The dissected formula for the lignin obtained in this fraction is, therefore, $C_{33}H_{25}O_8(OCH_3)_4(OH)_3$.

Summary

1. Two lignin fractions were isolated from oat hulls by extracting them successively and exhaustively, first with 2% alcoholic sodium hydroxide solution at room temperature, and then by refluxing with 4% aqueous sodium hydroxide solution. All the results agree with the dissected formula $C_{36}H_{31}O_9(OCH_3)_4(OH)_3$ for the first lignin fraction and $C_{38}H_{25}O_8(OCH_3)_4(OH)_3$ for the second lignin fraction.

2. The alkoxyl groups present in both lignin fractions were definitely proved to be methoxyls. A method for proving this is described.

3. The melting point of trimethylphenylammonium iodide was found to be 231.6° (corrected) and not 211–212° as recorded in the literature.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ILLINOIS]

THE EFFECT OF ETHYLENE UPON THE ACTIVITY OF DIASTASE AND INVERTASE

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With the increasing use of ethylene for the artificial ripening of fruits and vegetables much interest is manifested in the nature and mechanism of the action. In a recent bulletin emphasizing the practical application of the use of the gas, Harvey[1] has appended a very complete bibliography of the literature of the subject. A difference of opinion seems to prevail as to whether the change in the fruits and vegetables induced by the ethylene is chiefly a color change or a genuine ripening in which not only color but the normal accompanying changes in composition take place. Chace and Church,[2] after carrying out a large number of experiments and analyses, conclude that there is no difference in the composition of the edible portion of citrus fruits subjected to the ethylene method of coloring when compared to untreated fruits under the same temperature conditions. Data on other fruits were too meager to permit definite conclusions and did not suggest any changes which would not be caused by heat alone. Harvey[3] insists that ethylene brings about a genuine ripening effect when applied at concentrations of 1 to 1000 of air at 65°F. He believes that the results obtained by Chace and Church were due to the lower concentration of ethylene used, 1 part in 5000 of air. Rosa[4] states that ethylene favors the rate of softening, color change and inver-

[1] Harvey, Bulletin 247, University of Minnesota Agr. Exp. Sta., October, 1928.

[2] Chace and Church, *Ind. Eng. Chem.*, **19**, 1135 (1927).

[3] Harvey, *Science*, **67**, 421 (1928).

[4] Rosa, *Hilgardia*, **3**, 421 (1928).

sion of sucrose in the later stages of development of certain melons and attributes the effect to the acceleration of enzymic activity. This opinion seems to be more or less general and is concurred in by Regeimbal, Vacha and Harvey,[5] who report that treated bananas have one-fifth to one-fourth more sugar and less starch than the untreated samples. They believe that ethylene increases the activity of diastase and thus favors the conversion of starch to sugar. The respiratory activity was found to treble in a few minutes after treatment and then dropped to a subnormal value. This was ascribed either to an increase in respiratory oxidation or an increase in the permeability of the membranes.

Apparently very little has been done to determine the effect of ethylene upon the activity of enzymes using pure substrate material. One experiment by Regeimbal and Harvey[6] has been carried out with the proteoclastic enzymes of the pineapple and a substrate of pure casein. When the amino nitrogen produced was measured by the Van Slyke method a slightly higher rate of hydrolysis was indicated for the treated material.

Our attention to the effect of ethylene upon the activity of diastase was prompted by the observations of Dickter[7] that sometimes toluene and other antiseptics, which were being investigated in this Laboratory for use in diastase studies, appeared to have a catalytic action upon the hydrolysis of the starch. In 1908 Butkewitsch[8] found that when twigs were cut and stored in water containing chloroform or toluene, the starch-dissolving enzymes came rapidly into action and removed the starch, although in water alone the starch was not affected. These facts seemed to indicate the possibility that the effect of ethylene might be of similar nature. Accordingly, it was planned to determine its influence upon two enzymes concerned with the hydrolysis of carbohydrate material. The enzymes chosen were diastase and invertase.

