FORMOSE SUGARS FROM FORMALDEHYDE

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ABSTRACT
A mixture of sugars is produced by base catalysed condensation of formaldehyde. The sugars range from four to seven carbon atoms, mainly six. They are sufficiently toxic to kill rats at 50% of the diet level, possibly due to the multiplicity of species as well as the presence of branched sugars. Manipulation of the reaction conditions can effect a 75% selectivity to racemic glucose, but its economic recovery is improbable. The formose sugars were tested for cancer tumor growth suppression activity, but none was found.

THE FORMOSE REACTION SYSTEM
When an aqueous solution of formaldehyde is mixed with a base such as calcium hydroxide, a complex set of exothermic reactions proceeds autocatalytically. The product of the formaldehyde reaction is a mixture consisting mostly of carbohydrates ranging in carbon number from four to seven, with C_6 sugars forming the major fraction. This network of reactions is known as the formose reaction.

nHCHO + HOCH_2(CHOH)_{n-2}CHO

The main reactions involved are aldol condensations of formaldehyde with sugars to produce higher molecular weight sugars.

HCHO + HOCH_2(CHOH)_nCHO + HOCH_2(CHOH)_{n+1}CHO

Sugars of a given molecular weight can also break down to lower molecular weight
species in the alkaline medium.

\[ \text{HOCH}_2(\text{CHOH})_{n+m} \text{CHO} \stackrel{+}{\rightarrow} \text{HOCH}_2(\text{CHOH})_{n-1}\text{CHO} + \text{HOCH}_2(\text{CHOH})_{m-1}\text{CHO} \]

Important side reactions are the Cannizzaro reaction

\[ \text{HCHO} + \text{HCHO} + \text{OH}^- + \text{CH}_3\text{OH} + \text{HCOO}^- \]

and to a small extent the crossed Cannizzaro reaction

\[ \text{HCHO} + \text{HOCH}_2(\text{CHOH})_n\text{CHO} + \text{OH}^- \rightarrow \text{HOCH}_2(\text{CHOH})_n\text{CH}_2\text{OH} + \text{HCOO}^- \]

producing formate, methanol and polyols as byproducts.

The alkaline conditions also promote isomerization reactions. Aldoses are isomerised to ketoses by the Lobry de Bruyn-Alberda van Ekenstein rearrangements, e.g. in the case of glyceraldehyde and dihydroxyacetone

\[ \text{HOCH}_2(\text{CHOH})_n\text{CHO} \rightarrow (\text{HOCH}_2)_n\text{CO(CH}_2\text{OH}) \]

Sugars are also isomerized to a significant extent to the corresponding saccharinic acids

\[ \text{OH}^- + \text{HOCH}_2(\text{CHOH})_n\text{CHO} \rightarrow \text{HOCH}_2(\text{CHOH})_{n-1}\text{CH}_2\text{COO}^- + \text{H}_2\text{O} \]

This complex formose reaction system has been studied as a possible source of carbohydrates for human consumption during extended space flights. The carbon source for formaldehyde in a space craft would be exhaled carbon dioxide, which would then be converted to HCHO and then formose sugars, providing a closed-loop food synthesis metabolism system(1-4).

For a large scale production of carbohydrates, synthesis gas from petroleum or coal gasification processes would serve as the raw material for formaldehyde via methanol

\[ \text{CO} + 2\text{H}_2 \rightarrow \text{CH}_3\text{OH} \]
\[ \text{CH}_3\text{OH} + \frac{1}{2}\text{O}_2 \rightarrow \text{HCHO} + \text{H}_2\text{O} \]

McPherson (5) has discussed the possibility of solving world food problems by means of chemical synthesis. Block diagrams showing schemes for production of edible C6 carbohydrates from the formose reaction products have been presented and discussed (6,7).

Because the reaction is autocatalytic it is very conveniently controlled and studied in a continuous stirred tank reactor. Kinetic studies using this technique have been reported(8,9). If the reactor is operated near complete formaldehyde conversion, Cannizzaro reaction is minimized to less than 2%. However, at these conditions, saccharinic acid formation becomes very important.

The mixture of sugars formed in the formose reaction is illustrated in Figure 1, which is a gas chromatogram of the trimethylsilyl ether derivatives of formose products obtained at 73.4% formaldehyde conversion in a batch reactor at 98°C. The products found under these conditions are somewhat less complex than those identified by Partridge et al (12) in the product from a plug flow reactor at 60°C and complete conversion.
FIGURE 1 Gas chromatogram of trimethylsilyl ether derivatives of formose reaction products at 75% conversion in a batch reactor at 98°C. Initial concentrations: HCHO = 2.07M, Ca(OH)$_2$ = 0.2M.

TOXICITY OF FORMOSE SUGARS

The body metabolizes D-sugars. Formose sugars are racemic DL mixtures, so at best a 50% selectivity towards formose as food can be envisioned. When formaldehyde is completely converted to products the yield of sugars which are edible, let alone D-sugars, is low. Akerlof and Mitchell (10) found that formose sugar syrup was a poor carbon source for the growth of baker’s yeast, possessing approximately 4.5% of the activity of glucose. They also found that the formaldehyde-free mixture contained components that were highly toxic to rats, but they did not identify these species.

