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Prerequisites for the Enhancement of Formose Formation in the Presence of Hydroxy Oxo Compounds

Tomoya SAKAI,* Masahiko ISHIZAKI, Katsuaki KOBAYASHI, and Masafumi GOTO

Department of Chemical Reaction Engineering, Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467

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The enhancement of formose formation in the presence of several hydroxy oxo compounds was examined in an aqueous $Ca(OH)_2$ solution. Four terminal hydroxy oxo compounds, 2-hydroxyacetophenone, 1,3-dihydroxyacetone, DL-glyceraldehyde, and D-glucose, were confirmed to accelerate the reaction, but two internal hydroxy oxo compounds, 2-hydroxypropiophenone and acetoin, were found not to be effective. The acceleration efficiency was larger for a system in which hydroxy oxo compounds were introduced to an aqueous mixture of $Ca(OH)_2$ and formaldehyde than for a system in which formaldehyde was introduced to an aqueous mixture of $Ca(OH)_2$ and the hydroxy oxo compounds. The difference in the acceleration efficiencies between the two procedures of reagent introduction was large for 2-hydroxyacetophenone and 1,3-dihydroxyacetone. This is accounted for by the rapid conversions of the added hydroxy oxo compounds in an aqueous $Ca(OH)_2$ solution.

The typical formose formation in the absence of accelerators takes place in an aqueous mixture of $Ca(OH)_2$ and formaldehyde(C_{1A}) at about 50 °C. The initial heterogenous suspension of $Ca(OH)_2$ turns into a homogeneous solution as the reaction proceeds. The consumption rate of C_{1A} for the formose formation shows a characteristic feature of an auto-catalysis, accompanied by a long induction period. After the maximum rate of C_{1A} consumption, the reaction mixture is tinged with pale yellow. At this "yellowing point," the yield of formose sugars reaches its maximum; after that the dehydration of sugars proceeds gradually. The main products at the "yellowing point" have been reported to be C_2—C_8 sugars centering around C_6, including reducing sugars and sugar alcohols.[1] The reaction scheme proposed by Mizuno and Weiss[1] is summarized in a series of reactions: (1) the slow dimerization of C_{1A} to glyceraldehyde (C_{2A}), (2) the rapid aldol addition of C_{1A} to C_{2A} and higher carbohydrates, (3) Lobry de Bruyn-Alberda van Ekenstein(L-V) transformation, and (4) Cannizzaro and cross-Cannizzaro reactions among the products and C_{1A}.

The presence of such hydroxy oxo compounds as C_{2A} and D-glucose (Glu) in the reaction system has been known to shorten or to eliminate the induction period and to cause the reaction to proceed rapidly through C–C chain growth. Most of the hydroxy oxo compounds so far examined have the terminal 1-hydroxy-2-oxo structure: the order of the acceleration efficiency has been reported by Kusin[2] as D-fructose (Fru) > Glu > maltose; by Langenbeck,[3] as 1,3-dihydroxyacetone (C_{3K}) > C_{2A} > Fru, and by Langenbeck[4] and Monozov and Lavanevskii,[5] as will be described in a later section.

This study deals with the efficiencies of seven hydroxy oxo compounds, 2-hydroxyacetophenone (HAP), C_{3K}, DL-glyceraldehyde (C_{3A}), Glu, 2-hydroxypropiophenone (HPP), and acetoin, as accelerators of the formose formation. Among them, C_{3A} and C_{3K} represent the initial products of the typical formose formation. Glu was used because the major products at the "yellowing point" are hexoses. One of the most efficient accelerators, HAP, is unique because it is not involved in the typical formose formation. HPP and acetoin have the internal hydroxy oxo structure and are employed to examine whether or not these compounds show the acceleration effect.

