxide. The addition of 100 ml. of 2.5 *N* acetic acid to this solution resulted in the precipitation of a nearly white solid which was collected on a filter, dissolved in 10 ml. of glacial acetic acid, reprecipitated in dry ether, collected on a filter, washed free of acetic acid and dried forty-eight hours at 50° and two hours at 105°; yield, 4.3 g. It was soluble in acetic acid and 70% ethanol, but did not react with sodium hydroxide, silver nitrate, or potassium iodide.

Anal. N, 14.04; Br, 5.30; C, 49.02.

Control Experiment with Zein.—Five grams of zein was dissolved in 45 ml. of 90% acetic acid, allowed to stand twenty-four hours at 25° in diffuse daylight, and then poured into 2 liters of 0.25% aqueous sodium chloride with vigorous stirring. The zein was washed and dried as described in the preparation of bromozein: yield, 4.51 g.

Anal. N, 16.04.

The first supernatant liquid from the precipitation of zein was concentrated to 100 ml. and filtered. The addition of sodium hydroxide to this concentrate did not produce ammonia before or after digestion with sulfuric acid.

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Summary

1. A bromozein and its hydrobromide have been prepared.
2. Bromozein hydrobromide is acidic, reacts with silver nitrate and liberates iodine from potassium iodide. Sodium hydroxide converts it to bromozein.
3. Bromozein is neutral and does not react with silver nitrate or potassium iodide.
4. Bromozein and its hydrobromide have only a slight tendency toward gel formation.

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The Preparation of Stable Ketimines from 2,2,6-Trimethylcyclohexanecarbonitrile

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In 1942 Horeczy isolated the very highly hindered 2,2,6-trimethylcyclohexanecarboxylic acid from California petroleum.⁴ Shortly thereafter an attempt was made to synthesize the corresponding ketone by reaction of phenylmagnesium bromide with the nitrile of this acid. The usual

Grignard synthesis yielded a very stable ketimine instead of the ketone.⁵

Since this ketimine is not hydrolyzed even on prolonged heating with acids or bases either in aqueous or alcoholic solution, and since it possesses other interesting properties, the corresponding methyl ketimine was prepared.⁶ This ketimine is equally stable to hydrolysis, thus indicating that the abnormal stability is due mainly to the 2,2,6-

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(3) Du Pont Fellow 1947-1948.

(4) Shive, Horeczy, Wash and Lochte, *THIS JOURNAL*, **64**, 385 (1942).

(5) The observation that reaction of the nitrile with phenylmagnesium bromide produced a basic compound instead of a ketone was first made by William Shive in this Laboratory.

(6) P. L. Pickard, Ph.D. Dissertation, University of Texas, 1947.