Unfermentable Reducing Substances in Molasses

Volatile Decomposition Products of Sugars and Their Role in Melanoidin Formation

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About 10% of the reducing power of the unfermentable substances in cane molasses has been found to be due to volatile constituents. To identify these, the unfermentable, dehydrated residue of a heated fructose sirup was prepared as described previously, dissolved in hot anhydrous methanol, and the fructose anhydrides were removed by precipitation with anhydrous ethyl ether. The methanol-ether solution was evaporated in vacuo at a low temperature. Qualitative tests showed the presence of hydroxymethylfurfural, acetoin, levulinic and formic acids, all known as thermal or fermentative decomposition products of sugars. The color and osazone reactions of methylglyoxal also were obtained, but all these are likewise given by acetol. The Baudisch and Deuel reaction with *o*-aminobenzaldehyde definitely proved that the methanol-ether extract contained considerable quantities of acetol. The presence of small quantities of methylglyoxal was established by the reaction of 5,6-diaminouracil sulfate. It was concluded also that there are several types of melanoidins whose composition depends on the nature of the sugars and impurities present in the raw material, and on other factors. Some melanoidins, not fermented by yeast, yield fermentable sugars on acid hydrolysis, and must be formed from the sugars themselves.

IN PREVIOUS papers (*35, 37, 50, 51*) it was shown that the so-called glutose (*34*) in cane molasses consists principally of fructose anhydrides and condensation products formed from the reducing sugars and the amino acids and amides present in sugar cane juice. About 10% of the total reducing power of the unfermented residue was found to be due to volatile constituents, and the object of the present study was to identify these and to ascertain whether they contribute to melanoidin formation.

FORMATION OF VOLATILE DECOMPOSITION PRODUCTS FROM HEXOSES

The phenylosazone of glutose, prepared from the unfermentable residue of cane molasses or of heated fructose sirup, and melting at 165°, was found to be glucosazone contaminated with an impurity soluble in acetone. This suggested that it was the osazone of methylglyoxal. The work of Fischler (*19–21*), Enders (*13, 15, 16*), Neuberg (*29, 30*), and others (*18*) indicated that it is practically impossible not to obtain methylglyoxal when simple sugars, maltose, dextrin, or soluble starch are heated in aqueous solution, but it is no easy matter positively to identify it because even under favorable conditions the concentration of methylglyoxal is small. Enders (*14*) found only 30 mg. of it in 1 liter of a 10% maltose solution heated to 100° C. Access of air is unnecessary because, according to the same author, methylglyoxal is formed even when an aqueous sugar solution is heated with carbon dioxide–free water in an atmosphere of nitrogen. He postulates that a so-called triose-X, which is in equilibrium with the sugar, is split off. If the heating is carried out under conditions which permit the triose-X to be removed as methylglyoxal, its rearrangement product, as for instance by distillation at constant volume, equal quantities of the distillate contain the same amount of it, and all the sugar is ultimately converted into methylglyoxal. In this way Fischler (*19*) found 0.3825 gram of methylglyoxal in 2125 ml. of the distillate, as determined by titration with iodine.

The phenylosazone and the 2,4-dinitrophenylosazone have been prepared by the writers from such distillates of glucose, fructose, and pure maltose solutions, and were found to be those of methylglyoxal. The maltose had been purified through the octaacetate by the method of Zemplén (*49*). Clark and Hung Kao (*9*) have identified the phenylosazone by x-ray analysis. But it is well known that acetol gives the same osazones. It is, in fact, a difficult problem to differentiate between methylglyoxal and acetol when present at great dilution, because the sense of smell cannot be resorted to, and the color reactions used by various investigators to detect or identify methylglyoxal likewise are given by acetol. Among these color reactions may be mentioned particularly those with arsenophosphotungstic acid and sodium cyanide (*1*), with sodium nitroprusside (*30*), with codeine phosphate (*10*), with pyrrole (*3*), and with carbazole (*11*).

