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A SPECTROPHOTOMETRIC INVESTIGATION OF THE YELLOW COLOR THAT ACCOMPANIES THE FORMATION OF FURAN DERIVATIVES IN DEGRADED-SUGAR SOLUTIONS

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ABSTRACT

The nature of the yellow color that accompanies the degradation of sugars was investigated by studying the spectral characteristics of the tail-end absorption of yellow samples of 5-(hydroxymethyl)-2-furaldehyde (HMF) and 2-furaldehyde. The spectra of these furan derivatives were compared to those of the parent furan and of colorless benzaldehyde. The results obtained led to the conclusion that the yellow color is due to the formation of γ-unsaturated, dicarbonyl compounds during decomposition; the yellow color associated with these dicarbonyl compounds comes from the extension of their minor, broad absorption bands into the violet region of the visible spectrum. These broad bands are hidden under the intense absorption of the carbonyl groups of the unreacted, parent compounds. Thus, the tail-end absorption at the long-wavelength end of the spectra of HMF and 2-furaldehyde belongs to the hidden band.

INTRODUCTION

The degradation of sugars under acidic and other conditions is extensively covered in the literature[1–7a]. When heated under acidic conditions, solutions of D-glucose develop a yellow color, followed by the precipitation of a brown material of acidic nature, and, finally, a black resinous material.

Despite the abundance of literature on this problem, the nature of the color and of the precipitated materials, as well as the mechanism of their formation, have not yet been established. The main progress in this connection has been in establishing that 2-furaldehyde, from the pentoses[8,9], and 5-(hydroxymethyl)-2-furaldehyde (HMF), from the hexoses[6,7,10–15], are the main degradation products and the main precursors of the colored and resinous materials[13–16].

The purpose of this study was to interpret, by spectrophotometry, the yellow color that develops in HMF, 2-furaldehyde, and furan, and to extend the inter-

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pretation to account for the color observed in degraded D-glucose solution. The present spectral investigation was limited to the near-ultraviolet and visible regions of the spectrum, and was conducted on solutions of relatively high concentration.

Although a study was made of the spectral characteristics of degraded D-glucose solutions, the emphasis was on inspection of the tail-end of the absorption band of yellow samples of HMF and 2-furaldehyde, as the yellow impurities that are formed in these substrates lead to the color of degraded sugar solutions. When freshly prepared, HMF is a colorless solid, but it rapidly develops a creamy yellow color on storage, or on exposure to atmospheric conditions. Similarly, pure 2-furaldehyde is a colorless liquid when freshly distilled, but becomes yellow after a very short period of storage. The spectra of both furan derivatives were compared to those of the parent furan and benzaldehyde (the benzene analog of 2-furaldehyde).

EXPERIMENTAL

Materials. — D-Glucose (anhydrous powder) was reagent grade, from Allied Chemical Corp. (Morristown, N. J.). 5-(Hydroxymethyl)-2-furaldehyde was obtained from Aldrich Chemical Co. (Milwaukee, Wis.). 2-Furaldehyde and methanol were certified A.C.S. grade from Fisher Scientific Co. (Fair Lawn, N. J.). Furan was A.C.S. reagent grade from Eastman Organic Chemical Co. (Rochester, N. Y.). The water was doubly distilled.

Methods. — *1. Heating D-glucose in acidic, aqueous solution.* D-Glucose (16 g) was dissolved in 0.06M hydrochloric acid (100 ml) in a flask provided with a water condenser, and the solution was brought to the boil on an electric hot-plate. At intervals, aliquots (5 ml) were withdrawn, cooled in ice-water, filtered, and examined at 250 to 360 nm (in the u.v. range), and in the visible range up to 750 nm. Samples taken at the later times were diluted to give absorbances within the readable range of the recording chart.

Spectral measurements were made with a Beckman DK-1 Spectrophotometer having a hydrogen-discharge lamp for the u.v. range, and a tungsten lamp for the visible range; detector, photomultiplier 20×; sensitivity, 26; time-scale constant, 0.2; path length, 1 cm; type of chart, log paper.