Experimental

Effect of Ethylene upon Diastase. Materials and Method.—Since it is probable that maltase is present universally in plants,[9] it seemed desirable to select as materials for study some which are reported to contain this component of the amylase system in relatively large amounts. Accordingly, Taka-diastase (Parke-Davis), alfalfa meal and corn meal were selected. Soluble starch (according to Lintner) in a concentration of 2% was used as the substrate.

The optimum P_H for malt amylase has been found[10] to be in the region

[5] Regeimbal, Vacha and Harvey, *Plant Physiology*, **2**, 357 (1927).

[6] Regeimbal and Harvey, THIS JOURNAL, **49**, 1117 (1927).

[7] Dickter, Bachelor of Science "Thesis," University of Illinois, **1927.**

[8] Butkewitsch, *Biochem. Z.*, 10, 314 (1908).

[9] Davis, *Biochem. J.*, 10, 31 (1916).

[10] Sherman, Thomas and Baldwin, THIS JOURNAL, **41**, 231 (1919).

of 4.4–5, and it is likely that other plant diastases have a similar optimum. In order to obtain a value near this region a concentrated buffer containing one mole each of sodium acetate and acetic acid per liter was prepared. By diluting this solution with water in a ratio of 1 to 100, the P_H of the resulting solution as determined by the hydrogen electrode was 4.66. In all the experiments the buffer was added to the substrate material so that the final dilution was of the order to give this value.

Experiment I

Procedure.—A liter of 2% starch solution was prepared as directed by Sherman and Thomas.[11] After cooling to room temperature 10 cc. of the acetate buffer was added and the solution mixed thoroughly before making to volume. Then two 450-cc. portions were withdrawn. One was saturated with ethylene by passing the gas into it through a Folin aeration tube. Both portions were warmed to 40° in an electric oven.

Twenty-five one-hundredths g. of Taka-diastase was dissolved in 100 cc. of the acetate buffer of P_H 4.66. To each treated and untreated starch solution 25 cc. of Taka-diastase solution was added and the solutions were allowed to digest at 40°. At the end of the periods indicated 100-cc. portions were removed and added to flasks containing 3 g. of dry sodium carbonate to stop the enzymatic action. These solutions were analyzed for total reducing sugar by the Munson and Walker method. A blank was also run in which the starch solution was added to the sodium carbonate before the Taka-diastase was introduced. The results are given in Table I. This experiment indicates that there is no significant difference between the ethylene-treated and untreated samples.

TABLE I

EFFECT OF ETHYLENE UPON THE SACCHARIFICATION OF SOLUBLE STARCH BY TAKA-DIASTASE

EXPERIMENT I

Taka-diastase, 0.0139 g. per 100 cc. of substrate. Substrate, 2% soluble starch. Temperature, 40°

25-Cc. portions		Cu_2O, mg.	Apparent maltose, mg.
Blank		20.3	14.5
Portion A untreated	One-half hour	90.0	75.0
	One hour	152.5	125.1
Portion B, treated with ethylene	One-half hour	88.5	71.6
	One hour	152.9	125.4

EXPERIMENT II

Taka-diastase, 0.00695 g. per 100 cc. of substrate. Substrate, 2% soluble starch. Temperature, 40°

25-Cc. portions		Cu_2O, mg.		Apparent maltose, mg.	
Blank		23.8	24.0	17.7	17.9
Untreated	One hour	59.3	58.3	47.4	46.5
	Two hours	83.2	83.1	67.3	66.4
Treated	One hour	54.5	56.0	43.4	44.6
	Two hours	80.5	84.5	65.0	68.4

[11] Sherman and Thomas, THIS JOURNAL, **37**, 623 (1915).