Experimental. To obtain information on the nature of the low molecular compounds in the unfermentable residue, a 75% aqueous fructose solution was gently refluxed for 16 hours. The cooled solution was diluted to 8% solids and completely fermented with baker's yeast. After removal of the yeast the filtrate was concentrated in vacuo at a low temperature to a thick sirup which was dewatered by azeotropic distillation with ethanol and benzene. The sirup was taken up in a minimum of hot, anhydrous methanol, and the filtered solution was poured into a tenfold volume of dry ethyl ether. After removing the precipitated sugar anhydrides by filtration, the bulk of the ether was distilled off at room temperature, and in this way a methanol-ether solution of the low molecular materials formed was obtained. Qualitative tests with the freshly prepared solution revealed the following:

1. It reduced Fehling solution in the cold, which is characteristic of acetol and acetoin, but not methylglyoxal or hydroxymethylfurfural.
2. The color reagents mentioned above gave a positive test for methylglyoxal (or acetol).
3. The aniline acetate test showed the presence of hydroxymethylfurfural.
4. The solution was strongly acid, indicating levulinic acid, formic acid, or both.
5. It did not give a precipitate with hydroxylamine hydrochloride and nickel chloride. This showed the absence of diacetyl, but not of methylglyoxal, because the nickel salt of methylglyoxime is slightly soluble even in cold water (*23*).
6. When the solution was first oxidized with ferric ion (*33*), a precipitate with hydroxylamine hydrochloride and nickel chloride was obtained. By treatment with dilute ammonium hydroxide, this could be separated into an insoluble portion, consisting of the

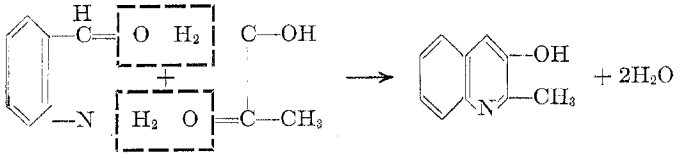
nickel salt of dimethylglyoxime, derived from acetoin, and a soluble portion, consisting of the nickel salt of monomethylglyoxime, derived from acetol (*23*).

7. Treatment with phenylhydrazine yielded a crude material from which three distinct osazones were separated by fractional crystallization. By means of x-ray analysis, Clark and Hung Kao identified one of these as the osazone of methylglyoxal (or acetol), and the second as that of acetoin. The third, melting at 173°, and analyzing C = 58.44, H = 6.70, gave a pattern different from that of the osazones of all expected low molecular carbonyl compounds examined. The diagram did not show the lines of the osazones of either methylglyoxal or diacetyl, but this does not prove their absence because according to Clark 3% or less of methylglyoxal cannot be detected by the method. The osazone probably is derived from other carbonyl compounds, as yet unconsidered. The presence of such compounds, formed thermally in brewing operations, has been indicated by Enders (*14*). They have reducing properties similar to vitamin C, but do not possess antiscorbutic value. Glucic acid is an example of such compounds (*38*, *52*).

8. The solution reacted rapidly with 2,4-dinitrophenylhydrazine (*4*, *14*) pointing to methylglyoxal, acetol, or both.

Results. Summarizing the results of these qualitative tests, the presence of methylglyoxal, acetol, or both, of acetoin, hydroxymethylfurfural, and its secondary products, levulinic and formic acids, was indicated. The formation of the last three, as thermal decomposition products of fructose in acid solution, and of acetoin, as a product of yeast fermentation, was known and to be expected. However, the question whether the methylglyoxal osazone obtained was derived from methylglyoxal or acetol was still to be settled. The failure to obtain nickel methylglyoxime without previous oxidation with ferric iron raised doubts as to the validity of Fischler's and Enders's opinion that methylglyoxal was the actual volatile material in their distillates, and of Enders's report (*13*) that methylglyoxal is obtained by distilling sugar solutions the pH of which ranged from strongly acid to strongly alkaline.

From the time of Pinkus (*33*) to the present day (*32*) it has been generally accepted by chemists that glucose in alkaline solution is broken down to methylglyoxal. But Baudisch and Deuel (*5*) have shown that when a solution of glucose in a 5% sodium bicarbonate solution is distilled, the volatile material is acetol and not methylglyoxal. Acetol combines with *o*-aminobenzaldehyde to form 3-hydroxyquinaldine, but methylglyoxal does not react with it.



