JOURNAL OF CATALYSIS 62, 107–116 (1980)

Formose Reactions

IX. Selective Formation of Branched Sugar Alcohols in a Modified Formose Reaction and Factors Affecting the Selectivity[1]

YOSHIHIRO SHIGEMASA, MIKIO KAWAHARA, CHIKAHIRO SAKAZAWA,
AND RUKA NAKASHIMA

Department of Industrial Chemistry, Faculty of Engineering, Tottori University, Tottori 680, Japan

AND

TERUO MATSUURA

Department of Synthetic Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606, Japan

Received April 16, 1979; revised September 13, 1979

In the course of kinetic studies of the calcium hydroxide-catalyzed formose reaction, which usually gives a complex mixture of sugar products, a selective formose reaction was found to occur giving three kinds of branched sugar alcohols, 2-C-hydroxymethyl glycerol, 2,4-C-dihydroxymethyl pentitol, and most probably a diasteremeric mixture of 3-C-hydroxymethyl pentitol, when the major part of the dissolved calcium ions were removed as sparingly soluble salts or chelate complexes at the end of the induction period.

Oxalic and phosphoric acids were found to be effective for removing the dissolved calcium ion as well as a chelating agent such as ethylenediamine-tetraacetic acid (EDTA). Various factors affecting this selective formose reaction were investigated. The addition of $Mg(OH)_2$, $Fe(OH)_3$, and FeO gave the same selectivity, while the addition of $Pb_2O(OH)_2$ resulted in a different type of selectivity. Other factors, such as the reaction pH and the time of adding the acid, were also found to be important for the selective reaction.

INTRODUCTION

In our previous reports (*1*, *2*), a convenient method for analyzing the course of the caclium hydroxide-catalyzed formose reaction in aqueous media was developed. The method involves measurement of the oxidation–reduction potential (ORP) changes during the reaction, which can distinctly discriminate between the induction, sugar-forming, and sugar-decomposing periods by an ORP minimum and a maximum appearing at the end of the induction period and the end of the sugar-forming period, respectively. The ORP maximum appears very near the so-called yellowing point at which the color of the reaction mixture becomes yellow and the yield of formose sugars reaches a maximum (*1*, *3*). the symbols $T_{\min}$ and $T_{\max}$ have been used for the times that the induction and the sugar-forming periods are respectively terminated. Although the exact nature of the ORP changes is not yet known, we have proved the ORP method to be useful for the kinetic studies of the formose reaction (*2*).

The formose reaction has a very complex nature as seen from the number of sugar products exceeding 30 (4). To our best knowledge, no one has succeeded in discovering a selective formose reaction and isolating the products in spite of numerous attempts, except our previous finding that a photochemical formose reaction gives rise

---

[1] Part VIII: Shigemasa, Y., Taji, T., Sakazawa, C., Nakashima, R., and Matsuura, T., *J. Catal.* 58, 296 (1979).

0021-9517/80/030107-10$02.00/0
Copyright © 1980 by Academic Press, Inc.
All rights of reproduction in any form reserved.

to the considerably selective formation of 2-C-hydroxymethyl glycerol (12% yield) and pentaerythritol (38%) in addition to other minor formose sugars (*5*). Recently, Likholobov *et al.* (*6*) reported that at 18% conversion at 98°C the formose reaction had a 75.4 wt% selectivity to glucose and no branched species were identified, although glucose was not isolated from the reaction mixture. In the course of our recent kinetic studies on the calcium hydroxide-catalyzed formose reaction we found that a number of branched sugar alcohols 2-C-hydroxymethyl glycerol (**1**), 2,4-C-dihydroxymethyl pentitol (**2**), and 3-C-hydroxymethyl pentitol (**3**) are obtained with a high selectivity when most of the dissolved calcium ions are removed as hardly soluble salts or stable chelates at the end of the induction period ($T_{\min}$) (*7*). The present paper describes how we came to discover this selective formose reaction and how the reaction is controlled.

EXPERIMENTAL

General. The apparatus and the preparation of aqueous formaldehyde were virtually the same as those described previously (*1, 2*). The oxidation–reduction potential (ORP), pH, and concentration of the dissolved calcium ion species were measured by the method described in the previous papers (*1, 2*). Melting points were obtained by packing capillaries and were uncorrected. The ir, CIMS, and MS spectra were recorded with a Hitachi EPI-G2 grating spectrometer, a JASCO JMS-D-300 double focus spectrometer, and JEOL-01SG-2 double focus mass spectrometer, respectively. ^{1}H and ^{13}C-NMR spectra were taken on a Varian T-60A and JEOL-FX-100 spectrometer, respectively, and chemical shifts were given by ppm from TMS as an internal or external standard.

Procedure. The formose reaction was started by controlling a 1.0 *M* aqueous formaldehyde solution in the presence of calcium hydroxide (0.1 mole/liter) at 60°C. The progress of the reaction was followed by the ORP measurement. Under these conditions, $T_{\min}$ and $T_{\max}$ usually appeared after 21 and 27 min, respectively. Work-up of the reaction mixture as previously reported gave a complex mixture of formose sugars (Fig. 1). For the selective formose reaction, however, at $T_{\min}$ the dissolved calcium ions were precipitated as calcium oxalate or phosphate by adding an equivalent amount of the corresponding acid, or chelated with EDTA or nitrilotriacetic acid (NTA). Then, two major procedures were used as follows.