Experiment II

Ford and Guthrie[12] have found that by using a papain digestion with ground barley malt, a much higher activity was obtained than without it. This was believed to be due to the bringing of endocellular enzymes into solution and preserving them as well. The preserving effect of protein derivatives has been observed and studied by Sherman and Walker.[13]

With the idea that some phase of starch hydrolytic action might have been lessened in the first experiment, 0.2 g. of asparagin was added as an aid in stabilizing the enzyme solution in the second experiment. The quantity of the enzyme was reduced and the incubation period increased. The results are also given in Table I, and confirm the results of the first experiment.

Experiment III

In order to test another source of diastase, alfalfa meal was added to the starch in place of Taka-diastase and the reaction allowed to proceed at room temperature. The solutions were filtered after inhibition of the enzyme action. The results given in Table II also show no apparent differences in treated and untreated samples.

TABLE II

EFFECT OF ETHYLENE UPON THE SACCHARIFYING ACTION OF THE DIASTASE OF ALFALFA MEAL

EXPERIMENT III

Enzyme source, alfalfa meal, 0.5 g. per 100 cc. of substrate. Substrate, 2% soluble starch. Temperature, 25°

25-Cc. portion		Cu_2O, mg.		Apparent maltose, mg.	
Blank		36.2	36.4	28.1	28.2
Untreated	One hour	68.0	70.0	54.6	56.3
	Two hours	88.1	85.0	71.4	68.8
Treated	One hour	67.2	65.8	53.9	52.8
	Two hours	86.1	82.5	69.7	66.7

In Expt. 4 corn meal was used as a source of the diastase and no additional starch was added. The general procedure devised by Rumsey[14] was followed.

The meal was added to distilled water in the untreated series and in the treated series to water previously saturated with ethylene. The results are given in Table III.

TABLE III

EFFECT OF ETHYLENE UPON THE SACCHARIFYING ACTION OF THE DIASTASE OF CORN MEAL

Enzyme source, corn meal, 10 g. per 100 cc. of water. Substrate, starch of corn meal. Temperature, 26°

		Cu_2O, mg.				Apparent maltose, mg. per 25 cc.			
Untreated	Blank		16.5	16.8			11.7	12.0	
	Sample, one hour	68.2	68.4	67.5	65.2	54.8	55.0	54.2	52.3
Treated	Blank		17.0	16.5			12.1	11.7	
	Sample, one hour	61.7	65.0	64.5	65.0	49.4	52.1	51.7	52.1

[12] Ford and Guthrie, Abs. *J. Soc. Chem. Ind.*, **27**, 239 (1908).

[13] Sherman and Walker, THIS JOURNAL, **43**, 2469 (1921).

[14] In Morrow, "Biochemical Laboratory Methods," John Wiley and Sons, Inc., New York, 1927, p. 286.

If the ethylene had the property of altering the permeability of the cell walls, freeing the endocellular enzymes and giving an increase in saccharifying power, it should have been apparent in Expts. 3 and 4.

It is unfortunate that the experiments cannot be carried over a longer digestion period, but if antiseptics be added to prevent bacterial action, there is the uncertainty as to the effect of the antiseptic itself.

Ethylene and Invertase. Materials and Method.—Invertase was prepared from Fleischmann's yeast according to the method given by Morrow.[15] It was dialyzed in collodion bags as directed.

The sucrose solution was made from pure Domino cane sugar, was of 10% concentration and was buffered with the acetate buffer to a *P*H of 4.66. It was divided into two equal portions, one of which was saturated with ethylene. The enzyme was added to each and the progress of hydrolysis followed by the polarimeter. The procedure was also that of Morrow,[16] with the exception that the action was inhibited and equilibrium conditions of the mutarotating mixture were attained by introducing the aliquot withdrawn into a dry flask containing enough solid sodium carbonate to give a concentration of about 1%. Five experiments were run. In the second a slight increase in rate of hydrolysis seemed to occur with the ethylene treated sample. However, it was slight and was never obtained again so it was doubtless due to some error or contamination. Only the results of the third run (See Table IV) will be given, as it is typical of the series.

