BRANCHED-CHAIN CARBOHYDRATE STRUCTURES RESULTING FROM FORMALDEHYDE CONDENSATION

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ABSTRACT

Sugars prepared by condensation of formaldehyde under catalysis by calcium hydroxide (the formose reaction) were analyzed by combined gas–liquid chromatography and mass spectrometry of the trimethylsilyl ethers; and when reduced to the corresponding alditols, as their *O*-trimethylsilyl and *O*-trifluoroacetyl derivatives Both branched-chain and straight-chain species are produced by formaldehyde condensation, and definitive spectra are provided that characterize the unique branched-chain products

INTRODUCTION

The formose reaction, namely the autocatalytic condensation of formaldehyde to sugars in the presence of alkaline catalysts, was first reported by Butlerow[1] in 1861, and has been studied intermittently since then, mainly to identify the reaction products The reaction gives a complex mixture of aldoses and ketoses ranging from a two-carbon product (glycolaldehyde) through three, four-, five-, six-, and seven-carbon, and perhaps even higher species, by the general process

$$(n+2)\mathrm{HCHO} \xrightarrow{\mathrm{Ca(OH)_2}} \mathrm{HOCH_2(CHOH)_nCHO}$$

The formose reaction is catalyzed both by divalent metal bases, such as calcium and barium hydroxides, and lead oxide, and by monovalent bases such as thallium hydroxide The condensation to sugars occurs in competition with the simple Cannizzaro reaction of formaldehyde, the latter course is the sole initial reaction observable when sodium or lithium hydroxides are used

The chemistry of the formose reaction has been reviewed recently by Akerlof and Mitchell[2] and by Frankenfield[3] in connection with the possibility of using the formose reaction to produce edible carbohydrates in sustained space flights Metabolic body wastes such as carbon dioxide would be hydrogenated to methane, and the latter

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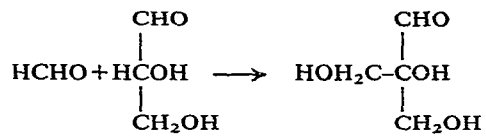
partially oxidized to formaldehyde and subsequently converted into formose sugar Akerlof[4] and Shapira[5] have shown that the complex formose sugar is toxic, and interest from the view of feeding during space travel has been directed to using the formose reaction, coupled to a reductive step, to produce glycerol instead of sugars[5,6]

There has also been major Soviet interest in the utilization of the formose reaction[7] The potential of the formose reaction for large-scale manufacture of sugars has been considered[8], and the mechanism and kinetics of the reaction have been studied[9]

To explain the known products of the formose reaction, the following processes suffice (*1*) An initiating — and autocatalytic — condensation of two molecules of HCHO to give $OH–CH_2CHO$ (glycolaldehyde), (*2*) successive aldol condensations involving HCHO and hydrogen atoms alpha to a carbonyl group, (*3*) Lobry de Bruyn–van Ekenstein interconversion, and (*4*) a crossed-Cannizzaro reaction to produce alditols and formate ion

It is to be noted that branched chain sugars — rarely found in Nature and not sought by earlier investigators of formose — would be expected to be major components of formose Furthermore, if the structural features required for processes (*2*) and (*3*) were absent in a product, then that product is a terminal one For example, if glyceraldehyde undergoes aldol condensation with HCHO, the product, 2-*C*-(hydroxymethyl)glyceraldehyde, would be expected to either accumulate or undergo crossed-Cannizzaro reduction to the tetritol



Apparently, the reverse aldol condensation is not favored under conditions of the formose synthesis, as large proportions of such terminal, branched products have now been found in formose

The crossed-Cannizzaro reaction seems to be minor in formose synthesis, as only a small proportion of alditols form Gas–liquid chromatographic analysis or appropriate derivatives [per(trimethylsilyl = Me_3Si and per(trifluoroacetyl) = CF_3CO] of formose led to very complex patterns Prior reduction of formose by sodium borohydride to convert carboxyl groups into alcohols, followed by derivatization, led to far simpler patterns[10]

Many carbohydrate derivatives have been studied by mass spectrometry[11], but only a few of these are amenable to analysis by g l c The mass spectra of a number of alditol per(trifluoroacetates) have been reported[12]

Whereas many known sugars have previously been identified as components of formose, apparently the finding of branched-chain sugars in the course of our work is new

To what degree the toxicity of formose is due to the L-forms (presumably not easily metabolized) of sugars present, and to what degree it is due to the unnatural branched-chain sugars present, remains to be seen