The accelerators with terminal 1-hydroxy-2-oxo structure all shared large acceleration efficiencies with regard to the formose formation. It was also noted that the procedure of reagent introduction, *i.e.*, either the reaction was started with the introduction of accelerators into an aqueous mixture of $Ca(OH)_2$ and C_{1A} or it was started with the introduction of C_{1A} into an aqueous mixture of $Ca(OH)_2$ and hydrox oxo compounds, affected the efficiency of each accelerator. The phenomenon was attributed to the rapid transformation of the hydroxy oxo compounds in an aqueous $Ca(OH)_2$ solution in the absence of C_{1A}.

Experimental

Materials. A commercially available 37% C_{1A} solution (Wako) was used for the C_{1A} stock solution without further purification. This contained 3 to 4% methanol as a stabilizer. Commercially available guaranteed-reagent-grade calcium hydroxide (Katayama), C_{3K} (Wako), and C_{3A} (Tokyo Kasei) were used without further treatment. The C_{3K} and C_{3A} are dimers, but these have been known to dissociate to the respective monomers immediately in an aqueous $Ca(OH)_2$ solution.[6]

HAP was prepared according to the method of Cebrian:[7] mp 76—78 °C (lit, 75—76 °C). HPP was prepared according to the method of Temnikowa;[8] the material thus obtained was found by GLC measurements to contain an isomer, 1-hydroxy-1-phenyl-acetone, in a 27% amount, but it was used without further purification: bp 115—120 °C/12 Torr (lit,[8] for HPP 123—124 °C/13 Torr) (1 Torr= 133.322 Pa).

Product Analysis. The products of formose formation have been reported to consist of formose sugars, including reducing sugars, sugar alcohols, and formic acid, produced by the side Cannizzaro reaction.[1] The amounts of remaining C_{1A} and formic acid were measured on a Kyowa K-880 high-performance liquid (HPL) chromatograph, using a column of Shodex Ionpak C-811 and an aqueous eluent of 0.1% phosphoric acid under a pressure of 50 bar at room temperature, and with an UV monitor (Kyowa KUV-254)

at 254 nm and a refractive index monitor (Shodex RISE-11) connected in series. The accuracy of the quantitative measurement of C_{1A} was confirmed to be in agreement with the photometric method with chromotropic acid[9] within a 2—3% error. Higher formose sugars, such as C_5 and C_6, were not fully separated in this HPL chromatogram, though.

The spectral change between 210—300 nm of an aqueous HAP and $Ca(OH)_2$ solution was recorded at 5-min interval with a Shimadzu UV-210A spectrophotometer with a Shimadzu WP-1 wavelength programmer and an X-Y recorder (Rika Denki) attached. The amounts of HPP and its isomer were measured with a postulation of equal molar sensitivities for the two isomers on an Ohkura Model 802 gas-liquid chromatograph, with FID by means of a silicone grease (SE-30) column at 150 °C.

Formose-formation Procedures. In a three-necked, round-bottomed flask, either an aqueous or a 40% methanol aqueous solution of $Ca(OH)_2$ was placed and thermostated at 35—50 °C. The reaction was initiated with two different procedures. In the normal procedure, first a thermostated C_{1A} solution was mixed with the $Ca(OH)_2$ solution; then after 1 or 3 min, a reaction was initiated by the introduction of a thermostated aqueous accelerator solution. In the reverse procedure, first, a thermostated aqueous accelerator solution was mixed with the $Ca(OH)_2$ solution; then, after 1 or 3 min, a reaction was initiated with the introduction of a thermostated C_{1A} solution. The reaction mixture was stirred magnetically and thermostated in a water bath. Aliquots of the reaction mixture were pipetted out at appropriate time intervals and were then dissolved in a dilute hydrochloric acid to quench the reaction (litmus). The amounts of remaining C_{1A} and formic acid were determined with the HPL chromatograph. The conversion of C_{1A} to formose was calculated by Eq. 1, where the formation of formic acid was assumed to be caused through the cross-Cannizzaro reaction between C_{1A} and aldoses.