2. Investigation of the tail-end absorption of yellow HMF and yellow 2-furaldehyde and furan. Solutions of the compound were examined in a Beckman DU Spectrophotometer (320 to 500 nm): light source, tungsten lamp; slit width, 0.06–1.3 mm; path length, 1 cm.

DISCUSSION OF RESULTS

1. *Spectral features of the degraded D-glucose solutions.* — The heated solution attained a yellow color after being boiled for 4 h; this deepened to an orange color after 22 h. After this time, brown turbidity appeared in the heated solution, which had a reddish-brown color. Later on, a black, flocculent solid precipitated from the solution; filtration of the suspension gave a bright-yellow filtrate.

In Fig. 1, the absorption curves of the aliquot samples are shown in the range from 250 to 360 nm. A broad absorption maximum occurs at 285 nm, the intensity of which increases with time. Also, the absorption in the range from 330 to 360 nm increases in intensity with time, although no distinct peaks appear. However, the tail-end of the bands at the later times (after 8.5 h) shows a slight hump.

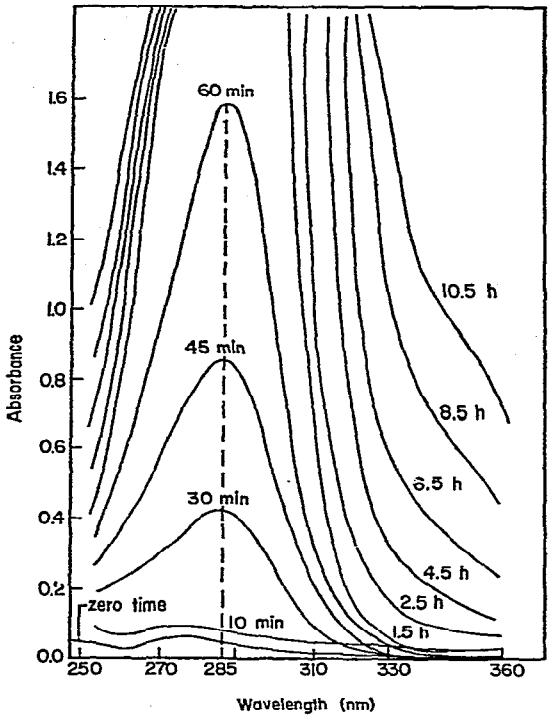


Fig. 1. Effect of heating time on absorption (u.v. region) of heated, 16% D-glucose in 0.06M hydrochloric acid.

In Fig. 2 are shown the absorption curves of the aliquots in the visible region from 360 to 750 nm. No absorption maximum appears in the visible region, the tail-end of the absorption curves extending smoothly throughout the region. The extension made by the absorption curves into the visible region (the shaded area in Fig. 2) is, apparently, the sole spectral explanation of the color that accompanies the degradation of D-glucose in acidic solution.

2. *Spectral characteristics of HMF.* — It has been reported[17] that HMF in water has a peak absorption at 282.5 nm (ε 16,900), but the value of 285 nm is more generally accepted. The absorption spectrum of HMF as given in the literature[15] is shown in Fig. 3; it is characterized by a major band at 285 nm (ε 16,500) and a minor band at 228 nm (ε 3,620). The major band is the π–π^* band, which is characteristic of the carbonyl group in HMF; this band is also characteristic of the spectrum of

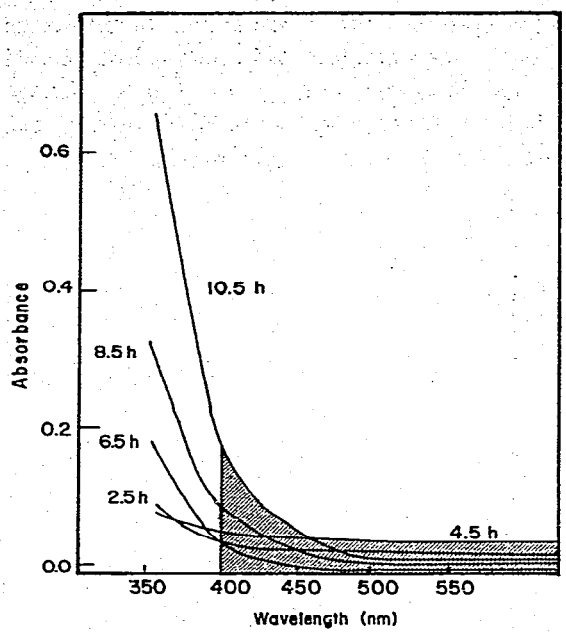


Fig. 2. Effect of heating time on absorption (visible region) of heated, 16% D-glucose in 0.06M hydrochloric acid.