Procedure A. Without removing the precipitated calcium salt and calcium chelate from the mixture, the reaction was restarted by immediately adjusting the pH to a given value with an aqueous potassium hydroxide and the temperature to 60°C. In some cases a certain metal hydroxide such as $Mg(OH)_2$, $Fe(OH)_3$, FeO, and Al_2O_3 (0.1 mole/liter) was added immediately after adjusting the pH. When a chelating agent was used, the reaction mixture was homogeneous.

Procedure B. After the reaction mixture was immediately cooled with an ice bath, the precipitated calcium salts were removed by centrifugation at 0°C. The tem-

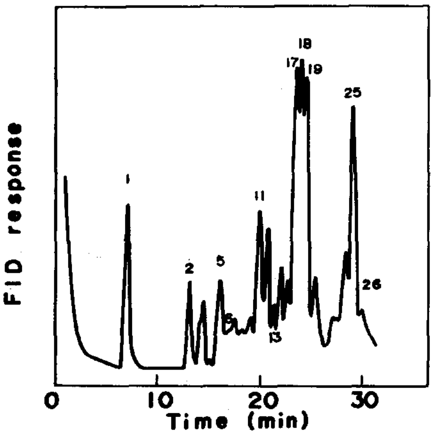


FIG. 1. Gas chromatogram of TMS derivatives. The usual calcium hydroxide-catalyzed formose reaction starting from [HCHO] = 1.0 *M* and $[Ca(OH)_2]$ = 0.1 *M* at 60°C (*5, 7*).

perature of the decanted supernatant solution was quickly set to 60°C and the reaction was restarted in the same manner as in procedure A. In same cases, a certain metal hydroxide was added immediately after adjusting the pH. The supernatant solution stored in a refrigerator (2°C) for a few days could also be used for further reactions.

For both procedures, the reaction was stopped at T_{max} by acidifying (pH 5–6) the mixture with 9 *N* HC1, which was then analyzed for formaldehyde, total sugar, organic acids, and product distribution by glc of trimethylsilylated products. The formaldehyde consumption was determined by the method of Bricker and Johnson (*8*) and the total sugar yield by the phenol-sulfuric acid method (*9*). The amount of organic acids were determined as follows. An aliquot (5 ml) of the reaction mixture was passed through a column of Amberlite IR 120(H) (40 ml) and washed with water to obtain 100 ml of an organic acid solution, which was titrated with 0.1 *N* KOH.

glc of sugar products. An aliquot (1 or 2 ml) of the neutralized reaction mixture was evaporated to dryness *in vacuo* at 40°C by the repeated additions and concentrations of water in order to remove the remaining formaldehyde. The residue trimethylsilylated in the usual manner (*10*) was extracted with chloroform and the chloroform extract was submitted to glc on a Shimazu GC-5A chromatograph equipped with a hydrogen-flame ionization detector under the following conditions: coiled glass column of 3m × 3 mm o.d.; adsorber, 5% silicon gum SE-30 on 60–80 mesh Chromosorb-W; nitrogen flow rate, 60 ml/min; temperature, 100–250°C, rising at the rate of 4°C/min. Yield of products was given by measuring peak area as glc% or by comparing the peak area with that of pentaerythritol as an internal standard.

Separation of products. The formose reaction was carried out starting from formaldehyde (30.0 g) according to Procedure A without removing the calcium oxalate precipitate. The consumption of formaldehyde, the sugar yield, and the yield of organic acid (as formic acid) were determined to be 95.6, 27.0, and 17.8%, respectively, based on the starting formaldehyde. The glc pattern shown in Fig. 2 indicated a very selective formation of products corresponding to peaks 2, 19, and 26. The reaction mixture was then passed through a column of Amberlite IR 120(H) (100 ml), and the column was eluted with water (6000 ml). The whole eluate was concentrated *in vacuo* to a sirup which was placed on the top of a column (42 × 6 cm o.d.) packed with Amberlite IRA 410(OH) (1200 ml). The column was eluted successively with water (4000 ml), 0.1 *N* KOH (1000 ml), 0.5 *N* KOH (5000 ml), and 1.0 *N* KOH (3000 ml). The KOH solution was effective for rapid elution of polyol products. The eluates were collected in 300-ml portions and were analyzed by glc. The eluates were than divided into the following five fractions by combining eluates showing same glc peaks and evaporated *in vacuo* at 40°C to dryness. Fraction A, a light yellow sirup, consisted mainly of a product corresponding to the glc peak 2 (1.4 g, 4.7% based on the starting formaldehyde); fraction B, a