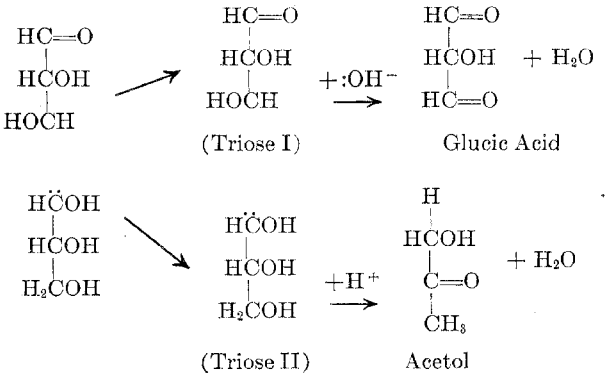
o-Aminobenzaldehyde, although commercially available, is expensive and unstable (*2*). The conversion of *o*-nitrotoluene to *o*-nitrobenzaldehyde (*45*) which is then reduced to the amino compound, is troublesome. It was thought that 2,4-dinitrobenzaldehyde, which is comparatively cheap, could be reduced to 2,4-diaminobenzaldehyde. This compound could be substituted for the monoamino compound and condensed with acetol to 3-hydroxy-7-aminoquinaldine. But the reduction product of the dinitro compound, condensed with chloroacetone (*25*), instead of the more expensive acetol, gave 3-hydroxyquinaldine (melting point, 259°, uncorrected; found: C = 75.01, H = 5.81; calculated C = 75.45, H = 5.70). The loss of the nitro group in para position to the aldehyde group can be accounted for by the inductive effect of the groups on the benzene ring.

In all subsequent experiments *o*-aminobenzaldehyde secured commercially was used as the reagent for acetol.

The 3-hydroxyquinaldine can be extracted with ether or ethyl acetate, and crystallizes on evaporation of the solvent in white needles (*5*). Its solution exhibits a brilliant blue fluorescence in ultraviolet light.

The test with *o*-aminobenzaldehyde (*6*) was applied to the methanol-ether extract of the fermented fructose extract, and the characteristic fluorescence was obtained. It was found also that aqueous solutions of glucose, fructose, and maltose, distilled at constant volume, give a strong positive test for acetol.

The formation of acetol from glucose may be explained by a rupture between carbons 3 and 4, with the retention of the electron pair on carbon 4:



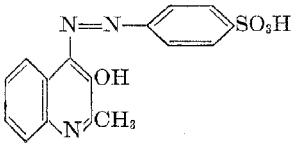
Triose fragment I can act as an acceptor of an OH group, and by the loss of water yield glucic acid. Triose fragment II, acting as a hydrogen acceptor, can exchange H and OH between carbons 5 and 6, and by loss of water yield acetol. Theoretically, acetol may be formed also from methylglyoxal by a Cannizzaro reaction, as explained in a previous paper (*38*), but since Baudisch and Deuel have shown that the distillate from 1 gram of methylglyoxal gives only an insignificant acetol test, whereas 5 mg. of glucose under the same conditions gives a decided test, it must be concluded that acetol, and not methylglyoxal, is the primary product.

A similar mechanism as that described for glucose would hold in the case of fructose, but dihydroxyacetone should form initially from triose fragment I, instead of glucic acid. According to Sigurdsson (*40*) (Table 1) fructose yields about three times as much methylglyoxal (actually acetol plus methylglyoxal) as glucose. This is probably due to the inductive effect of the carbonyl group in position 2 instead of 1.

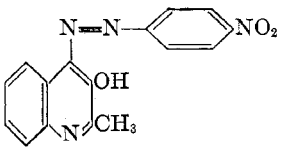
Table I. Relation between Concentration and Amount of Methylglyoxal in Distillate

	Methylglyoxal in Distillate, Mg./100 Ml.	
	Concentration, 2 grams/liter	Concentration, 20 grams/liter
Sucrose	0.14	0.19
Maltose	0.25	0.55
Galactose	0.26	0.54
Mannose	0.22	0.54
Glucose	0.22	0.54
Fructose	0.51	1.67

The quantitative determination of acetol through fluorometric measurement of the 3-hydroxyquinaldine has not been studied as yet. A very sensitive colorimetric method might be developed, based on the observation that 3-hydroxyquinaldine is capable of yielding azo dyes. With diazotized sulfanilic acid it readily forms a dye which has an intense red color in alkaline solution. Its structure is presumably:



On the addition of diazotized *p*-nitroaniline to 3-hydroxyquinaldine a dye of the probable structure:

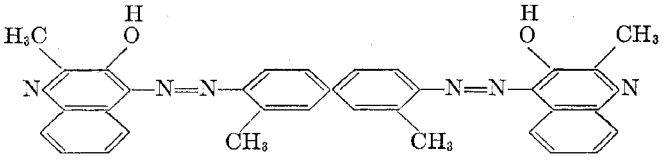


is obtained. In the dry state it has the color of concord grapes; in acid solution the color is golden yellow; and in alkaline solution, deep purplish red. Berlingozzi (*7*) has reported the corresponding compound prepared with diazotized aniline.