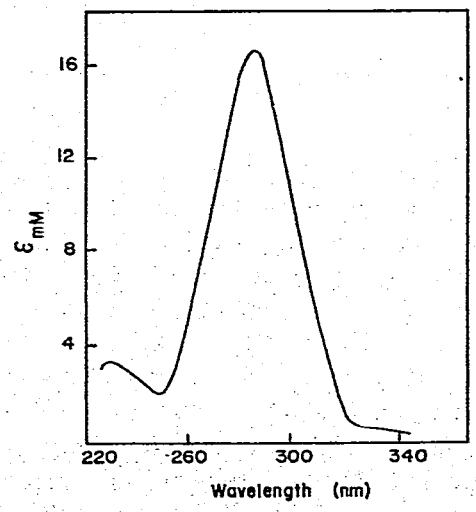


Fig. 3. Absorption spectrum[15] of HMF (concentration, 63.6 μM).

degraded D-glucose solutions, a fact which led to establishing that HMF is the main degradation product of D-glucose.

The nature of the minor band, on the other hand, has not been definitely established. Wolfrom and co-workers[15] indicated that the compound responsible for the band at 228 nm is not HMF, because this band appears first in the absorption spectrum of heated D-glucose solutions and is not accompanied by any absorption at 285 nm. This spectral evidence led them[15] to suggest that the band at 228 nm is due to the presence of an intermediate structure in the dehydration reaction of D-glucose that affords HMF. A possibility, advanced later in this work, is that HMF readily oxidizes to form a γ-unsaturated dicarbonyl compound having a major band near 228 nm, and a minor, broad band at higher wavelengths in the ultraviolet range which extends into the visible range.

Presumably, the tail-end of the π–π^* band of HMF includes a hidden n–π^* band, which is also characteristic of the carbonyl group. Direct evidence that the tail-end absorption in HMF belongs to an n–π^* absorption is shown in Fig. 4. In this Figure, the hypsochromic effect characteristic of the n–π^* transition on changing the medium from nonpolar to polar can be seen in the region beyond 327 nm. In the region below 327 nm, the reverse effect that is observed is that expected for the π–π^* transition[18]; this means that the point where the two curves cross indicates, roughly, the location of the hidden, n–π^* maximum.

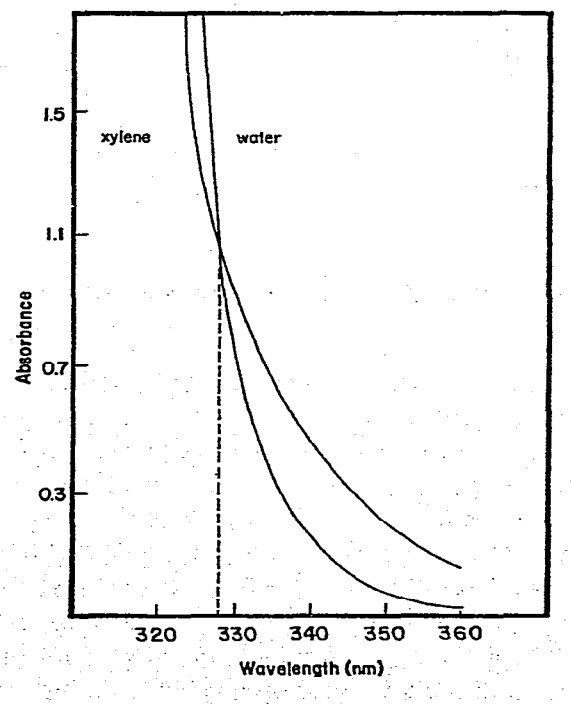


Fig. 4. Effect of solvent on tail-end absorption of HMF (fresh solutions). Concentration: 5.08mM.