C_{1A} conversion to formose

$$= 1 - \frac{[C_{1A}]_{remained} - [HCOOH]}{[C_{1A}]_{initial}} \quad (1)$$

Deactivation of Accelerators. An aqueous solution of an accelerator was added to a $Ca(OH)_2$ solution in the absence of C_{1A}. At appropriate time intervals, aliquots of the reaction mixture were pipetted out and examined by means of an HPL chromatograph after acid treatment.

Results and Discussion

Essential Structure for Acceleration. C_{1A} consumption in a typical formose formation in the absence of accelerators is shown in Fig. 1. This shows the autocatalytic nature of the formose formation. The change in the HPL chromatogram with reaction periods is illustrated in Fig. 2. The arrow indicates the retention time of each authentic sample, measured separately under the same conditions of HPL chromatography. C_{4A} and C_{5A} denote D-erythrose and D-arabinose respectively. The final chart at 60 min shows product peaks distributing between C_{5A} and Glu, suggesting that they are mostly aldo- and ketohexoses including sugar alcohols, in accordance with the literature.[1] Figure 3 shows a change in the HPL chromatogram in the presence of an accelerator, C_{3A} (0.01 mmol cm^{-3}), with the normal procedure. The products of formose formation gave similar HPL chromatograms irrespective of the introduction of a small amount of C_{3A} (compare Figs. 2 and 3), although the consumption of C_{1A} was rapid for the reaction with added C_{3A}. The reaction shown in Fig. 3 was carried out in an aqueous solution.

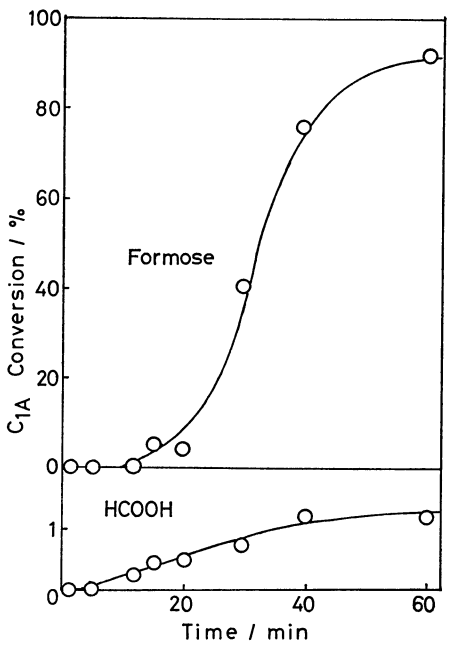


Fig. 1. Autocatalytic nature of formose formation in the absence of accelerators.
50 °C, 20 vol% methanol–H_2O, $Ca(OH)_2$: 0.10 mmol cm^{-3}, C_{1A}: 2.45 mmol cm^{-3}.

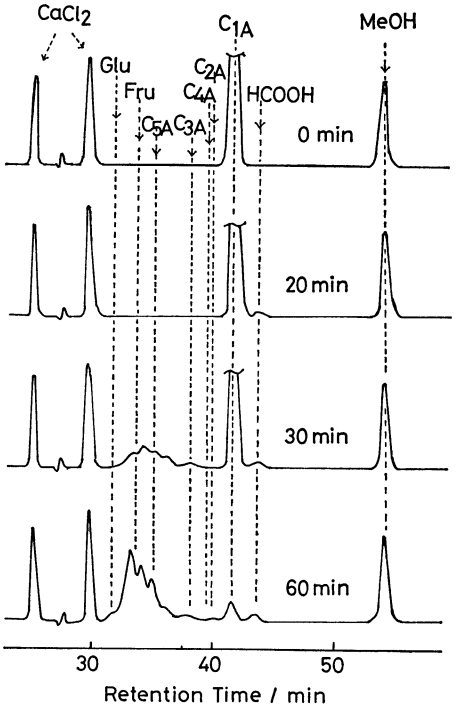


Fig. 2. Change in HPL chromatogram with reaction period in the absence of accelerators.
50 °C, 20 vol% methanol–H_2O, $Ca(OH)_2$: 0.10 mmol cm^{-3}, C_{1A}: 2.45 mmol cm^{-3}.