Mizuno and Weiss (2), in a review of formose chemistry, described various feeding studies, including a report by Mizuno and coworkers (11) that when formose was substituted for 50% of the diet of adult rats, their body weight decreased linearly with time and all the rats died after 32±5 days. The rats suffered profuse diarrhoea, blisters in the intestine and abnormalities in other organs.

Formose syrups were also tested by Bok and Demain (6) as carbon sources for various yeasts, bacteria and moulds. The best results were obtained with strains of Aerobacter aerogenes, which produced a dry cell mass of 1.3 g l$^{-1}$ in a supplemented
medium containing 10 g l\(^{-1}\) of formose solids. The same strains yielded about 3.7 g l\(^{-1}\) dry cell mass from 10 g l\(^{-1}\) of glucose. Bok and Demain point out that even if a process could be developed which could produce 5 g dry cell mass from 10 g of formose solids, this process would have trouble competing with one based on methanol, a substrate which could be produced much more easily from carbon dioxide by reduction.

The poor utilization and toxicity of formose are probably related, inter alia, to the presence of branched chain sugars, saccharinic acids, and to the racemic nature of the mixture. It has been suggested that these unnatural species, being similar in structure to natural sugars, bind to and block glucose oxidase sites (6).

Based on the possibility that this blocking hypothesis is correct, formose sugars were recently tested for anti-tumor activity by the Drug Evaluation Branch of the National Cancer Institute, National Institutes of Health (13). The actual testing was carried out in the laboratories of A.D. Little, Inc. Groups of female mice, previously inoculated with lymphocytic leukemia tumors, were treated with doses of formose sugars dissolved in saline solution injected intraperitoneally. The results, summarized in Table 1, show no anti-tumor activity, and consequently it is not certain about the blocking activity. The particular formose sugar used for these tests was prepared by the late Dr. J. Shapira, NASA Ames Research Center, Moffett Field, CA 94035, and was used for the study described in (12).

### TABLE 1

<table>
<thead>
<tr>
<th>Size of injection (mg/kg mouse)</th>
<th>Frequency of injection (times/day)</th>
<th>Total number of injections</th>
<th>Weight gain of mice (g) Control</th>
<th>Survivors after four days</th>
<th>Tumor Evaluation Control Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1</td>
<td>9</td>
<td>2.0</td>
<td>1.0</td>
<td>10.8</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>9</td>
<td>2.0</td>
<td>0.8</td>
<td>10.8</td>
</tr>
<tr>
<td>200</td>
<td>1</td>
<td>9</td>
<td>2.0</td>
<td>1.0</td>
<td>10.8</td>
</tr>
</tbody>
</table>

*This is related to tumor progression. The ratio of test to control evaluation must be greater than 1.25 for the agent to be considered worthy of further study as an anti-tumor agent.*

It is clear that even if the toxic components of formose were identified and eliminated, the problem of low yield of useable carbohydrates would remain. As mentioned, not the least of the problems is that the body only metabolizes D-sugars, while racemic DL mixtures are produced in the formose reaction. The long-term effects of ingestion of L-sugars would have to be assessed.

A possible solution is the hydrogenolysis of formose products to glycerol (1), which is metabolized by the body to D-glucose. This hydrogenolysis reaction is complicated by the formation of saccharinic acids from the sugars at the severe alkaline conditions used. The result is relatively low selectivity to glycerol or
even to sugar polyols, as well as the formation of species such as ethylene glycol and propylene glycol, etc. If Ca(OH)$_2$ is used to enhance carbon-carbon bond cleavage during hydrogenolysis, as suggested by Vasynina et al (17), saccharinic acids are formed at even higher rates and the selectivity to glycerol is decreased.

Table 2 lists product distributions from sucrose reported by Van Ling et al (14) in a continuous process operating at 200 - 225°C and 200 atm. Figure 2 shows a possible production scheme for producing polyols via the formose reaction. Formaldehyde is mixed with Ca(OH)$_2$ and nickel catalysts and reacted to almost complete conversion in a CSTR-plug flow reactor combination. The formose product, as well as recycled C$_4$ and higher polyols, then passes to a battery of batch hydrogenolysis autoclaves. Product from these is filtered to recycle insoluble Ca(OH)$_2$ and nickel, and the filtrate is neutralized with sulphuric acid, precipitating soluble calcium as CaSO$_4$, which is discarded. A battery of stills is used to separate the various products. Saccharinic acids present in the bottoms are recycled with C$_4$ and higher polyols to hydrogenolysis.