3. *Comparison of the spectra of HMF, 2-furaldehyde, and benzaldehyde.* — A fresh solution (0.48M) of HMF in methanol had a light-yellow color, whereas benzaldehyde at the same concentration was colorless. The tail-end spectra of these two solutions are shown in Fig. 5, from which it is clear that the spectrum of HMF differs from that of benzaldehyde in two respects: the absorption of HMF is shifted toward the red, and HMF absorbs significantly above 390 nm, whereas benzaldehyde does not. The yellow color of HMF solution is obviously caused by the extension of the tail-end absorption into the violet region of the spectrum.

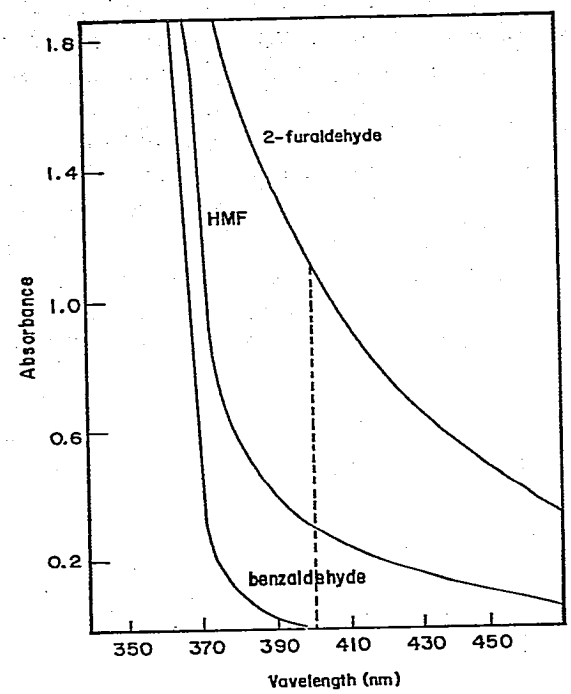


Fig. 5. Tail-end absorption of benzaldehyde, HMF, and 2-furaldehyde (concentration) 480mM, in methanol).

The spectrum of yellow 2-furaldehyde may also be compared with the spectra of benzaldehyde and HMF. 2-Furaldehyde, the main degradation product from pentoses, is the main precursor of the color that develops in degraded solutions of pentoses[8,9]. The top curve in Fig. 5 shows the spectrum of a solution (0.48M) of 2-furaldehyde in methanol, which had a deep yellow color. The 2-furaldehyde sample used in preparing the solution had a deep brown color as a result of being stored for more than a year. The intense color of the 2-furaldehyde solution, as compared to that of HMF solution of the same molarity, is obvious from the strong absorption in the region above 390 nm. It may tentatively be suggested that the yellow color of 2-furaldehyde originates in the same way as the yellow color of HMF in solution.

In simple carbonyl compounds, the absorption due to the $n-\pi^*$ transition of the

carbonyl group, although it occurs in the near-u.v. region, is not intense enough to cause an overlap into the visible region. Thus, simple carbonyl compounds containing one carbonyl group (including such aromatic carbonyl compounds as benzaldehyde, acetophenone, and benzophenone) are colorless.

2-Furaldehyde and HMF, each having one carbonyl group, would be expected to behave like the rest of the aromatic carbonyl compounds and to attain no color. Therefore, the development of the yellow color in 2-furaldehyde and HMF, which is associated with the extension of the tail-end absorption into the visible, cannot be attributed to the n–π^* transition of the carbonyl group in these two compounds. It seems likely that, for HMF and 2-furaldehyde, absorption in the visible region is associated with another band, whose absorption merges with the intense absorption of the carbonyl group, so that the maximum of the band is hidden. The existence of this band would explain the peculiar spectral differences between HMF and 2-furaldehyde and the aromatic carbonyl compounds.

4. *Nature of the hidden band. — A. Spectra of furan.* The discussion in the previous section postulated that, for HMF and 2-furaldehyde, there is a band hidden under the tail-end absorption of the carbonyl group in these compounds. In other words, the intense absorption of the carbonyl groups veils the presence of any other band that could be responsible for the absorption in the range above 390 nm. Consequently, this band ought to be revealed by a spectroscopic investigation made on a compound lacking a carbonyl group. One statisfactory candidate would be the parent furan.