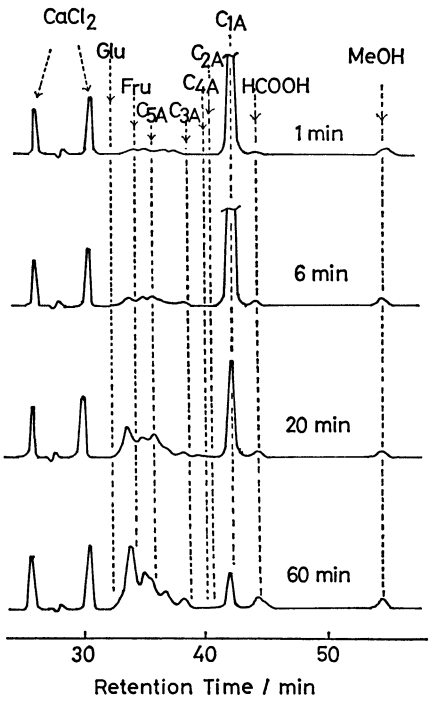


Fig. 3. Change in HPL chromatorgam with reaction period in the presence of C_{3A}.
50 °C, H_2O, $Ca(OH)_2$: 0.10 mmol cm^{-3}, C_{1A}: 2.45 mmol cm^{-3}, C_{3A}: 0.01 mmol cm^{-3}.

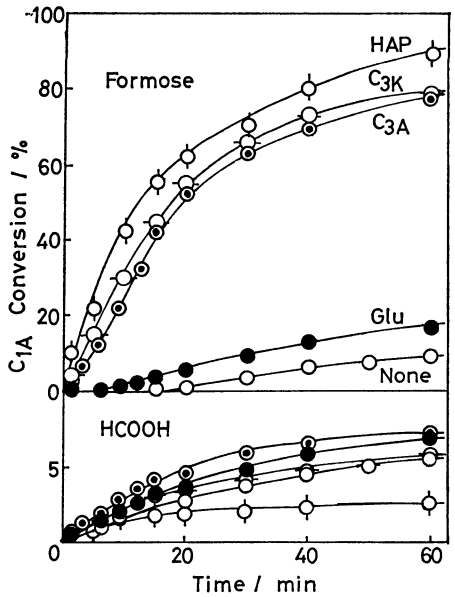


Fig. 4. Acceleration efficiencies of HAP, C_{3K}, C_{3A}, and Glu.
50 °C, H_2O, $Ca(OH)_2$: 0.10 mmol cm^{-3}, C_{1A}: 2.45 mmol cm^{-3}, accelerator: 0.01 mmol cm^{-3}.

The efficiencies of the acceleration of various hydroxy oxo compounds, HAP, C_{3K}, C_{3A}, or Glu, are compared in Fig. 4 with the normal procedure. The concentration of each accelerator is 0.01 mmol cm^{-3}, while that of C_{1A} is 2.45 mmol cm^{-3}. The conversion of C_{1A} amounted to 80% at 60 min for HAP, C_{3K}, and C_{3A}, so that the molar amounts of the converted C_{1A} are more than 200 times those of the introduced accelerators. A comparison of the results shown in Fig. 4 with those in Fig. 1 suggests that the steep rising of the conversion of C_{1A} in the middle and the leveling off in the later stage of the reaction periods in Fig. 1 correspond to the rapid C_{1A} additions to lower carbohydrates, such as C_{3A} and C_{3K}, and to the slow C_{1A} additions to higher carbohydrates, such as Glu, respectively, based on the different acceleration efficiencies of the compounds shown in Fig. 4.