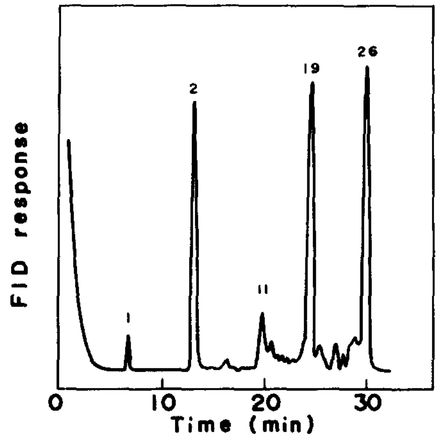


FIG. 2. Gas chromatogram of TMS derivatives. The selective formose reaction starting from [HCHO] = 1.0 *M* and [Ca(OH)$_2$] = 0.1 *M* at 60°C followed by precipitating the dissolved calcium ions by the addition of oxalic acid at the end of the induction period (Procedure A).

light yellow sirup, consisted of a product corresponding to the glc peak 19 (3.4 g, 11.3%); fraction C, a light brown sirup, consisted of a product corresponding to the glc peak 26 (6.6 g, 22.0%); fraction D, a colorless sirup, consisted of products corresponding to the glc peaks 1, 11, and other minor peaks (2.1 g, 7.0%); fraction E, a dark red sirup consisted of products the TMS derivatives which were not detected in glc (7.0 g, 23.3%) due to their nonvolatility.

Peak 2 product was obtained as a colorless and hygroscopic sirup by repeating column chromatography of fraction A on Amberlite IRA 410(OH) and identified as 2-C-hydroxymethyl glycerol (1) by a direct comparison with an authentic sample (*5*). ^{1}H-NMR (D_2O), 4.08s ppm; ir (KBr) 3350, 2930, 1400, 1110, and 1040 cm^{-1}.

Peak 26 product [2,4-C-dihydroxymethyl pentitol (2)] was obtained by crystalization from the methanol–acetone solution of fraction C as colorless crystals, mp 117°C, 4.0 g, ir (KBr), 3400, 2950, 1450, 1390, 1160, 1120, 1040, and 920 cm^{-1}; CIMS (isobutane), 213(QM$^+$, relative intensity 100), 195(QM$^+$-18, 37), 177(9), 159(14), and 91(29) *m/e*; ^{13}C-NMR (D_2O), 63.25t (2C), 64.71t (2C), 72.85d (1C), and 77.50s (2C) ppm; MS of the trimethylsilylate, 307(relative intensity 69), 217(100), 205(17), and 103(59) *m/e*. Found: C, 39.17; H, 7.53%. Calcd for $C_7H_{16}O_7$: C, 39.62; H, 7.60%. The acetate of (2) was prepared as follows. A mixture of acetic anhydride (0.5 g), 0.7 g of dry pyridine, and 0.1 g of (2) cooled with ice bath was stirred at 0°C until (2) was all dissolved. The solution was allowed to stand 20 hr at room temperature. The cooled solution was stirred with 3 ml of ice and water and neutralized with an excess of sodium hydrogen carbonate. The solution was extracted with three 5-ml portions of chloroform. The extract was washed with water, dried with anhydrous sodium sulfate, and evaporated under reduced pressure to a pale yellow sirup, acetate of (2). ^{1}H-NMR of the acetate ($CDCl_3$), 2.0–2.2m (21H; including 7 CH_3–CO peaks), 4.1–4.7m (8H; CH_2OAc), and 5.3s (1H; *CH*) ppm.

Peak 19 product was purified by repeating column chromatography with anion exchange resins [Amerlite IRA 410(OH)] from fraction B as a colorless sirup and its structure was assigned as a mixture of three diastereomers (two *meso* and a *dl* isomers) of 3-C-hydroxymethyl pentitol (3). ir (KBr), 3400, 2950, 1440, 1120, and 1040 cm^{-1}; CIMS (isobutane), 183(QM$^+$, relative intensity 100), 147 (QM$^+$ − 18, 22), 129 (14), and 99 (21) *m/e*; ^{13}C–NMR (D_2O), 3C at 62.88t, 63.31t, 63.73t, 64.09t, 64.29t, and 64.76t, 2C at 70.70d, 71.36d, and 72.47d, and 1C at 76.87s, 77.06s, and 77.58s ppm; MS of the trimethylsilylate, 307 (34), 217 (78), 205 (57), 103 (100) *m/e*; Found: C, 37.79; H, 8.21%. Calcd for $C_6H_{14}O_6 \cdot 1/2H_2O$: C, 37.65; H, 7.86%. The hexaacetate of (3) was synthesized by the same method as in case of the acetate of (2) as a pale yellow sirup. ^{1}H–NMR of the acetate ($CDCl_3$), 2.0–2.2m (at least 7 CH_3–CO– peaks; 18H), 4.0–4.7m (6H; CH_2OAc), and 5.31 broad t (2H, J = 3.5 Hz; –*CH(OAc)*–CH_2–).