The optical density–wave length curve for the sulfanilic acid derivative at pH 9.0 has a maximum at 500 mμ, and drops off rapidly between 600 and 680 mμ to zero, while the *p*-nitroaniline derivative at pH 11 has two sharp peaks at 335 and 515 mμ and two sharp minima at 420 and 775 mμ.

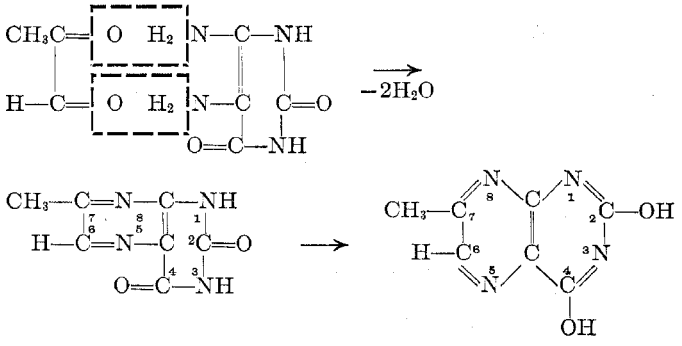
The above structures are proposed because it has been found that the same diazotized compounds can be coupled also with 3-hydroxypyridine.

With tetrazotized *o*-tolidine a brown, water-insoluble product forms, probably:



Such azo dyes may be of interest to bacteriologists or biologists.

The presence of acetol in the methanol-ether extract having been established, the question arose whether the extract contained also methylglyoxal. Neuberg and Scheuer (*31*) have found that methylglyoxal reacts with 1,2-naphthalenediamine, and Kuhn and Cook (*26*) have shown that 5,6-diaminouracil reacts with methylglyoxal, and have named the resulting product methyllumazine. According to Sharefkin (*39*) it has the methyl group in the 7 position:



It is prepared as follows (*39*):

Add 5 grams of 5,6-diamino uracil sulfate (*8*) to a mixture of 300 ml. of water, 13 ml. of commercial 30% methylglyoxal, and sufficient 10% sulfuric acid to bring the mixture to a pH of 1.5 to 2. Boil down to one half of the original volume in 1.5 hours. Filter hot, and add sodium hydroxide solution to pH 9 to 9.5. Chill in an ice bath to 2°. Collect the crystals on a Büchner funnel and wash with ice water. The yield is 3.7 to 3.8 grams of shiny, yellow crystals. Purify by recrystallization from hot water, with filtration through Nuchar C.

The solution of methyllumazine shows a brilliant fluorescence when illuminated in the dark room with ultraviolet light. The authors found, however, that this property is not specific for this compound, and hence for methylglyoxal, but that 5,6-diaminouracil itself gives a fluorescent solution, alone or when acetol is substituted for methylglyoxal in the above procedure. Therefore, recourse was taken to a study of the ultraviolet absorption spectra of acetol, methylglyoxal, and of distillates of glucose solutions prepared according to the methods of Enders (aqueous solution), and of Baudisch and Deuel, each after reaction with 5,6-diaminouracil. Sufficient 5,6-diaminouracil sulfate was added to each distillate (about 3 liters) to combine with all the methylglyoxal expected to be present according to Fischler (about 400 to 500 mg.). The solutions then were concentrated to about 50 ml. The solutions of acetol and of methylglyoxal were treated analogously with 5,6-diaminouracil sulfate. The pH of the final solutions was adjusted to 2.5. For comparison, the absorption curve of 5,6-diaminouracil sulfate alone was determined under similar