The diene structure of furan and its simplest derivatives has been reported[19,20] not to show selective absorption beyond 220 nm. However, furan has been stated[21] to have a very weak absorption at 290 nm (ε 1.4). As the freshly distilled liquid, furan is colorless, but it develops a yellow color after a short period of storage in the air.

The lower curve in Fig. 6, which represents the tail-end absorption of pure furan, indicates that furan has very weak absorption in the near-u.v. region below

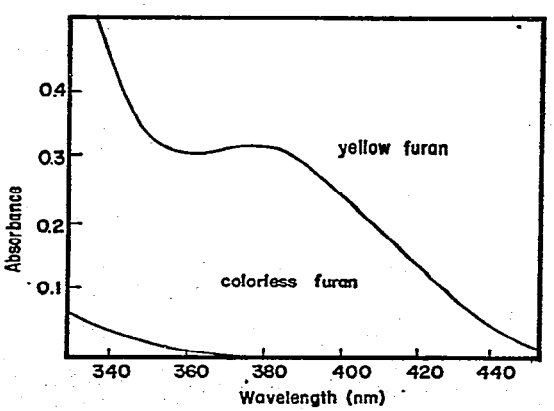
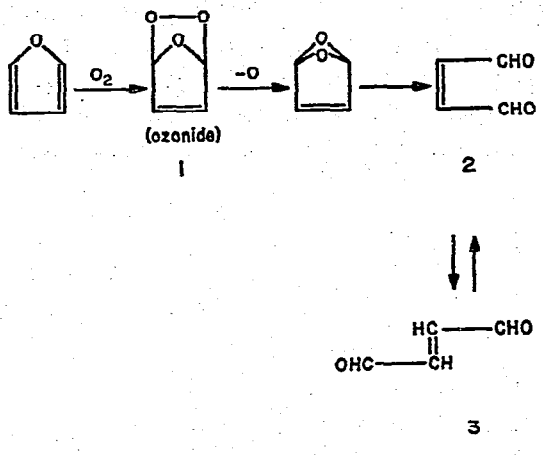


Fig. 6. Tail-end absorption of yellow furan and colorless furan.

380 nm, and no absorption above 380 nm; this explains the lack of color of freshly distilled furan. The top curve in Fig. 6 shows the tail-end absorption of a yellow sample of furan. The yellow color was produced on bubbling oxygen through a colorless sample of furan and then storing for 5 days. As may be seen in Fig. 6, the yellow color of the furan sample is accompanied by the appearance of a broad shoulder starting at ~350 nm, the tail-end of which extends into the visible region.

A carbonyl group is not present in furan, so that the absorption peak of the chromophore that is responsible for the color is not completely obscured by the carbonyl absorption band, as is the case with HMF and 2-furaldehyde. It is reasonable to expect that the yellow color that develops in HMF and 2-furaldehyde derives from the formation of a compound having the same chromophore as that which is formed from furan.

B. The action of oxygen on furan. In order to understand the spectral results presented in the preceding section, it is necessary to know the structural changes that accompany the oxidation of furan. Oxidation of furan is known to involve an attack of oxygen on the furan nucleus to form a peroxide intermediate, which then affords an unsaturated, dicarbonyl compound; this oxidation mechanism, suggested by Schenk[22], is shown. In this mechanism, oxygen adds to the furan nucleus as in a Diels–Alder addition, the furan nucleus acting as the diene, and the oxygen molecule as the dienophile. The intermediate peroxide (1) is commonly referred to as an ozonide. The product of the oxidation was found to be an equilibrium mixture of maleic dialdehyde (2) and fumaric dialdehyde (3).



Schenck mechanism for the oxidation of furan

Among the products of the oxidation of furan, the unsaturated dialdehyde would, from a consideration of the interaction between the two carbonyl groups through the ethylenic double bond, be expected to show absorption in the near-u.v.

region. The spectrum of pure fumaric dialdehyde, reported by Hufford *et al.*[23], shows a major band at 227 nm (ε 1,700) and a minor band having a broad maximum at 354 nm (ε 70) (see Fig. 7). The extension of the band into the visible region is responsible for the fact that fumaric dialdehyde is yellow.