RESULTS AND DISCUSSION

Effects of the Solid Ca(OH)$_2$ on the Formose-Forming Step

In a previous paper (*2b*), it was pointed out that the active catalytic species in the $Ca(OH)_2$-catalyzed formose reaction are different between the induction and the formose-forming steps; dissolved calcium ions (mainly $CaOH^+$) in the former and dissolved calcium ions and $Ca(OH)_2$ (or OH^-) in the latter. In order to see the effects of the solid $Ca(OH)_2$ on the time length ($T_{max} - T_{min}$) of the formose-forming step, the dissolved calcium ions, which are the effective catalyst in the induction period, were precipitated as calcium oxalate by adding an equivalent amount of oxalic acid to the starting $Ca(OH)_2$ at the end of the induction step (T_{min}). Then without removing the precipitate of calcium oxalate

(Procedure A), a given amount of $Ca(OH)_2$ was added immediately into the reaction mixture adjusted to pH 12.0 at which the dissolved calcium ions are scarcely present (*2b*). The results are shown in Fig. 3.

The formose-forming period ($T_{max} - T_{min}$) was steeply shortened with increased amount of the solid $Ca(OH)_2$ but became constant as the solid $Ca(OH)_2$ amounts of above 0.05 mole/liter. The sugar yield increased with an increase of $Ca(OH)_2$ amount below 0.1 mole/liter of the solid $Ca(OH)_2$ and decreased with larger amounts of $Ca(OH)_2$. The difference between the sugar yields at T_{max} and at 3 min after T_{max}, which is an approximate guide to the extent of sugar decomposition, increased with increased amount of the solid $Ca(OH)_2$. The results indicate that the solid $Ca(OH)_2$ is effective for accelerating both formose-forming and the product decomposition rates, and most probably that the surface of the hydroxide is effective for the catalysis in the formose-forming step.

Selective Formose Reaction and Product Analysis.

As seen in Fig. 3, it was of interest that the formose reaction occurred in the absence of $Ca(OH)_2$ in the formose-forming step, though at a slower rate than that in the presence of $Ca(OH)_2$. This finding prompted us to investigate product analysis of the former formed in the absence of $Ca(OH)_2$. The reaction was restarted by adjusting the pH of the reaction mixture to 12.0 with KOH without readding $Ca(OH)_2$ after precipitating the dissolved calcium ions with oxalic acid at T_{min} (Procedure A). glc analysis of the trimethylsilylated products obtained under these reaction conditions showed the selective formation of three products corresponding to peaks 2, 19, and 26 (Fig. 2), in sharp contrast to the nonselective product formation in the usual $Ca(OH)_2$-catalyzed formose reaction. A similar selectivity was observed when the restart of the reaction was done with a supernatant solution decanted from the calcium oxalate precipitates (Procedure B).

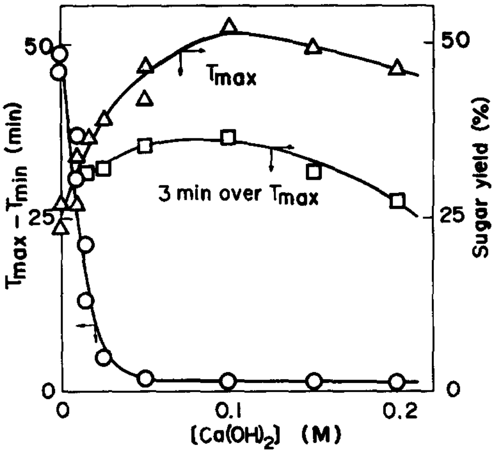
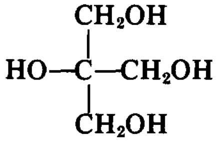


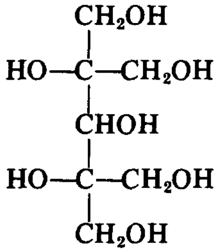
FIG. 3. Effect of the solid $Ca(OH)_2$ amount on the formose-forming rate and the sugar yield. Starting conditions: [HCHO] = 1.0 *M*; [$Ca(OH)_2$] = 0.1 *M*; Temperature, 60°C. After oxalic acid (0.1 *M*) was added at ORP_{min}, the formose-forming reaction was restarted by adjusting the mixture to pH 12.0 by concn KOH rapidly and adding solid $Ca(OH)_2$ successively (Procedure A).

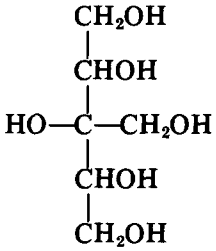
These three produces were isolated by repeating column chromatography with ion-exchange resins. Peak 2 product was identified as 2-C-hydroxymethyl glycerol (1) by a direct comparison (ir and the glc retention time of trimethylsilyl derivative) with authentic sample (*5*). Peak 26 product obtained in a crystalline form has a molecular formula $C_7H_{16}O_7$ which was deduced by its elemental analysis and chemical ionization mass spectral peak at *m*/*e* 213 (QM^+). The ^{13}C-NMR spectrum showed a pair of two equivalent CH_2 (63.25t, 64.71t ppm), a methine CH (72.85d ppm), and two equivalent tertiary C (77.50s ppm). The mass spectrum of its trimethylsilyl derivative showed an intense peak at *m*/*e* 307 compared with peaks at *m*/*e* 103 and 205, supporting the presence of $-(OH)C(CH_2OH)_2$ group in the parent compound (*11*). These spectral data led us to assign structure (2) [2,4-C-dihydroxymethyl penitol] for peak 26 product.