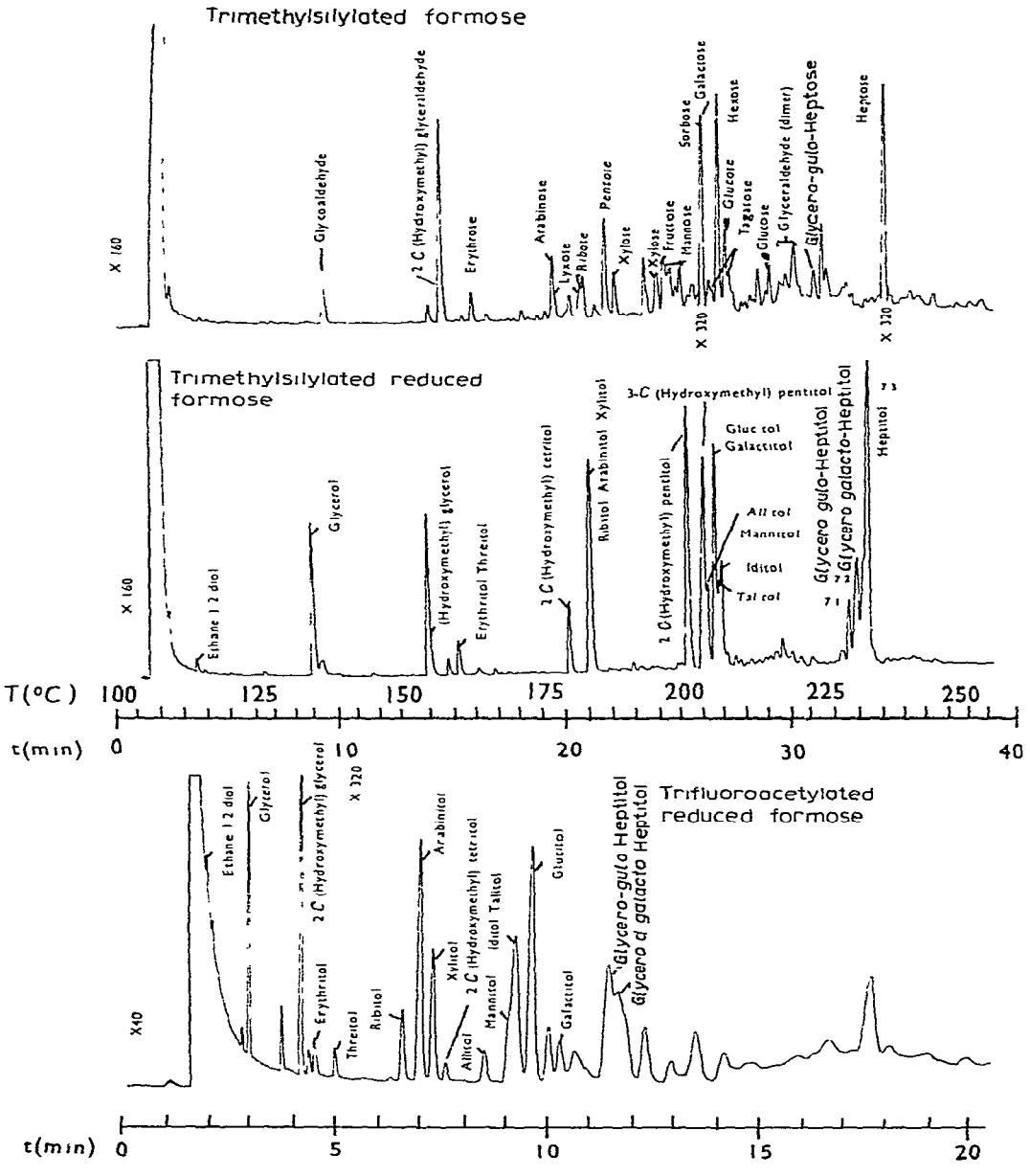


Fig 1 Gas–liquid chromatograms of derivatives of formose and reduced formose Formose syrup produced at complete conversion in a plug-flow reactor

RESULTS AND DISCUSSION

The chromatogram in Fig 1 shows the complex product-distribution of the Me_3Si ethers of the sugars in formose, together with the greatly simplified chromato-

grams obtained after borohydride reduction of the formose before silylation The branched-chain alditols are resolved from the straight-chain components as the Me_3Si ethers Better resolution of the straight-chain species was accomplished by using the alditol trifluoroacetates, but there was incomplete separation of the branched from the straight-chain alditols as the trifluoroacetates

The mass spectra of the silylated alditols showed fragmentation patterns resulting primarily from simple cleavage of the molecule with a minimum of rearrangement. The molecular ion fragments initially by loss of $OSiMe_3$ (*m/e* 89), followed by $OSiMe_3$ molecules (*m/e* 90) in a manner similar to that of the trifluoroacetates[12] Major fragments at *m/e* 147 and 191 have been found in all Me_3Si derivatives reported to date, and are explained by rearrangement[16]. A base peak of *m/e* 73 can be explained by the loss of formaldehyde from the *m/e* 103 fragment[16] Molecular ions were observed only with the trimethylsilylated tetritols

The mass spectra (of both the Me_3Si and CF_3CO derivatives) of diastereomeric straight-chain alditols coincided, within the limits of error The mass spectra of branched-chain alditols differed from those of straight-chain species in the relative intensities of tertiary versus secondary fragments

Identification of tetritol — Fig 2 presents an analysis of the region corresponding to four-carbon products in the silylated reduced formose, and shows possible structures for the alditols and their fragmentation patterns There are three possible four-carbon alditol structures one branched-chain and two diastereomeric, straight-chain forms The chromatogram of the silylated, reduced formose showed three peaks in this region The last peak of the chromatogram from formose corresponded in retention time to the trimethylsilyl ethers of both erythritol and threitol, and its mass spectrum matched that of authentic erythritol or threitol tetrakis(trimethylsilyl) ether, both derivatives gave identical spectra The first two peaks thus remained to be identified.