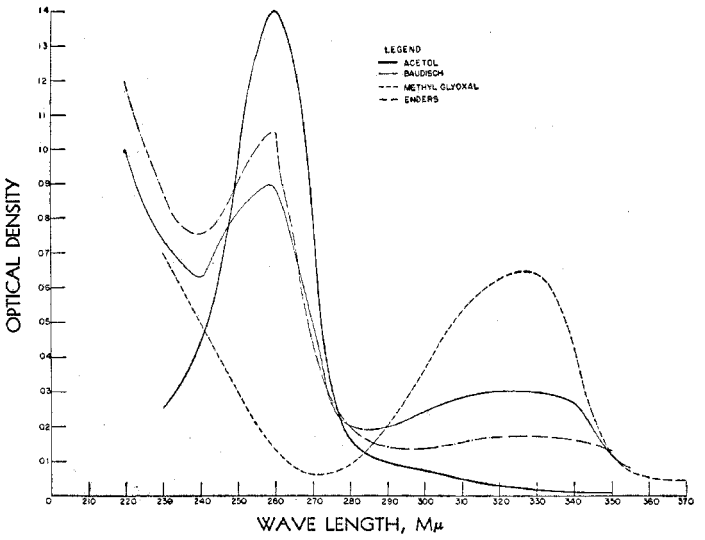


Figure 1. Comparison of Volatile Sugar Fragmentation Products with Acetol and Methylglyoxal

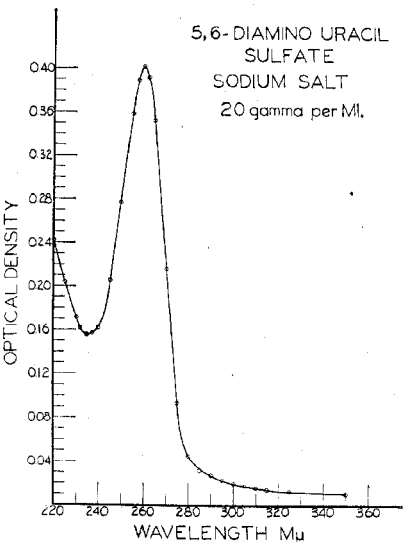


Figure 2. Light Absorption Properties of 5,6-Diaminouracil Sulfate Sodium Salt in Acid Solution

conditions. The transmittancy measurements in the ultraviolet were made on diluted aliquots with a Beckman spectrophotometer and checked independently by H. A. Frediani. In the curves shown in Figure 1 (triose fragments plus 5,6-diaminouracil sulfate) and in Figure 2 (5,6-diaminouracil sulfate alone) the optical densities are plotted against wave lengths.

The 5,6-diaminouracil curve has a minimum at 235 mμ, rises to a maximum at 260 mμ and then drops rapidly to 270 to 280 mμ, followed by a gradual drop between 280 and 350 mμ. The curve for acetol plus 5,6-diaminouracil sulfate shows the same characteristics, indicating that this curve is really that of 5,6-diaminouracil sulfate and that apparently no reaction with acetol has taken place. It is possible that if the curves were extended beyond 350 mμ toward the visible spectrum, a difference may be found between them. The curve for methylglyoxal plus 5,6-diaminouracil sulfate is entirely different. It has a minimum at around 272 mμ, in about the same region where the acetol plus 5,6-diaminouracil curve has its maximum. Conversely, it has a maximum at about 328 mμ, where the optical density of the acetol curve is near zero. The portion of the Enders and Baudisch curves from 290 to 345 mμ indicates the presence of methylglyoxal, as is also shown by the lowering of the acetol maximum at 260 mμ. Since about 3 liters of distillate were used, which would contain around 400 to 500 mg. of triose capable of reacting with 5,6-diaminouracil, a rough estimate shows that about 1 mg. of methylglyoxal was present in the total distillate. In other words, the ratio of acetol to methylglyoxal would be of the order of 500 to 1.

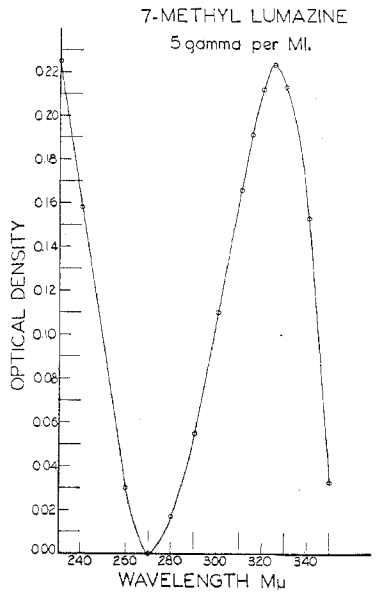


Figure 3. Sensitivity of Determining Methylglyoxal as 7-Methyllumazine in Acid Solution

The sensitivity of determining methylglyoxal as 7-methyllumazine sulfate is shown in Figure 3 where 5 γ per ml., equivalent to about 2 γ of methylglyoxal, clearly displays its characteristic absorption curve in acid solution. In water solution the sodium salt of 7 methyllumazine sulfate exhibits different absorption properties; this is shown in Figure 4.