The peak 19 product appears to be a

mixture of three diastereomeric compounds, since its ^{13}C–NMR spectrum showed three sets of signals, namely, six signals (62.88t, 63.31t, 63.73t, 64.09t, 64.29t, 64.76t ppm) assignable for two equivalent and another CH_2 group, three signals (70.70d, 71.36d, 72.47d ppm) for two equivalent CH groups, and three signals (76.87s, 77.06s, 77.58s ppm) for a tertiary C group. The data are very consistent with 3-C-hydroxymethly pentitol (**3**) structure (a mixture of two *meso* and a *dl* isomers) for the peak 19 product. The structure assignment is also supported by the ^1H–NMR spectrum of its acetate and the fact that the mass spectrum of its TMS derivative showed a relatively weak peak at *m/e* 307 compared with peaks at *m/e* 103 and 205 (*11*).


(1)


(2)


(3)

Effects of Added Metal Hydroxides and Oxides on the Selectivity

In order to gain further insight into the nature of catalyst affecting the product selectivity in the formose-forming step, the effects of various metal-hydroxide or -oxide catalysts in the formose-forming step were investigated. The results are shown in Table 1. The remarkable features in these results are summarized as follows. (i) Regardless of the kind of the added metal hydroxides, $T_{max} - T_{min}$ is reduced with increased pH. These results are consistent with the important catalytic role of hydroxide ion on the formose-forming step (*2*). (ii) Comparing the reactions at pH 12.0, at which most of the metal hydroxides have very low solubility, $T_{max} - T_{min}$ decreases in the order of the metal hydroxide, $Fe(OH)_3$, FeO, Al_2O_3 > $Pb_2O(OH)_2$, $Mg(OH)_2$ > $Ba(OH)_2$ > $Ca(OH)_2$. (iii) In contrast to the nonselectivity with $Ca(OH)_2$ catalyst, $Mg(OH)_2$, $Fe(OH)_3$, FeO, $Ba(OH)_2$ and Al_2O_3 catalysts were found to give a similar selectivity yielding mainly three branched sugar alcohols, (**1**), (**2**), and (**3**), although less and slightly different selectivities were observed with $Ba(OH)_2$ and Al_2O_3. (iv) Among three catalysts ($Mg(OH)_2$, $Fe(OH)_3$, and FeO), $Mg(OH)_2$ gives the best selectivity with good reproducibility. Without adding any metal hydroxides or oxides at T_{min} (except KOH added for pH control), the formose-forming step was so sensitive to the pH and the procedure for removing the dissolved calcium ions that the reproducibility for product selectivity was somewhat difficult to obtain. The poor reproducibility due to the lack of the added catalyst is unaccountable at the present. (v) Interestingly, addition of $Pb_2O(OH)_2$ resulted in a different selective reaction giving peak 18 product as the major one. Although the isolation and the structure elucidation of this product are under investigation, the result encourages us for challenging discovery of other selective formose reactions. (vi) The best total sugar yield was obtained with $Pb_2O(OH)_2$. As has been previously pointed out (*1, 2*), the yield of formose sugars is highly de-

TABLE 1

Effect of Various Hydroxides in the Formose-Forming Step[a]

Metal hydroxide[b] catalyst added	pH	$T_{max} - T_{min}$ (min)	Total sugar yield at T_{max} (%)	Products[c] (glc%)							
				1	2	11	17	18	19	25	26
—[d]	12.0	49.0	29.8	2.0	24.5	4.4	<1.0	<1.0	22.6	3.0	12.1
$Ca(OH)_2$	11.0	5.0	55.1	2.7	3.9	8.3	7.8	12.0	7.8	20.3	3.4
$Ca(OH)_2$	12.0	1.5	41.3	5.7	3.3	6.4	9.4	9.9	10.1	13.1	1.6
$Mg(OH)_2$	11.0	64.0	42.2	2.4	18.5	<1.0	<1.0	<1.0	27.4	3.5	27.4
$Mg(OH)_2$	12.0	31.5	46.4	1.0	22.7	<1.0	<1.0	<1.0	23.6	1.0	42.4[e]
$Fe(OH)_3$	12.0	50.0	39.2	<1.0	15.5	1.0	<1.0	<1.0	19.1	<1.0	52.9
FeO	12.0	51.0	36.9	2.3	17.7	2.7	<1.0	<1.0	20.0	2.7	38.5
$Ba(OH)_2$	12.0	3.5	41.1	2.5	6.0	2.5	6.3	5.0	19.8	3.8	18.1
Al_2O_3	12.0	47.5	31.5	<1.0	13.4	1.0	<1.0	<1.0	25.7	18.0	23.4
$Pb_2O(OH)_2$	10.5	67.0	60.7	1.9	2.8	<1.0	8.3	27.3	8.6	19.2	1.7
$Pb_2O(OH)_2$	11.0	54.3	64.1	<1.0	1.3	1.2	5.3	22.8	8.7	12.3	1.8
$Pb_2O(OH)_2$	12.0	34.5	50.5	3.0	2.4	<1.0	3.1	34.5	7.1	9.5	3.1

[a] Starting conditions: [HCHO] = 1.0 *M*; $[Ca(OH)_2]$ = 0.1 *M*; temperature, 60°C. In all experiments, the consumption of formaldehyde at T_{max} was over 95%.