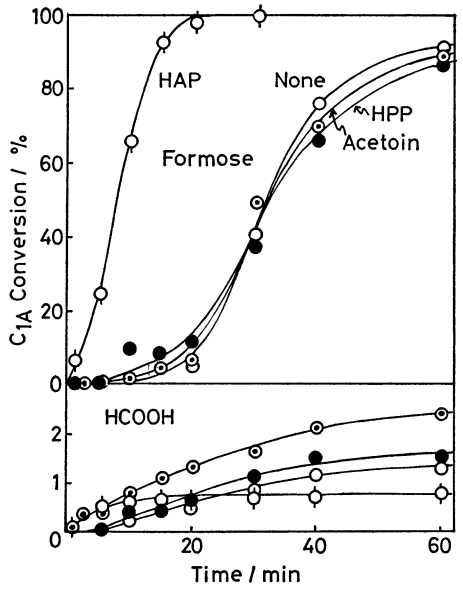


Fig. 5. Non-effectiveness of internal hydroxy oxo compounds, HPP and acetoin, as accelerator.
50 °C, 20 vol% methanol–H_2O, $Ca(OH)_2$: 0.10 mmol cm^{-3}, C_{1A}: 2.45 mmol cm^{-3}, accelerator: 0.01 mmol cm^{-3}.

HAP is a unique compound in that it belongs to the most effective class of accelerators for the formose formation and in that it is not formed in a typical formose reaction. Langenbeck[4] has reported that the efficiency of the acceleration of hydroxy oxo compounds decreases in the following order; HAP > 2-hydroxyacetonaphthone > 1-hydroxy-2-propanone > C_{3K} > C_{2A} > 5-(2-hydroxyacetyl)acenaphthene > Fru > Glu. Further, Monozov and Levanevskii[5] have reported that the efficiencies of HAP derivatives follow a Hammett-type LFER; *i.e.*, 2′,5′-dichloro-HAP>4′-chloro-HAP=3′-chloro-HAP>HAP>4′-methyl-HAP>4′-methoxy-HAP. These compounds have, without exception, terminal 1-hydroxy-2-oxo structure. The accelerations by internal hydroxy oxo compounds deserve to be examined. Langenbeck[3] reported the acceleration efficiencies of benzoin, anisoin, and acetoin to be smaller than that of Glu. The present results with HPP and acetoin as internal hydroxy oxo compounds are shown in Fig. 5. A mixture of methanol and water (2:8 in volume) was employed in this case because of the poor solubility of these substances in water. It is concluded that internal hydroxy oxo compounds have no acceleration efficiency.

Influence of the Procedures of Reagent Introduction. The procedures of the reagent introduction for starting

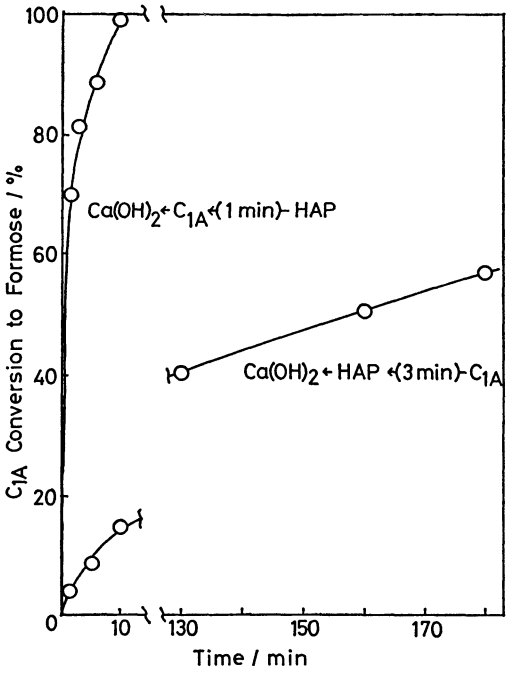


Fig. 6. Difference in acceleration efficiency of HAP with normal or reverse procedure.
35 °C, 40 vol% methanol–H_2O, $Ca(OH)_2$: 0.10 mmol cm^{-3}, C_{1A}: 1.0 mmol cm^{-3}, HAP: 0.20 mmol cm^{-3}.