The branched-chain tetritol, 2-*C*-(hydroxymethyl)glycerol*, was synthesized by diazotization of tris(hydroxymethyl)aminomethane (Tris) The silylated, total crude-product that resulted from this synthesis gave the three peaks shown in Fig 2 The peak among these identified as 2-*C*-(hydroxymethyl)glycerol was identical by retention time and mass spectrum (Fig 2) with the peak for that compound in the tetritol region of the formose The presence of a molecular ion at *m/e* 410 suggests that the first product eluted, which cannot be a straight-chain tetritol, is a branched-chain tetritol The mass spectrum of silylated 2-*C*-(hydroxymethyl)glycerol showed a low relative abundance of *m/e* 205 and a high relative abundance of *m/e* 307 when compared with the spectra of the straight-chain, silylated tetritols, indicative of fragmentation from the branched-chain species The structure of the branched, silylated tetritol indicates that there can be no direct formation of *m/e* 205 by cleavage Cleavage at C-1 does result in a very stable, tertiary fragment at *m/e* 307 The struc-

*A preparation of this compound, characterized by a carbon–hydrogen analysis, has been reported[13] The triacetate, tris(acetoxymethyl)methanol, has been reported in a patent[14] Ger 874, 774

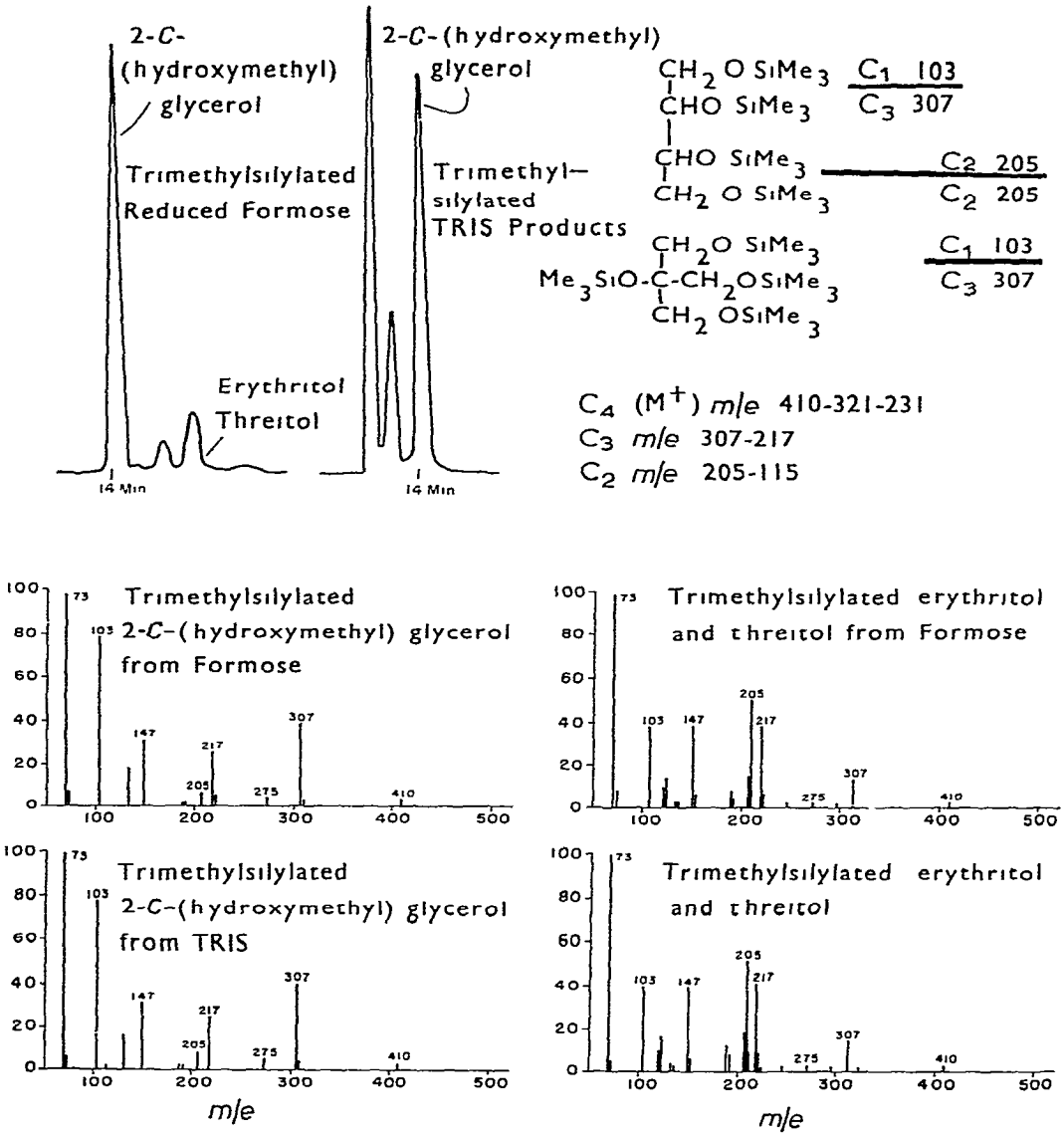


Fig 2 Identification by g l c–m s of species in the tetritol region of trimethylsilylated, reduced formose

tures of the straight-chain, silylated tetritols indicate the formation of *m/e* 205 proceeds by cleavage at C-2 and of *m/e* 307 by cleavage at C-1 The lower abundance of *m/e* 307 in the case of the straight-chain structures appears due to its lower stability compared with the tertiary fragment from the branched species