[b] After oxalic acid (0.1 *M*) and a metal hydroxide (0.1 *M*) were successively added at ORP_{min}, the reaction was immediately initiated by adjusting the mixture to a given pH by concn KOH (Procedure A).

[c] See Figs. 1 and 2.

[d] The result giving the best selectivity was given among several experiments.

[e] By compairing the peak area with that of pentaerythritol as an internal standard, the yield of product 26 was 21%, based on the starting formaldehyde.

pending on the Cannizzaro reaction with formaldehyde and the decomposition of formose sugars. In order to obtain further information on the effect of metal hydroxides on the total sugar yield, the rates of the decomposition of formose in the presence of a few metal hydroxides at pH 11.0 were compared. It was found that $Pb_2O(OH)_2$ decomposed the formose at the slowest rate: the rate increased in the order $Pb_2O(OH)_2 < Mg(OH)_2 < Ca(OH)_2$.

Effect of the Time of Adding Oxalic Acid

As described above, the removal of dissolved calcium ions by oxalic acid at T_{min} followed by adding certain metal hydroxides was proved to be a very useful technique for accomplishing a selective formose reaction. In Table 2, it is shown how the time of adding oxalic acid (T_{add}) influences the sugar yield, the length of the formose-forming period, and the product distribution. As T_{add} was set later than T_{min}, the time length ($T_{max} - T_{add}$) became shorter and some increase of the sugar yield was observed. When oxalic acid was added at T_{min} − 2 min or at T_{min} + 1 min, the product selectivity was not much altered. However, the addition of the acid later than T_{min} + 2 min caused nonselective formose formation. From these results, it is assumed that the quality and quantity of products at T_{add} might affect the rate and the product distribution in the formose-forming step. The results led us to conclude that it is necessary for obtaining a good selectivity to conduct the addition of oxalic acid followed by the addition of a metal hydroxide at or just before T_{min}. Earlier addition of oxalic acid resulted in a significantly lower rate in the formose-forming step giving a low sugar yield.

TABLE 2

Effect of the Time of Adding Oxalic Acid on the Selective Formose Reaction[a]

T_{add}[b] (min)	HCHO consumption at T_{add} (%)	Sugar yield at		$T_{max} - T_{min}$ (min)	Products[c] (glc%)								
		T_{add} (%)	T_{max} (%)		1	2	11	17	18	19	24	25	26
$T_{min} - 4$	21.6	0.8	10.3	118	Not determined								
$T_{min} - 2$	29.0	2.3	23.7	58	1.0	25.0	2.1	<1.0	<1.0	16.8	<1.0	<1.0	34.6
T_{min}	33.0	5.1	29.8	49	2.0	24.5	4.4	<1.0	<1.0	22.6	7.7	3.0	12.1
$T_{min} + 1$	44.5	11.5	40.3	9	4.3	26.4	21.	<1.0	3.4	17.5	<1.0	<1.0	19.2
$T_{min} + 2$	59.2	21.4	39.1	4	1.4	14.4	1.8	3.2	4.6	21.5	11.0	6.2	21.0
$T_{min} + 4$	65.4	23.3	39.3	3.5	2.0	16.4	3.3	2.0	3.9	19.0	13.1	5.3	16.7
$T_{min} + 6$	90.7	35.3	41.2	1	2.3	6.8	4.1	10.4	8.8	13.3	8.4	11.3	2.1

[a] The starting conditions were the same as for Fig. 3.
[b] T_{add} is the time from adding oxalic acid (0.1 M) followed by adjusting the pH to 12.0 with concn KOH (Procedure A).
[c] See Figs. 1 and 2.

Effect of Various Acids Added at T_{min} on the Selectivity

The effects of various acids which were added to the reaction mixture at T_{min} to precipitate the dissolved calcium ions or in hope of changing the nature of the calcium species, were studied as shown in Table 3. When HCl, HCOOH, CH_3COOH, and H_2SO_4 were added, $T_{max} - T_{min}$, the sugar yield, and the product distribution were very similar to those of the formose reaction which was carried out without adding any acid. When KH_2PO_4 and calcium-chelating agents, EDTA (ethylenediamine-tetraacetic acid) and NTA (nitrilotriacetic acid) were added (Procedure A), virtually the same selectivity was observed as in the case of oxalic acid, although $T_{max} - T_{min}$ became longer and the sugar yield was low compared to the above cases in which HCl, HCOOH CH_3COOH, and H_2SO_4 were used. NTA, however, gives a relatively high yield with similar selectivity.