Another method for the determination of methylglyoxal has recently been devised by Thornton and Speck (*43*), who have found that the reaction product with chromotropic acid in the presence of sulfuric acid fluoresces when irradiated with ultraviolet light. Freshly prepared acetol, treated in the same manner, fluoresces very little, but formaldehyde, and to some extent diacetyl, interfere in the determination of methylglyoxal by this method.

MECHANISM OF MELANOIDIN FORMATION

Enders (*14*) concluded from his researches that melanoidins are not formed through a reaction between the whole sugar molecule with amino acid, although he concedes this may happen in some cases of discoloration at low temperature. He believes that the primary decomposition product is a triose-X, which rearranges to methylglyoxal. This compound can undergo either aldol or acyloin condensations, and the resulting polyenals, which have reducing properties, combine with amino acids, and thus incorporate nitrogen into the molecule. The amino acids are considered to act as catalysts in what he calls polycondensation reactions. In the absence of amino acids the products are caramels, in their presence they are melanoidins (*27*, *52*).

TABLE II. COMPOSITION OF REACTION PRODUCTS OF METHANOL-ETHER EXTRACT WITH CARBONYL REAGENTS

Age of Extract, Months	Reagent Used	Analysis of Product	Carbon Content of Carbonyl Compound
Fresh	*p*-Nitrophenylhydrazine	C 53.91 H 4.28	4
1	Dinitrophenylhydrazine	C 46.86 H 4.78	5
3	Thiobarbituric acid	C 50.83 H 4.42	9
		N 7.32 S 10.25	..
13	Diphenylacethydrazide	C 66.54 H 5.67 N 3.86	26

Some of the aspects of Enders' speculations are supported by the following observations of the writers. While the fresh methanol-ether extract discussed previously reacted rapidly with dinitrophenylhydrazine, after standing for some weeks it required heat to initiate the reaction. This indicated that the carbonyl compounds present had undergone a change. The character of this change is illustrated by the analysis of the products obtained when the methanol-ether extract was treated with carbonyl reagents after the elapse of increasing time periods (Table II).

It is evident that increasingly larger molecules are being formed with time, and the slower reaction with dinitrophenylhydrazine, after the extract had stood for some time, is thus explained.

The product obtained with diphenylacethydrazide is particularly interesting because it is dark and noncrystalline, as shown by x-ray diffraction. The low molecular carbonyl compounds, such as methylglyoxal, acetol, acetoin, etc., yield white, crystalline materials.

Calculation of an empirical formula for the brown material gives $C_{46}H_{40}O_{11}N_2$ (calculated, C = 66.28, H = 5.56, N = 3.87, molecular weight, 725; found, C = 66.54, H = 5.67, N = 3.86, molecular weight over 640). Making allowance for one molecule of diphenylacethydrazide, as shown by the molecular weight of the condensation product, the empirical formula $C_{26}H_{28}O_{11}$ is ob-

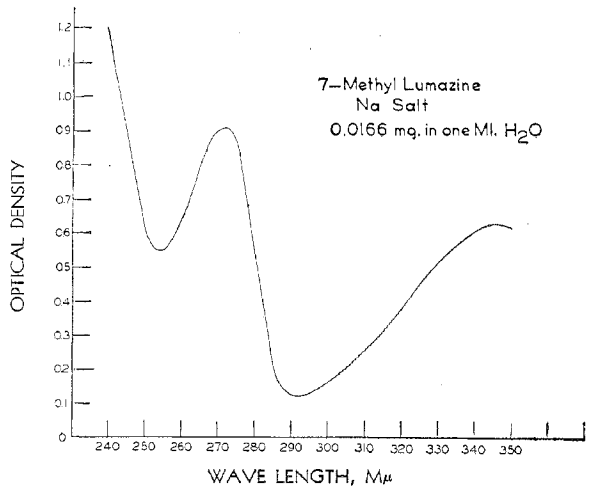


Figure 4. Light Absorption Properties of 7-Methyllumazine Sodium Salt in Water Solution

tained for the carbonyl compound. While no specific meaning is to be attached to this formula, it does show that a polymerization of simple molecules has taken place, and that one free carbonyl group is left at each stage; this is available for reaction with the amino group of the reagent. The C_{40} condensation product, though not a true melanoidin in the sense that an amino acid is required for its formation, is nevertheless of the same general character. The so-called catalytic action of amino acids is not involved in the formation of the product unless it is postulated that they hasten the polymerization of the simple carbonyl compounds.