The analysis of the formose tetritol trifluoroacetates corroborates the results with the trimethylsilyl ethers, as shown in Fig 3, The retention times of erythritol

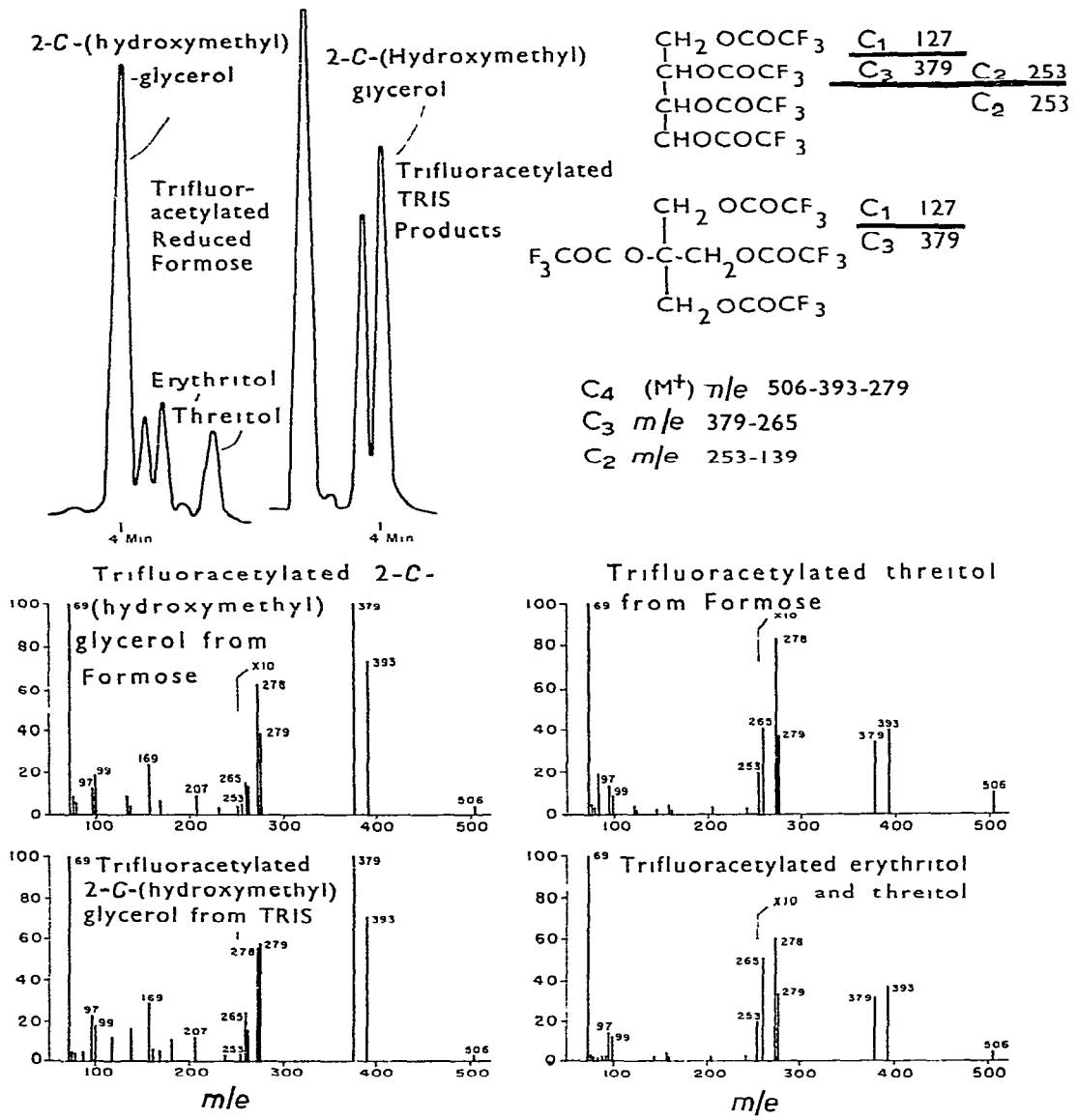


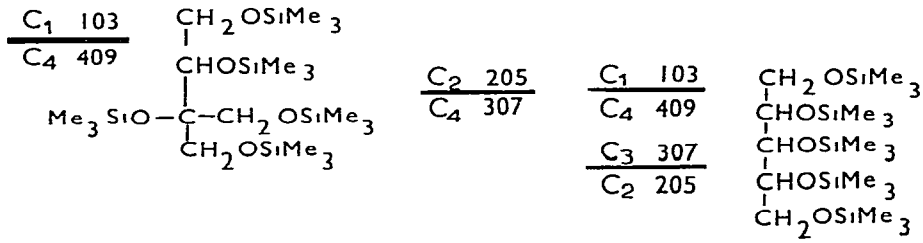
Fig 3 Identification by g l c–m s of species in the tetritol region of trifluoroacetylated, reduced formose