There is an interesting relationship between the concentration ([Ca]) of the dissolved calcium ions after adding an acid and the selective product formation. While at

TABLE 3

Effect of Acids Added at ORP_{min}[a]

Acid[b] added	$T_{max} - T_{min}$ (min)	Sugar yield at T_{max} (%)	[Ca][c] (M)	Products[d] (glc%)								
				1	2	11	17	18	19	24	25	26
—	1.5	49.0	4.7×10^{-2}	7.5	3.8	8.6	9.2	15.2	6.8	3.6	13.0	<1.0
$(COOH)_2H_2O$	49	29.8	5.2×10^{-4}	2.0	24.5	4.4	<1.0	<1.0	22.6	7.7	3.0	12.1
KH_2PO_4	61	18.2	1.0×10^{-4}	1.0	20.3	2.7	<1.0	<1.0	12.2	<1.0	<1.0	18.0
EDTA	10	30.0	1.2×10^{-6}[e]	2.3	18.1	<1.0	<1.0	<1.0	20.8	<1.0	<1.0	38.1
NTA	7.0	43.0	1.6×10^{-4}[e]	3.6	15.7	3.0	<1.0	<1.0	19.3	7.2	<1.0	25.9
H_2SO_4	2.0	46.2	3.4×10^{-3}	4.4	2.5	7.2	8.0	14.1	9.9	7.7	13.3	<1.0
HCOOH	1.5	47.3	5.2×10^{-2}	2.8	1.5	5.1	12.1	22.1	7.0	3.2	12.3	<1.0
HCl	1.5	45.2	4.7×10^{-2} }[f]									
CH_3COOH	1.5	48.8	4.3×10^{-2}									

[a] The starting conditions were the same as those for Fig. 3.
[b] An equivalent amount of the acid to the added $Ca(OH)_2$ was added at T_{min}, then the pH was immediately adjusted to 12.0 by KOH (Procedure A).
[c] [Ca] is the concentration of the dissolved calcium ion species after the addition of an acid.
[d] See Figs. 1 and 2.
[e] The reaction solution is homogeneous. The data show the calculated concentration of the free (not chelated) calcium ion species in the absence of formaldehyde (*13*).
[f] Qualitatively the same product distribution as that with HCOOH as obtained.

10^{-2} M concentrations of [Ca] no selectivity was observed, at [Ca] lower than 10^{-4} M a good selectivity was attained. The results suggest that the effective [Ca] may be lower than 10^{-3} M for results in the selective formose reaction. The concentration of $Ca(OH)_2$, even when it is very low, is supposed to affect the product distribution.

Effects of Other Factors on the Selective Formose Reaction

The effects of pH in the formose-forming step on the product distribution were examined for Procedure A (Table 4). As described before, the formose-forming period ($T_{max} - T_{min}$) was reduced and the sugar yield increased with increased pH. The product distribution became simpler at higher pH. The addition of $Mg(OH)_2$, however, maintained the high selectivity at relatively low pH such as 11.0.

As shown in Tables 4 and 5, $T_{max} - T_{min}$ was reduced and the sugar yield increased with an increase in $Mg(OH)_2$ amount added after the precipitation of calcium ions, showing that $Mg(OH)_2$ assisted the selective formose formation. However, at concentrations above 0.1 and 0.5 mole/liter in removing calcium ions with oxalic acid and KH_2PO_4, respectively, both $T_{max} - T_{min}$ and sugar yield remained constant. At concentrations below 0.1 mole/liter $Mg(OH)_2$, a difference of $T_{max} - T_{min}$ was observed between the additions of oxalic acid and KH_2PO_4. These results imply that the calcium oxalate may have better catalytic activity for the selective reaction than the calcium phosphate.

The selective formose reactions described above were mostly carried out by Procedure A. In order to ascertain whether or not the precipitated calcium oxalate acts as catalyst for the formose-forming step giving the selective products, the formose-forming step was started after the precipitate was removed by centrifugation (Procedure B). The product distribution was simple and similar to those (Table 1) obtained by adding $Mg(OH)_2$ without removing the precipitated calcium oxalate or phosphate from the mixture (Procedure A) at T_{min}. Without adding $Mg(OH)_2$, the supernatant solution from the precipitated calcium phosphate gave shorter $T_{max} - T_{min}$ (60 min) than that (90 min) from the calcium oxalate (Procedure B). On the other hand, for Procedure A without adding $Mg(OH)_2$ $T_{max} - T_{min}$ was shorter in the presence of