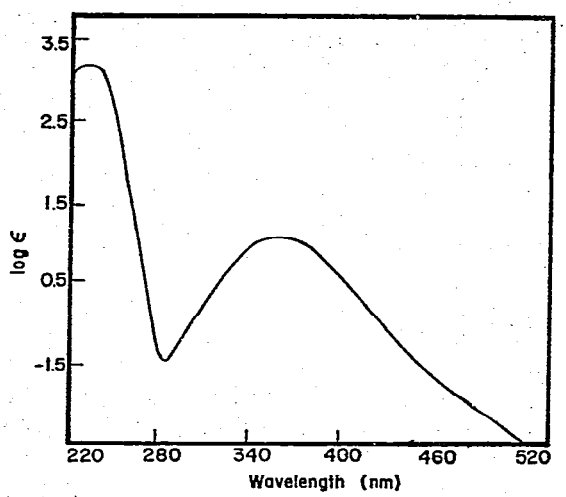


Fig. 7. Absorption spectrum[23] of fumaric dialdehyde.

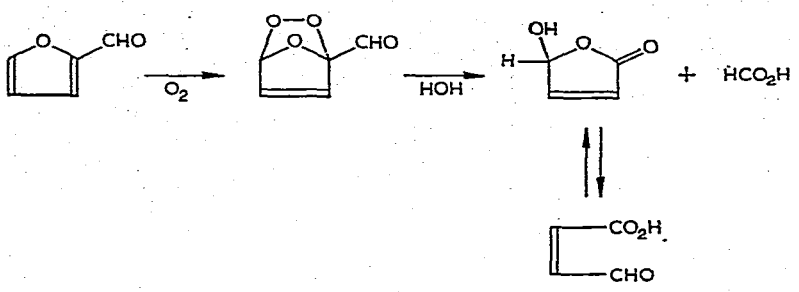
On the basis of the spectra depicted in Figs. 6 and 7, and the knowledge that unsaturated dialdehydes are formed by the oxidation of furan, it may be concluded that the yellow color of stored samples of furan is due to the formation of unsaturated dialdehyde as a result of the oxidation of furan during the storage.

Two comments may be made about Schenck's mechanism. *a.* In the 1,4 addition of oxygen to cyclic dienes[24], oxygen acts as a dienophile and must first be photosensitized from the triplet ground-state to a singlet; this means that the oxidation of furan to the intermediate ozonide is photochemically initiated. *b.* If the intermediate ozonide **1** is a Diels–Alder adduct of furan, it would be expected to be thermally unstable and to dissociate in solution. It is known that the Diels–Alder adducts of furan are unstable and undergo spontaneous dissociation, even after being isolated as pure solids[25]; because of this instability, furan adducts have been viewed as loose, molecular compounds.

In view of these considerations, the photochemical oxidation step (the first step in Schenck's mechanism) could more appropriately be represented as a photochemical equilibrium in which the rate of the formation of ozonide is balanced by the rate at which the ozonide dissociates as a result of the thermal (dark) reaction.

C. The action of oxygen on 2-furaldehyde. If, in the spectra of 2-furaldehyde and HMF, there is a hidden band corresponding to that of yellow furan, it may be attributed to unsaturated, dicarbonyl compounds formed by the action of oxygen on the parent furan derivatives.