and threitol trifluoroacetates from formose corresponded to those of the authentic derivatives, and both gave mass spectra essentially identical with one another and those of the pure products Again, the third peak of the product from Tris corresponded in retention time to the first and most abundant peak from the formose, that of the branched-chain tetritol The mass spectra were identical and again indicated the branched-chain species, having its molecular ion at *m/e* 506, a low relative

abundance of *m/e* 253 and high abundance of *m/e* 379 corresponded to the mass numbers of *m/e* 205 and 307, respectively, in the silylated tetritols

The longer retention times and the mass spectra of minor peaks in the silylated tetritol region and in the trifluoroacetylated tetritol region of Fig 2 and 3, respectively, suggest the presence of partially derivatized forms of the branched-chain tetritol No molecular ion corresponding to the completely derivatized species was present for either component, and the remaining spectra were very similar to those of the parent species Fig 1 shows peaks that correspond to similar incomplete derivatization for ethylene glycol and glycerol in the products from reduced formose

C_5 (M^+) *m/e* 512-423-333-243
C_4 *m/e* 409-319-229
C_3 *m/e* 307-217
C_2 *m/e* 205-115



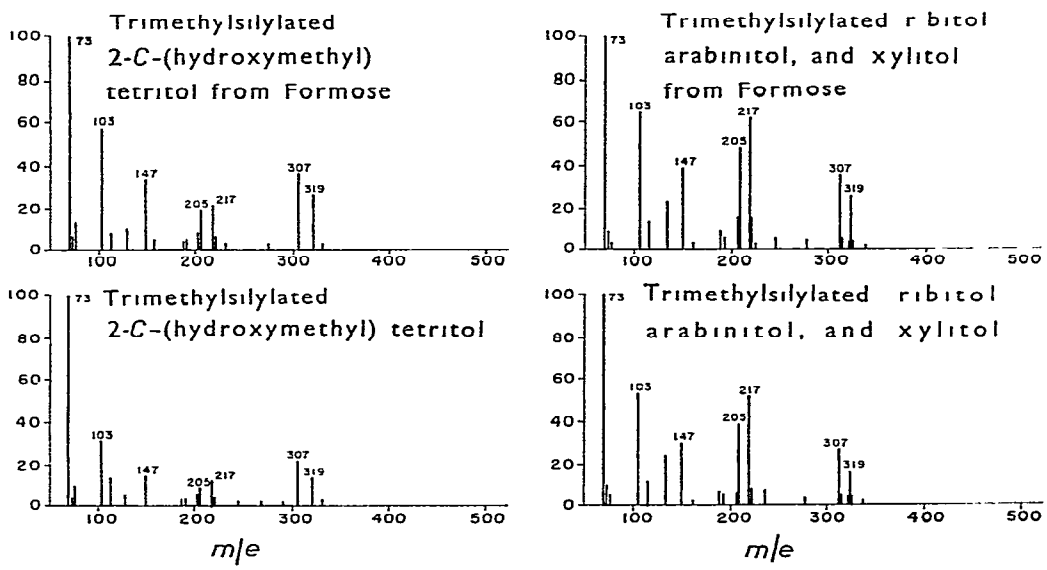


Fig 4 Mass spectra of species in the pentitol region of trimethylsilylated, reduced formose

Identification of pentitols — Fig 1 shows two peaks in the pentitol region of the silylated, reduced formose The first peak corresponded in retention time to a branched-chain, C_5 polyol, a 2-C-(hydroxymethyl)tetritol, as the per(trimethylsilyl) ether The second peak corresponded in retention time to the possible straight-chain species as their Me_3Si ethers*

Fig 4 compares the mass spectra obtained from the formose peaks and from the authentic pentitol species Again, the diastereomeric straight-chain pentitol trifluoroacetates gave identical spectra, as did the trimethylsilyl ethers[12]

The mass spectrum of the branched-chain silylated species is distinct from those of the straight-chain silylated pentitols, showing a lower relative abundance of *m/e* 205 and 217 and a greater abundance of *m/e* 307 and 319, since the tertiary *m/e* 307 fragment from the branched-chain species is more stable The mass spectrum of the silylated 2-*C*-(hydroxymethyl)tetritol from formose corresponded identically with that of the known branched pentitol (made by borohydride reduction of apiose) and the mass spectrum of the group of silylated straight-chain pentitols was identical with the spectra of the individual analogs

The pentitol trifluoroacetate region of the chromatogram from reduced formose (Fig 1) indicates by retention times the presence of all three straight-chain pentitols Overlap of the straight-chain and branched-chain species in this and higher carbon-number alditol regions interferes with the identification of structures by the mass spectra of their trifluoroacetates

Identification of hexitols — Fig 1 indicates the straight-chain species identified in the hexitol region of the silylated and trifluoroacetylated reduced formose All six of the straight-chain hexitols were identified by their retention times The first two major peaks in the trimethylsilylated hexitol region remained unidentified by retention time and are branched-chain hexitols