TABLE 4

Effect of pH and the Amount of $Mg(OH)_2$[a]

pH[b]	$Mg(OH)_2$ (M)	$T_{max} - T_{min}$ (min)	Sugar yield at T_{max} (%)	Products[c] (glc%)							
				1	2	4	11	19	24	25	26
11.0	0	180	13.2	3.1	8.1	10.0	6.7	17.1	4.0	14.6	5.9
12.0	0	49	29.8	2.0	24.6	2.7	4.4	22.6	7.7	3.0	12.1
13.0	0	8.5	42.4	4.1	6.5	<1.0	6.2	28.1	<1.0	<1.0	32.8
11.0	0.1	64	42.2	2.4	18.5	<1.0	1.1	27.4	<1.0	3.5	27.4
12.0	0.1	31.5	46.4	2.8	17.2	<1.0	1.4	25.8	1.1	3.3	32.9
13.0	0.1	9.0	42.4	<1.0	4.4	<1.0	1.1	38.6	4.2	7.2	19.9
10.5	0.5	180	36.3	<1.0	19.6	6.8	1.4	10.0	<1.0	<1.0	41.6
11.0	0.5	67	44.7	2.7	30.7	<1.0	<1.0	20.0	<1.0	4.0	32.0
12.0	0.5	32	45.6	8.8	16.7	<1.0	<1.0	25.9	<1.0	<1.0	33.0
13.0	0.5	8.0	44.5	3.3	10.4	<1.0	<1.0	30.1	<1.0	<1.0	21.7

[a] The starting conditions were the same as those for Fig. 3.

[b] The pH was adjusted by concn KOH after adding oxalic acid (0.1 M) and $Mg(OH)_2$ successively at T_{min} (Procedure A).

[c] See Figs. 1 and 2.

TABLE 5

Effect of the Amount of $Mg(OH)_2$ Added at T_{min}[a]

Acid[b] added	$Mg(OH)_2$ (M)	$T_{max} - T_{min}$ (min)	Sugar yield at T_{max} (%)	Products[c] (glc %)					
				1	2	11	19	23	26
KH_2PO_4	0	61	18.2	<1.0	20.3	2.7	12.2	<1.0	18.0
KH_2PO_4	0.1	46	21.2	<1.0	14.0	2.0	26.0	2.1	28.0
KH_2PO_4	0.5	32	32.8	3.5	20.5	1.5	24.5	2.0	26.5
KH_2PO_4	1.0	33	35.3	<1.0	20.7	<1.0	26.8	2.1	23.3

[a] The starting conditions were same as Fig. 3.

[b] After the equivalent amount of an acid to the added $Ca(OH)_2$ and the given amount of $Mg(OH)_2$ were successively added at ORP_{min}, the pH of the reaction mixture was adjusted to 12.0 by concn KOH as soon as possible (Procedure A).

[c] See Figs. 1 and 2.

the precipitated calcium oxalate than that of the phosphate. The above results and the fact that the supernatant solution from the precipitated calcium oxalate had more dissolved calcium ions than that from the calcium phosphate as shown in Table 3, lead us to assume that the calcium oxalate precipitated at T_{min} accelerates the formose-forming step.

In summary, the best way to carry out the selective formose reaction is by precipitating the dissolved calcium ion with oxalic acid at T_{min} followed by restarting it without removing the precipitated calcium oxalate in the presence of $Mg(OH)_2$ at pH 12.0, at which the product selectivity is higher than those at other pH.

REFERENCES

1. Matsuura, T., Shigemasa, Y., and Sakazawa, C., *Chem. Lett.* 1974, 713; Shigemasa, Y., Shimao, M., Sakazawa, C., and Matsuura, T., *Bull. Chem. Soc. Japan* **48**, 2099 (1975).
2. (a) Shigemasa, Y., Fujitani, T., Sakazawa, C., and Matsuura, T., *Bull. Chem. Soc. Japan* **50**, 1527 (1977); (b) Shigemasa, Y., Shimao, M., Sakazawa, C., and Matsuura, T., *Bull. Chem. Soc. Japan* **50**, 2138 (1977).
3. Mizuno, T., Mori, T., Shiomi, N., and Nakatsuji, H., *J. Agr. Chem. Soc. Japan* **44**, 324 (1970).
4. Mizuno, T., and Weiss, A. H., *Adv. Carbohydrate Chem. Biochem* **28**, 173 (1974).
5. Shigemasa, Y., Matsuda, Y., Sakazawa, C., and Matsuura, T., *Bull. Chem. Soc. Japan* **50**, 222 (1977).
6. Likholobov, V. A., Weiss, A. H., and Sakharov, M. M., *React. Kinet. Catal. Lett.* **8**, 155 (1978).
7. Preliminary accounts appeared in Shigemasa, Y., Nagae, O., Sakazawa, C., Nakashima, R., and Matsuura, T., *J. Amer. Chem. Soc.* **100**, 1309 (1978); Shigemasa, Y., Sakazawa, C., Nakashima, R., and Matsuura, T., *in* "Origin of Life," p. 211 (1978).
8. Bricker, C. E., and Johnson, H. R., *Ind. Eng. Chem.* **17**, 400 (1945).
9. Dubois, M., Gills, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F., *Anal. Chem.* **28**, 350 (1956).
10. Sweeley, C. C., Bentley, R., Makita, M., and Wells, W. W., *J. Amer. Chem. Soc.* **85**, 2497 (1963).
11. Partridge, R. D., Weiss, A. H., and Todd, D. *Carbohydrate Res.* **24**, 29 (1972).