**TABLE 2**

Sucrose hydrogenolysis yields (14)

<table>
<thead>
<tr>
<th>Product</th>
<th>Yield (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene glycol</td>
<td>15.6</td>
</tr>
<tr>
<td>1,2 Propylene glycol</td>
<td>17.9</td>
</tr>
<tr>
<td>Glycerol</td>
<td>31.3</td>
</tr>
<tr>
<td>Tetritols</td>
<td>4.9</td>
</tr>
<tr>
<td>Pentitols</td>
<td>2.9</td>
</tr>
<tr>
<td>Hexitols and higher</td>
<td>27.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

**SACCHARINIC ACIDS IN FORMOSE**

Figure 3 shows that, in the formose system, Cannizzaro reaction proceeds initially, when only HCHO and no significant amounts of sugar are present.

\[ \text{OH}^- + 2\text{HCHO} \rightarrow \text{CH}_2\text{OH} + \text{HCOO}^- \]

Near complete conversion, where there is no significant HCHO present but only sugars, isomerization to saccharinic acids occurs.

\[ \text{OH}^- + \text{RCH(OH)CHO} \rightarrow \text{HOH} + \text{RCH}_2\text{COO}^- \]

At intermediate conversions, when both HCHO and sugars are present, aldoses without α-hydrogens undergo crossed Cannizzaro reaction.

\[ \text{HCHO} + \text{OH}^- + \text{RC(R')(OH)CHO} \rightarrow \text{CH}_3\text{OH} + \text{RC(R')(OH)COO}^- \]

The solid points on Figure 3 are data collected at low formaldehyde conversions in the formose system at typical conditions. The open points are data from experiments using pure sugars to simulate high formaldehyde conversions to sugars. The two lines drawn are not fitted to the data, but merely represent 2:1 and 1:1 ratios of formaldehyde or carbohydrate conversion to acid production. It can be seen that the formaldehyde
case approximates the 2:1 line, while the sugars follow the 1:1 line. This indicates that the acid in each case is produced by a different reaction in the formose system. Indeed, as is seen above, the Cannizzaro reaction consumes two moles of formaldehyde to form one mole of acid, while the isomerization of glucose or arabinose to saccharinic acids transforms one mole of carbohydrate to one mole of acid.

Also, since all ketoses (as well as aldoses containing α-hydrogen atoms) do not undergo crossed Cannizzaro reactions, it can be concluded that most of the acid produced under conditions of high formaldehyde conversion is to saccharinic acid formation. Figure 4 shows that the rates of saccharinic acid formation are not inappreciable at formose conditions.

DIRECTING THE FORMOSE REACTION TO GLUCOSE

It has been an elusive goal to attempt to control or direct the formose reaction so that only glucose is produced. Recently, it was shown that at 18% formaldehyde
FIGURE 3 Simulation of the formose system using natural sugars shows that isomerization to saccharinic acids proceeds at high conversion levels. Cannizzaro reaction occurs at low conversion levels.

FIGURE 4 Kinetic curves for saccharinic acid formation.
FIGURE 5 Effect of conversion level on formose glucose selectivity at 98°C. Initial concentrations: HCHO = 1.82 M, Ca(OH)₂ = 0.112 M. Curve identification: A = total yield of C₆ carbohydrates, 1 = α-glucose, 2 = β-glucose, 3 = α- and β-mannose, 4 = α- and β-galactose, fructose and allose, 5 = C₇ carbohydrates, 6 = C₂ carbohydrates, 7 = C₃ + C₄ + C₅ carbohydrates, 8 = hydroxymethyl glycerol.

Conversion, hexoses can be produced at 84 wt% selectivity and that glucose is a major fraction of the C₆ species (4) amounting to 90%. In order to attain this selectivity, high temperature (98°C) was required. Under these conditions, in alkaline solution, the glucose initially formed quickly reacted to form other products, including saccharinic acids (16). However, if reaction is stopped at low conversions, and formaldehyde separated and recycled, it is, in principle, feasible to obtain a high overall yield of glucose. Figure 5 shows the change in selectivity of the reaction as a function of formaldehyde conversion at 98°C. These data were obtained in a batch reactor study in which the reaction was carried out under a nitrogen atmosphere in a 200 ml flask supported in an isothermal bath controlled within ± 2 degrees of nominal reaction temperature. Figure 6 shows the effect of temperature on the formose reaction product carbon number at 18% formaldehyde conversion. These results indicate that by the use of even higher temperatures (requiring pressures greater than atmospheric), it might be possible to increase the selectivity to six-carbon sugars even further. The problem of formaldehyde separation would still remain, however, if the low
CONCLUSIONS

It is evident that, at the present state of the art, formose sugars are neither directly edible nor efficiently used as a substrate for the growth of microorganisms. Further research directed towards increasing the conversion and selectivity to glucose to the point where formose edible sugars can compete with natural sugars or with cellulose hydrolysis is still necessary before the formose reaction can be
considered an economical potential source of food.

If the reaction over zeolites can be controlled to produce glycerol instead of glycolaldehyde, this would solve the problems not only of producing edible species but also of separation of DL mixtures. However, even the effects of ingesting large amounts of glycerol over extended periods of time would have to be evaluated.

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