Fig 5 shows the fragmentation patterns of the possible silylated hexitol structures resulting from the formose reaction, and a comparison of one mass spectrum of a straight-chain product taken in the formose hexitol region with that for the pure straight-chain species Again the diastereomeric straight-chain species gave essentially identical spectra

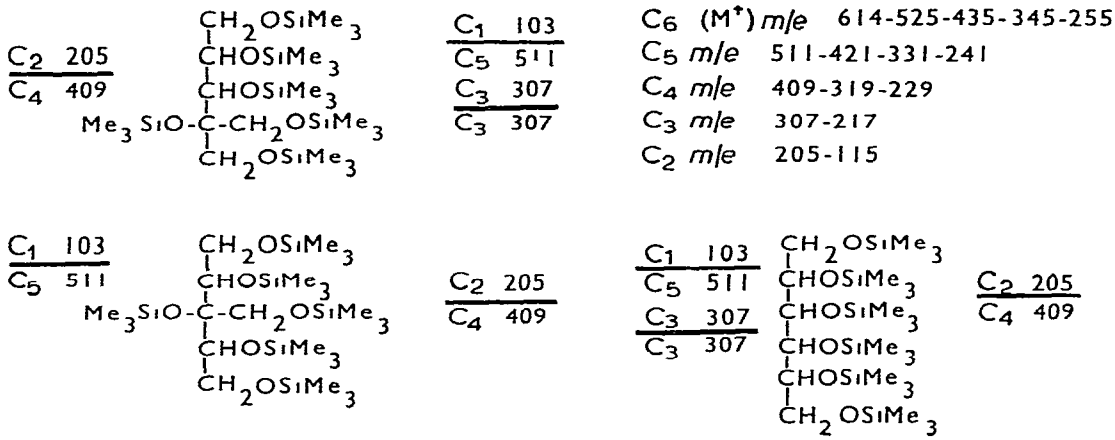
Scans of straight-chain, trimethylsilylated hexitol peaks from reduced formose, as shown in Fig 1, gave identical spectra corresponding to spectra of the pure species

The mass spectra from scans of the trimethylsilylated branched-chain hexitols gave fragments indicative of the silylated hexitols, but which were distinctly different from the fragments from the straight-chain species The shorter retention-times

*It should be noted that (considering only open chain structures) there are 8 possible straight-chain aldo- and keto-pentoses in formose that, following borohydride reduction, give three straight-chain pentitols Furthermore, the α- and β- anomers of sugars and also the pyranose and furanose tautomers generally show different retention times when subjected, as standard derivatives, to g l c It is thus possible to have 22 g l c -separable aldo- and keto-pentose derivatives from the original formose mixture

observed, combined with the spectral interpretations, indicate that the two peaks corresponded to branched-chain hexitol structures

Both spectra, reproduced in Fig 5, show a lower relative abundance of *m/e* 205 and a greater abundance of *m/e* 307 when compared with the abundance of these fragments in the straight-chain trimethylsilylated species The relative abundance of *m/e* 307 in the peak designated 2-*C*-(hydroxymethyl)pentitol is about twice that of *m/e* 307 in the peak assigned the 3-*C*-(hydroxymethyl)pentitol structure (as clea-



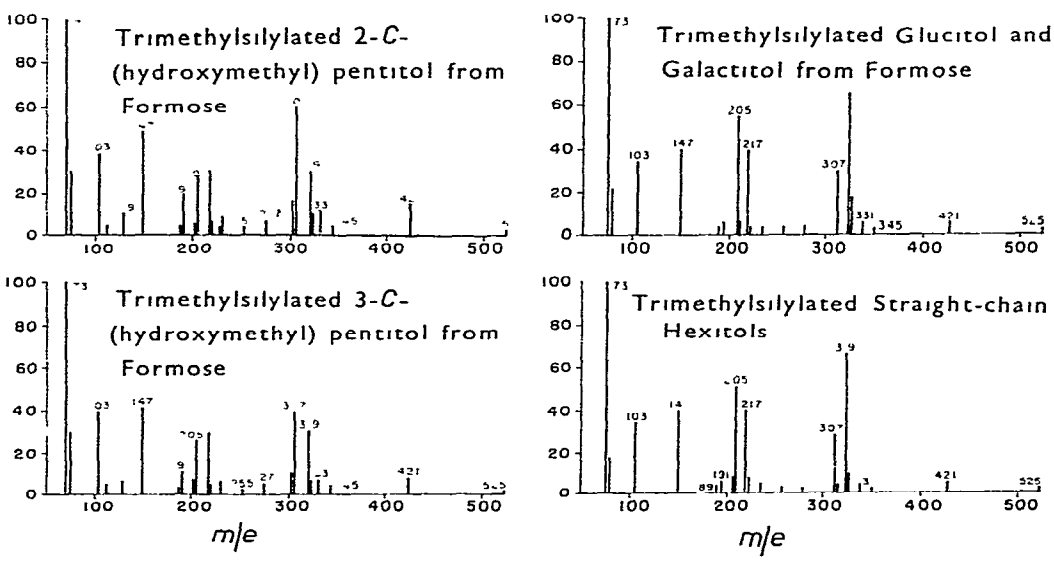


Fig 5 Mass spectra of species in the hexitol region of trimethylsilylated, reduced formose

vage at C-3 position of the 2-substituted species gives two fragments of *m/e* 307 with one being the stable tertiary fragment, and as there is no direct cleavage of the 3-substituted species to give *m/e* 307) Probably there are diastereomers of the two mono-branched structures present that are not resolved under the chromatographic conditions employed