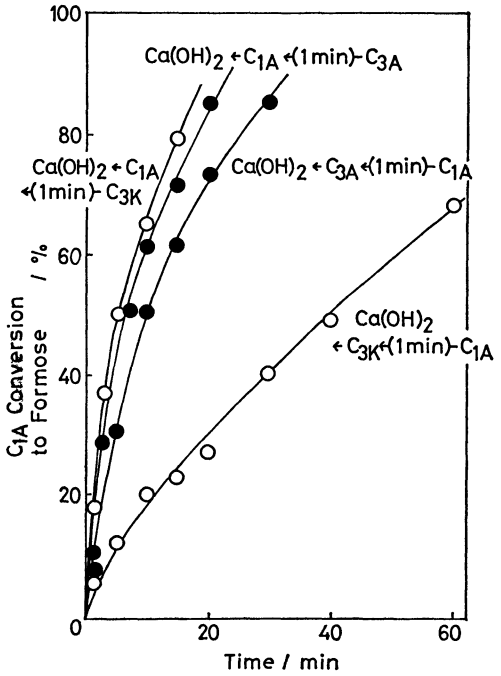


Fig. 7. Differences in acceleration efficiencies of C_{3K} and C_{3A} with normal or reverse procedure.
35 °C, H_2O, $Ca(OH)_2$: 0.10 mmol cm^{-3}, C_{1A}: 0.98 mmol cm^{-3}, C_{3A}: 0.20 mmol cm^{-3}, C_{3K}: 0.20 mmol cm^{-3}.

formose formation greatly influenced the efficiency of acceleration, whether by the normal procedure, $Ca(OH)_2$←C_{1A}←accelerator, or by the reverse procedure, $Ca(OH)_2$←accelerator←C_{1A}. A large difference in the acceleration efficiencies was observed for

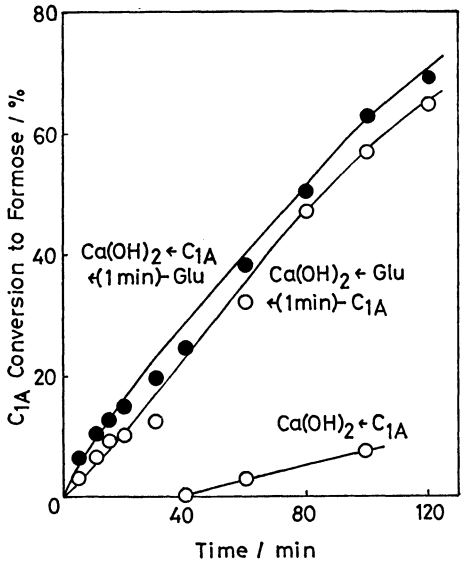


Fig. 8. Difference in acceleration efficiency of Glu with normal or reverse procedure.
35 °C, 40 vol% methanol–H_2O, $Ca(OH)_2$: 0.10 mmol cm^{-3}, C_{1A}: 1.0 mmol cm^{-3}, Glu: 0.20 mmol cm^{-3}.

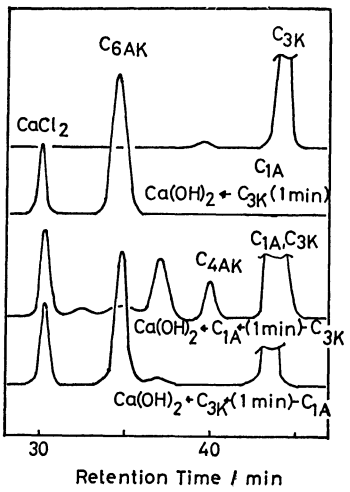


Fig. 9. HPL chromatographic change of C_{3K} by mixing with $Ca(OH)_2$ and C_{1A} in different procedures.
35 °C, H_2O, $Ca(OH)_2$: 0.10 mmol cm^{-3}, C_{3A}: 0.20 mmol cm^{-3}, C_{1A}: 1.0 mmol cm^{-3}.

HAP, as is shown in Fig. 6. The results for C_{3A} and C_{3K} are shown in Fig. 7. The difference is larger for C_{3K} than for C_{3A}. The difference for Glu was small, as shown in Fig. 8.