The substance with the empirical formula $C_{26}H_{28}O_{11}$ is apparently related to the material obtained by Weast and Mackinney (*46*) from discolored dried apricots. These authors report that the intense color of dried apricots must be caused by severe dehydration and that the uptake of halogen by the black apricot extract is most readily explained by saturation of the double bonds. This, and the evidence from other sources, points to the conclusion that melanoidins are unsaturated compounds. Enders (*14*) prepared some artificial melanoidins which had reducing properties, by heating known aldol condensation products formed from acetaldehyde, up to octatrienal, with glycine. The octatrienal did not react as vigorously as he expected, but this was attributed to the insolubility of this compound, resulting in a lower concentration and hence a lower reaction rate. He did not use any known methylglyoxal condensation polymers because they were nonvolatile and unavailable.

There seems to be no justification for the assumption of Enders that methylglyoxal is the one and only fragmentation product of sugars responsible for the formation of melanoidins or of caramels. The writers have found that an aqueous solution of acetol, when heated in the presence of glycine or asparagine, produces a discoloration comparable to that produced by methylglyoxal-glycine and methylglyoxal-asparagine mixtures. In the absence of amino compounds, acetol solutions, when heated at a high pH, produce caramellike substances, similar to those obtained with methylglyoxal. Speck (*42*) has reported the formation of diacetyl from reducing sugars and amino acids. Glucic acid, which is formed from invert sugar and lime (*28*, *47*), or from glucose and dilute sodium hydroxide (*24*), also may play a part in melanoidin formation. In acid solution, reducing sugars yield hydroxymethylfurfural, and the researches of Singh, Dean, and Cantor (*41*) have shown that a major portion of hydroxymethylfurfural and of coloring matter in starch hydrolyzates comes from the destruction of glucose by acid under pressure. The same authors found no evidence for the condensation between glucose and leucine under their experimental conditions, but Troje (*44*) observed that hydroxymethylfurfural condenses readily with aspartic or glutamic acid, with the formation of dark colored compounds.

Experiments with the anthrone reagent of Dreywood (*12*) have enabled the writers to throw some new light on the role of hydroxymethylfurfural in the darkening of sugar products. A few years ago samples of the melanoidin from dried apricots and of a synthetic melanoidin, prepared from fructose and aspartic acid, were obtained from Weast and Mackinney in connection with some other problem. When these products were treated with the Dreywood reagent the synthetic melanoidin gave a positive test for carbohydrate, but the result with the natural material was negative. The principal difference in the history of the two materials is that the natural product is formed under mild conditions at a relatively low temperature, whereas heat was used in preparing the synthetic material. The Dreywood test then was applied to some of the low molecular fragmentation products of sugars, and it was found that methylglyoxal and acetol do not give the reaction but hydroxymethylfurfural gives a strong positive test. Methylated sugars and even plastics which have a carbohydrate base give the test (*36*). Ascorbic acid produces not a blue, but a cherry red color. The answer to the problem was found by subjecting phenylglucosazone, phenylgalactosazone, glucosotriazole, and mannose phenylhydrazone to the test. The reaction was negative with the first three but positive for the fourth. This shows that hydroxymethylfurfural must be responsible for the Dreywood reaction of hexoses. According to the mechanism of the formation of this compound, postulated by Hurd and Isenhour (*22*) and confirmed by Wolfrom, Schuetz, and Cavalieri (*48*), a double bond between carbons 2 and 3 of the hexose is initially necessary for the formation of hydroxymethylfurfural. With phenylhydrazones this is possible but not with osazones or osotriazoles, which are converted into osones by the sulfuric acid in the Dreywood reagent. It follows that the positive reaction with the synthetic melanoidin was actually caused by hydroxymethylfurfural. The coloring matter in the natural melanoidin must have been formed from some other fragmentation product of sugars, such as acetol or methylglyoxal. Here the question arises whether sunlight to which the apricots had been exposed plays a part in the fragmentation of the sugar molecule during the earlier stages of melanoidin formation.