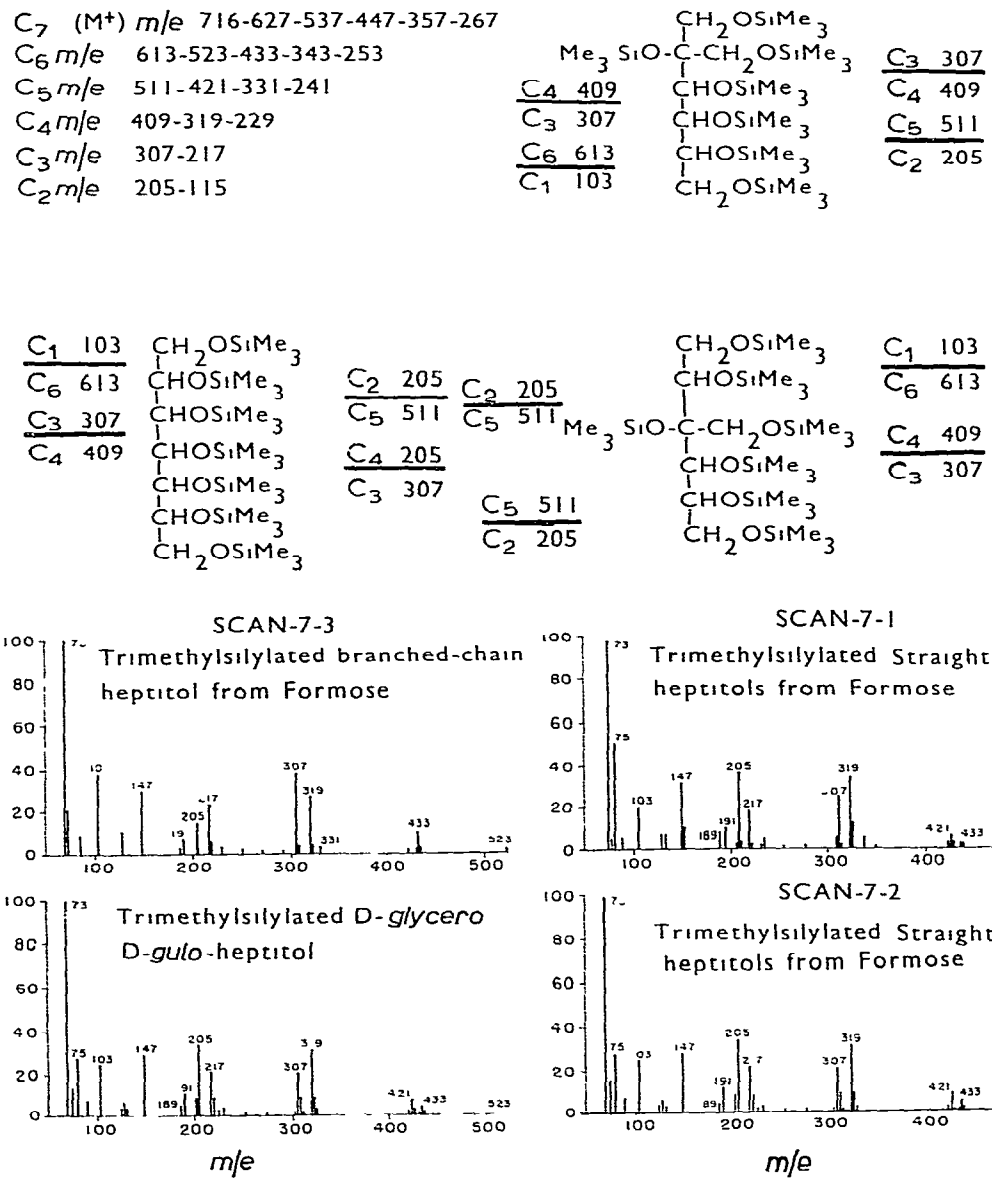


Fig 6 Mass spectra of species in the heptitol region of trimethylsilylated, reduced formose

The relative quantities and mass spectra of the hexitols in the reduced formose trifluoroacetate indicate overlap of the branched-chain and straight-chain species, and preclude comparison of the mass-spectral data Unfortunately, no known reference branched-chain hexitol was available

Identification of heptitols — The chromatograms of the trimethylsilylated and trifluoroacetylated derivatives of the reduced formose shown in Fig 1 indicate the presence in major proportions of a number of species having more than six carbon atoms The silylated, reduced formose gives a distinct band of peaks in the heptitol region Of the many possible straight-chain species possible, only D-*glycero*-D-*gulo*-heptitol and D-*glycero*-D-*galacto*-heptitol have so far been identified by retention times