To investigate the difference in the acceleration efficiencies between the two procedures, the HPL chromatographic change of the accelerator with a duration time of 1 min was examined for the case of C_{3K} in Fig. 9. The chromatogram of the mixture of C_{3K} and $Ca(OH)_2$ solution, 1 min after the mixing, showed a complete consumption of C_{3K} and the formation of C_6 sugars (C_{6AK}) containing DL-dendroketose, Fru, and D-sorbose.[10] A chromatogram obtained with the normal procedure (a C_{3K} solution was mixed 1 min

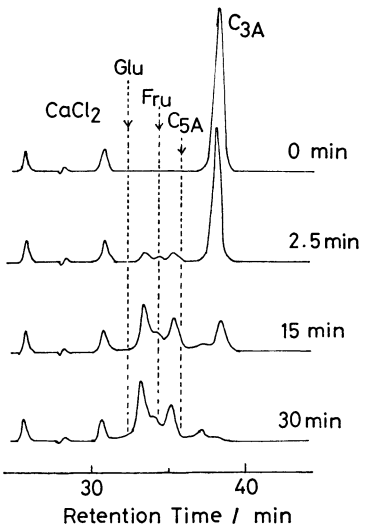


Fig.10. HPL chromatographic change of C_{3A} in an aqueous $Ca(OH)_2$ solution.
20 °C, H_2O, $Ca(OH)_2$: 0.05 mmol cm^{-3}, C_{3A}: 0.20 mmol cm^{-3}.

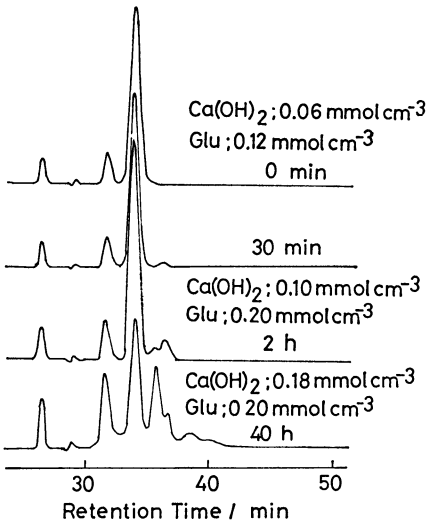


Fig.11. HPL chromatographic change of Glu in an aqueous $Ca(OH)_2$ solution.
18—20 °C, H_2O.

after the preparation of an aqueous mixture of C_{1A} and $Ca(OH)_2$) exhibited few dimerization products, but a peak corresponding to C_{4A} (D-erythrose), presumably a mixture of tetroses (C_{4AK}), appeared. On the other hand, the chromatogram of the reaction mixture with the reverse procedure (a C_{1A} solution was mixed 1 min after the preparation of an aqueous mixture of C_{3K} and $Ca(OH)_2$) showed that C_{3K} had already been converted to dimerization products and that no tetrose peak had appeared. Thus, the high efficiency of C_{3K} with a normal procedure, as is shown in Fig. 7, corresponds to the process which involves C_{1A} addition to C_{3K}, where the dimerization of C_{3K} is excluded. With the reverse procedure, however, the dimerization takes place prior to the addition of C_{1A} to C_{3K} and the weak acceleration efficiencies of such dimerization products, like that of Glu, govern

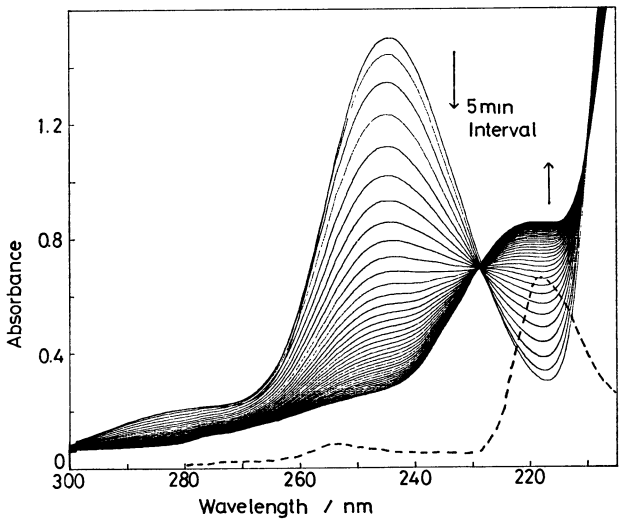
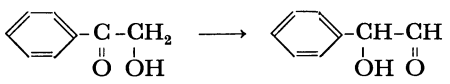