TABLE IV

EFFECT OF ETHYLENE UPON THE RATE OF INVERSION OF SUCROSE

Enzyme, invertase, 1 cc. per 100 cc. of sucrose. Substrate, 10% sucrose. Temperature, 25.5°

Interval, minutes		0	15	45	75	105	135	165	One day
Polarization in 4-dm. tube, Ventzke °	Treated	74.9	65.9	48.9	33.8	20.9	9.9	1.8	−21.9
	Untreated	74.9	66.0	48.6	34.0	21.0	10.1	1.8	−21.9

It seems safe to conclude that ethylene has little or no effect upon the diastases or invertase under the conditions of these experiments. While it does not necessarily follow that the same would be true in a fruit or vegetable with more or less interdependent reactions going on, the results are in harmony with the analyses of Chace and Church and tend to support their conclusion that the ethylene process is primarily concerned with coloration.

Summary

A study has been made of the effect of ethylene upon the activity of diastase and invertase with pure substrate materials.

No acceleration of the saccharifying action of Taka-diastase or alfalfa meal upon soluble starch was observed when the solutions were treated with ethylene. This was also true of the diastase of corn meal acting upon the starch of the grain.

A determination of the rate of hydrolysis of sucrose using the polarimetric method indicated no benefit due to ethylene.

[15] Ref. 14, p. 281.

[16] Ref. 14, p. 283.

These observations are in accord with the idea that the effect of ethylene in the ripening of fruits and vegetables is primarily concerned with color change rather than a true ripening process.

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[CONTRIBUTION FROM GATES CHEMICAL LABORATORY, CALIFORNIA INSTITUTE OF TECHNOLOGY, NO. 235]

A NEW TEST FOR DISTINGUISHING THE PRIMARY, SECONDARY AND TERTIARY SATURATED ALCOHOLS

BY HOWARD J. LUCAS

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The most convenient method of distinguishing the three classes of alcohols is that given by Kamm,[1] who makes use of the difference in the rate with which the alcohols react with hydrobromic acid.

The readiness with which tertiary butyl alcohol reacts with cold concentrated hydrochloric acid[2] suggests this reaction as a means of distinguishing the tertiary alcohols.

Secondary but not primary alcohols react at room temperature with concentrated hydrochloric acid containing zinc chloride in the mole ratio of one to two. This reagent, which was first employed by Norris and Taylor[3] at higher temperatures for the preparation of alkyl chlorides, has recently been used for preparing 2-chloro- and 3-chloro-3-ethylpentane from the corresponding alcohols at room temperature.[4]

Reagent.—The hydrochloric acid–zinc chloride reagent is made by dissolving 136 g. (1 mole) of anhydrous zinc chloride in 105 g. (1 mole) of concentrated hydrochloric acid with cooling. Either Baker's zinc chloride, fused sticks, or a technical powder may be used.

Procedure.—To 2 ml. of the alcohol in a vial or test-tube is quickly added 12 ml. of the hydrochloric acid–zinc chloride reagent at 26–27°. The mixture is shaken and the tube is closed with a cork. Alcohols lower than hexyl are soluble, but tertiary alcohols react so fast and the separation of the tertiary chloride proceeds so rapidly that two phases are observed from the time of mixing. On standing, within five minutes or less the clear solution becomes cloudy in the case of the secondary alcohols and undergoes no change other than darkening in the case of the primary. After one hour a distinct upper layer is visible in the case of all of the secondary alcohols except *iso*propyl. The results are shown in the table.

[1] Kamm, "Qualitative Organic Analysis," John Wiley and Sons, Inc., New York, 1923; Kamm and Marvel, THIS JOURNAL, **42**, 299 (1920).

[2] Davis and Murray, *Ind. Eng. Chem.*, **18**, 844 (1926); "Organic Syntheses," John Wiley and Sons, Inc., New York, 1928, Vol. VIII, p. 50.

[3] Norris and Taylor, THIS JOURNAL, **46**, 753 (1924).

[4] Lucas, *ibid.*, **51**, 248 (1929).