A mechanism proposed by Dunlop and Peters[26] postulates the oxidation of 2-furaldehyde as shown. Interestingly, according to this mechanism, oxygen attacks the furan nucleus, not the aldehyde group, in 2-furaldehyde. This was established



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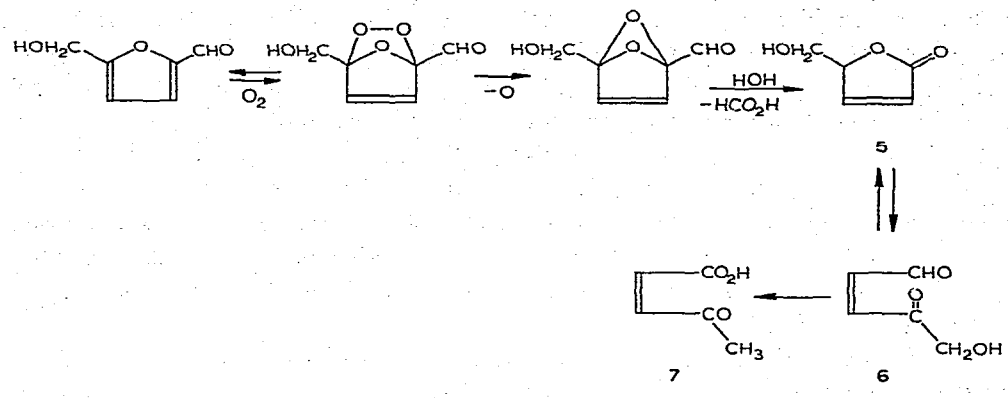
Dunlop and Peters mechanism for the oxidation of 2-furaldehyde

because furoic acid could not be detected or isolated under the conditions of the oxidation. The acidic ingredients that were detected and isolated were formic acid and 3-formylacrylic acid (**4**).

3-Formylacrylic acid, an unsaturated dicarbonyl compound, would be expected to show absorption at long wavelengths, and thus to be responsible for the yellow color of oxidized 2-furaldehyde. The spectrum of 2-furaldehyde, relative to that of benzaldehyde, would be expected to display a significant extension into the visible range, because of the presence of the dicarbonyl compound. The maximum of this band would, however, be hidden by the n–π^* absorption band of the carbonyl group of 2-furaldehyde.

The oxidation of 2-furaldehyde has been referred to as an autoxidation, and it is known to be subject to auto-inhibition[26], although no explanation of this inhibition has been given. However, in accordance with the foregoing discussion on the oxidation of furan, auto-inhibition observed during the oxidation of 2-furaldehyde may be taken to indicate the approach to equilibrium of the photochemical addition of oxygen to the furan nucleus. This photochemical equilibrium represents the stage at which the rate of the addition of oxygen is balanced by the thermal dissociation of the Diels–Alder adduct.

D. Action of oxygen on HMF. For the oxidation of HMF, a scheme similar to the Dunlop and Peters mechanism is now proposed that leads to the formation of 3-acetylacrylic acid (4-oxo-2-pentenoic acid) (**7**). This mechanism assumes that oxidation of HMF follows the same pattern as that for furan and 2-furaldehyde, that is, a nuclear attack by oxygen. As with the oxidation of 2-furaldehyde, formic acid is split out, leading to the formation of cyclic (**5**) and acyclic (**6**) isomers of **7**. The last step by which **7** is formed from the rearrangement of the acyclic isomer **6** is a disproportionation wherein the ketol group is reduced and the aldehyde group is oxidized. Such a rearrangement is known to occur on hydrolytic cleavage of the furan



Proposed mechanism for the oxidation of HMF

ring in furan derivatives; for example, in the formation of levulinic acid from the hydrolytic degradation of furfuryl alcohol[11,27] and of HMF[5]. Teunissen[11] suggested that the rearrangement involves a kind of internal, Cannizzaro reaction through the intermediate formation of a hemiacetal structure; he also stated that opening of the furan ring under oxidative conditions leads to the formation of acrylic acid derivatives.

The mechanism proposed leads to the formation of an unsaturated, dicarbonyl compound (7), and it is this compound that is considered responsible for the yellow color that forms from HMF. The dicarbonyl compound would contribute to the peak