Fig. 12. UV spectral change of HAP in an aqueous $Ca(OH)_2$ solution.
25 °C, H_2O, $Ca(OH)_2$: 2.0×10^{-3} mmol cm^{-3}, HAP: 1.3×10^{-4} mmol cm^{-3}.

the formose formation. For C_{3A}, the dimerization in the presence of $Ca(OH)_2$ is slower than for C_{3K}, as is shown in Fig. 10. The difference with different procedures for reagent introduction is not significant for this accelerator, as is shown in Fig. 7. The dimerization of either C_{3A} or C_{3K} has been reported by Gutche *et al.*[10] and Berl and Feazel.[11] Glu changed little in a $Ca(OH)_2$ solution, as is shown in Fig. 11; consequently, no apparent difference between the two procedures was observed.

The significant difference observed for HAP with the procedure of reagent introduction *cf.* Fig. 6 is not fully understood. A spectral change in the ultraviolet spectrum of HAP in an aqueous $Ca(OH)_2$ solution (shown in Fig. 12) exhibits a decrease in absorbance at the absorption maximum, 246 nm, and an increase in the region of wavelength shorter than the isosbestic point at 227 nm. This suggests a loss of absorption due to the carbonyl group conjugated with the phenyl group of HAP by the reaction below. However, the HPL chromatographic separation of these compounds was not attained.



This postulation is in agreement with the facts that no significant absorption appears around 246 nm for phenylethylene glycol, as is shown by the broken curve in Fig. 12, and that 2-hydroxybutyrophenone forms an equilibrium mixture with 1-hydroxy-1-phenyl-2-butanone in the presence of OH^-.[12] The addition of one molecule of C_{1A} to the isomerized product of HAP, *i.e.*, 2-hydroxy-2-phenylacetaldehyde (HPA), would yield 2-phenylglyceraldehyde, which has saturated α-carbon next to carbonyl group, and thus is not susceptible to the addition of further C_{1A}. Although HPA has not yet been isolated from the reaction mixture with HAP, the isomerization of HAP in a $Ca(OH)_2$ solution is a plausible reason why the

large difference was observed between the different procedures of HAP introduction.

On the Mechanism of Formose Reaction. The finding of the present study, that the acceleration does not originate from the internal hydroxy oxo structure, but from the terminal hydroxy oxo structure of the accelerator, indicates that the C–C chain growth in formose formation is attained by successive additions of C_{1A} to the α-carbon to the carbonyl group, accompanied by the successive L-V transformation of the carbonyl group to the terminal position. The internal hydroxy oxo compound exhausts the hydrogen attached to α-carbon by the addition of one molecule of C_{1A} and then terminates, provided that there is no cleavage of the C–C bond of the accelerator during the reaction. Another finding, that the procedures of reagent introduction considerably influence the efficiencies of the accelerators, also suggests the aldol addition mechanism for the C–C chain growth of formose formation, because the acceleration by the hydroxy oxo compound occurs through the reaction between the hydroxy oxo compound and C_{1A}, as is shown in Fig. 9 for C_{3K}.

Moreover, the findings, that the acceleration efficiencies of Glu and Fru are small compared with those of C_{3A} and C_{3K}[4] and that the molar amounts of the consumption of C_{1A} are more than 200 times those of the introduced accelerators, as is shown in Fig. 4, suggest a degradation by a retro aldol reaction in order to account for the high activity being retained throughout the reaction until most of the C_{1A} is consumed.

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