The evidence adduced by the writers and other workers in this field points to the conclusion that there is not just one type of caramel or melanoidin, as postulated by Enders, but several, each of which in turn may represent mixtures of compounds of different degrees of polymerization. The nature of the sugars and impurities present in a given sugar or food product, the temperature, concentration, pH, and probably still other factors will determine the type of coloring matter formed and the degree of polymerization. Erb and Zerban (*17*) have shown definitely that a portion of the unfermentable reducing substances in cane molasses yields fermentable reducing sugars on acid hydrolysis, and therefore must have been formed directly from the sugars and not from substances such as methylglyoxal, acetol, or hydroxymethylfurfural.

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Regeneration of a Cation Exchange Resin

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At moderate flow rates, upflow regeneration of Dowex 30 cation exchange resin is only slightly less efficient than downflow. Upflow regeneration has the advantage of permitting a high initial removal in the exhaust phase, without the use of the large excess of acid required to complete regeneration. Variation of the flow rate between 0.5 and 4 gallons per square foot per minute has little effect on downflow regeneration. With upflow regeneration, efficiency decreases when noticeable lifting of the bed occurs, between 1 and 2 gallons per square foot per minute. Variation of the concentration of sulfuric acid regenerant has little effect on total capacity for sodium chloride, but the more dilute acid results in better maximum removal of sodium in the subsequent exhaustion phase of the cycle. Sulfuric acid is as efficient as hydrochloric acid for regenerating a resin in the sodium form. A resin in the calcium form is apparently less efficiently regenerated by sulfuric acid; a major part of the reduction in capacity is due to subsequent exhaustion by the calcium sulfate which is precipitated during the regeneration and dissolved by the following rinse water.

IN EVALUATING a cation exchange resin for a particular use, a thorough study of the regeneration requirements is of prime importance since the chief operating cost is for regenerating chemicals. Thompson and Roberts (*4*) pointed out the necessity for data on amounts of regenerant, strength of regenerant, and flow rates in engineering considerations, and gave break-through capacity figures for six resins. Other data on regeneration are scattered through the literature. Kunin (*3*) gave an extensive bibliography of this literature.

The study described here gives the regeneration requirements for the commercial cation exchanger Dowex 30, a resin of the phenol-formaldehyde type containing nuclear sulfonic acid groups, described by Bauman (*1*) New Dowex 30 was wet-screened to remove particles smaller than 50 mesh. The resin was supported in a Pyrex tube of 35-mm. outside diameter, and the regenerated, rinsed, backwashed, and drained volume was adjusted to 300 cc. The resin was completely exhausted with sodium chloride solution (0.5 gram per 100 ml.), run downflow at 25 ml. per minute. A 500-ml. rinse at 50 ml. per minute and a 1500-ml. backwash at 250 ml. per minute followed. All water used was purified by ion exchange. The regeneration was carried out with sulfuric acid under variations which follow. A 500-ml. rinse completed the regeneration cycle. The regeneration effluent was collected at 100-ml. intervals and titrated against 0.1 *N* sodium hydroxide solution.

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EFFECT OF AMOUNT OF REGENERANT

The effect of the amount of regenerant used was determined in a series of runs using 0.17 *N* sulfuric acid at a flow rate of 25 ml. per minute. Both downflow and upflow trials were made. Table I gives data for six runs. At this flow rate, upflow regeneration is only slightly less efficient than downflow. (Efficiency is the percentage of total acid used which is absorbed by the resin.) Since the ion exchange reaction is reversible,

$$ResNa + {}^1/_2H_2SO_4 \rightleftharpoons ResH + {}^1/_2Na_2SO_4$$

with K for the reaction as written approximately 1, it is evident that high capacity can be attained only with a large excess of regenerant.

When the resin is only partially regenerated, downflow regeneration leaves the lower portion of the resin in the sodium form. As the sodium chloride solution runs through the bed, the acid

TABLE I. EFFECT OF AMOUNT OF REGENERANT ON CAPACITY AND EFFICIENCY

Vol. of 0.17 *N* H_2SO_4, Liters	H_2SO_4 Absorbed, Equivalent		Efficiency of Regeneration, %		Max. Na Removal, %	
	Down	Up	Down	Up	Down	Up
0.5	0.084	0.084	98	98	44	78
1.0	0.153	0.150	90	88	62	89
2.0	0.239	0.232	70	68	84	98
3.0	0.286	0.277	56	54	94	99
4.0	0.305	0.298	45	44	96	98
6.0	0.303	0.304	30	30	99	99