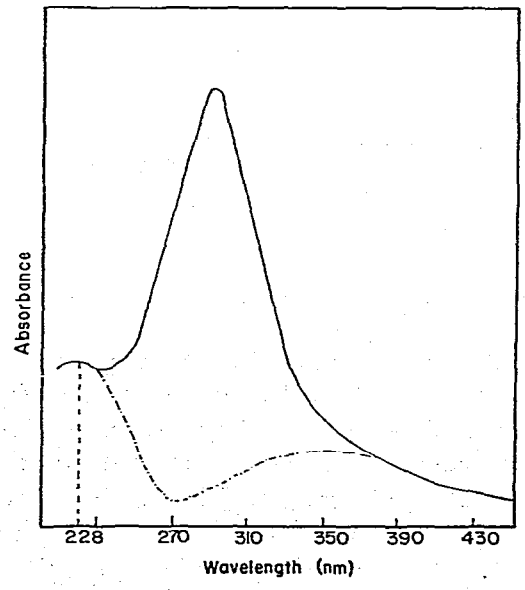


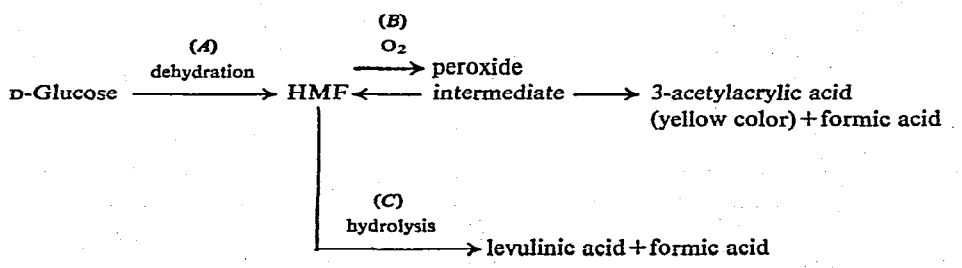
Fig. 8. Absorption spectrum of HMF, hiding the expected absorption of the γ-dicarbonyl compound that results from the oxidation of HMF (arbitrary curves). (–.–.–. hidden spectrum; ——— observed spectrum.)

which is found at 228 nm in the spectrum of HMF (see Fig. 3). The dicarbonyl compound that is produced by the oxidation of furan has a major peak in this region (see Fig. 7). Fig. 8 shows how the absorption spectrum of a compound such as 7 might be hidden by the spectrum of the major component, HMF.

E. Change in pH during the degradation reaction. Ever since the early investigations on the problem of the degradation of D-glucose, it has been noticed[3,7,28] that the degradation reaction under the various conditions is associated with a drop in pH, usually explained as due to the formation of a soluble acidic product, namely, levulinic acid, together with formic acid at a late stage (from the hydrolytic decomposition of HMF[1,5,11]).

Neil and co-workers[7] reported, however, that a small decrease in pH occurs during the early stage of the degradation reaction, when only a very small concentration of HMF is present. As levulinic acid has not been detected at such an early stage of the degradation, the lowering in pH is obviously caused by some other product. It is reasonable that this early acidity is related to the appearance of the compound absorbing at 228 nm. Absorption at this wavelength is observed in the spectrum of degraded D-glucose solutions at an earlier stage than the absorption at 285 nm, which marks the appearance of HMF. Thus 3-acetylacrylic acid accounts for both the acidity and the absorption at 228 nm in the early stages of the degradation reaction.

5. *Mechanism of the degradation reaction.* — In view of the foregoing considerations, and with reference to what is known about the degradation reaction of D-glucose, the process by which the yellow color is formed from D-glucose may be represented as follows:



A. Early stage. As HMF, any of the dehydration intermediates suggested[15], and levulinic acid were not detected at the early stages of the degradation (and if it is assumed that both the absorption at 228 nm and the early acidity are due to the formation of 3-acetylacrylic acid), step (*A*) may be regarded as being the rate-determining step of the early degradation reaction of D-glucose. That is, although HMF is formed from D-glucose, it is oxidized as fast as it is formed.

B. Later stage. As the degradation of D-glucose continues, step (*B*) slows down, probably due to the photochemical equilibrium, and this results in the accumulation of HMF in the degraded solution, which manifests itself by the rapid growth of intensity of the absorption band at 285 nm. When a sufficiently large concentration of

HMF has accumulated, decomposition through step (*C*) becomes important. This mechanism explains the induction period that has been kinetically established[7] with respect to the production of HMF in degraded D-glucose solutions; it also explains the achievement of a limiting concentration[7] of the compound responsible for the absorption at 228 nm.

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