Mass-spectral scans were taken in the *O*-trimethylsilylated heptitol region of the reduced formose Fig 6 shows the spectra obtained and the possible fragmentations of a number of the heptitol structures The mass spectra of scans 7-1 and 7-2 compare favorably with one another and with that of the pure, straight-chain hepta-*O*-trimethylsilyl-D-*glycero*-D-*gulo*-heptitol Scan 7-3 shows a lower relative abundance of *m/e* 205 and a greater relative abundance of *m/e* 307 compared with the other spectra, suggesting that this peak contained a branched-chain species The longer retention time does not necessarily indicate that the product giving the peak is not branched The trend noted for the earlier retention times of singly branched species with respect to their straight-chain isomers might imply that the species shown by scan 7-3 may have multiple branching This suggestion is not ruled out for the seven-carbon species by the reaction mechanism postulated for formose[13], but it is only speculative, all that can be stated with any degree of certainty is that the region is composed of heptitols having both straight- and branched-chain structures

The mass spectra of the trifluoroacetylated, reduced formose in the regions greater than six carbon atoms also indicate the presence of C_7 species

Identification of aldoses and ketoses — Although the alditols of formose give a good indication of carbon number and distribution of branched-and straight-chain species, they also simplify the actual formose reaction mixture. Attempts have been made to identify the species present in the original formose mixture by g l c of the (unreduced) silylated derivatives A number of peaks are identified by retention time in Fig 1 (see ref 15), but there is overlap in a number of instances Not only is there overlap within a given carbon-number distribution, but also, for example, an overlap in the C_6 region by dimers of 1,3-dihydroxy-2-propanone and glyceraldehyde[17 15] A number of peaks could not be identified by comparison of retention times with those of known species

Fig 7 shows an attempt to simplify the interpretations of the mass spectra taken The top chromatogram of the figure is the normal flame-ionization chromatogram of silylated formose Simultaneously, a "mass" chromatogram was obtained by setting the mass spectrometer to monitor a single ion and recording the intensity of this ion versus time Two masses, *m/e* 204 and 217, were examined in this manner The ratio of the two mass numbers chosen has been shown to be an effective measure

of the ring size of a number of carbohydrates[16]; the fragments giving rise to these peaks are shown in Fig 7

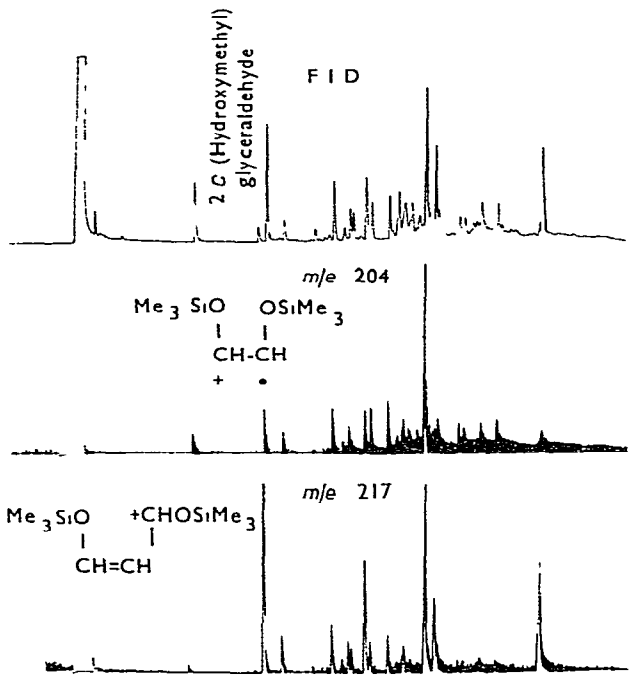


Fig 7 Gas and "mass" chromatograms of trimethylsilylated formose, mass numbers *m/e* 204 and *m/e* 217 were monitored

The major peak in the 2-*C*-(hydroxymethyl)glyceraldehyde region of the chromatogram of silylated formose designated in Fig 1 has not been previously identified As the major component of this particular formose in the tetritol region of the chromatograms of silylated and of trifluoroacetylated, reduced formose is the branched chain tetritol, 2-*C*-(hydroxymethyl)glycerol, we were led to suspect that the major component of the four-carbon region of the silylated formose might be 2-*C*-(hydroxymethyl)glyceraldehyde (Fig 1) There is no possible keto-analog of this sugar and thus it is unlikely to dimerize or form any ring structures

Fig 7 indicates that this peak contains very little component *m/e* 204 as compared with *m/e* 217 Generally, for the higher sugars[18 16], this observation would imply a furanoid ring-form, but when the formation of ring forms is ruled out, a branched species is indicated The complete mass spectrum of this peak is shown in Fig 8 and is compared with the mass spectrum of trimethylsilylated erythrose The scan for 2-*C*-(hydroxymethyl)glyceraldehyde in Fig 8 again shows the small relative amount of *m/e* 204 compared with *m/e* 217 The large relative abundance of *m/e* 307 indicates a stable, tertiary C_3 fragment, characteristic of the branched species, as previously noted The mass spectrum of the erythrose as the Me_3Si derivative,

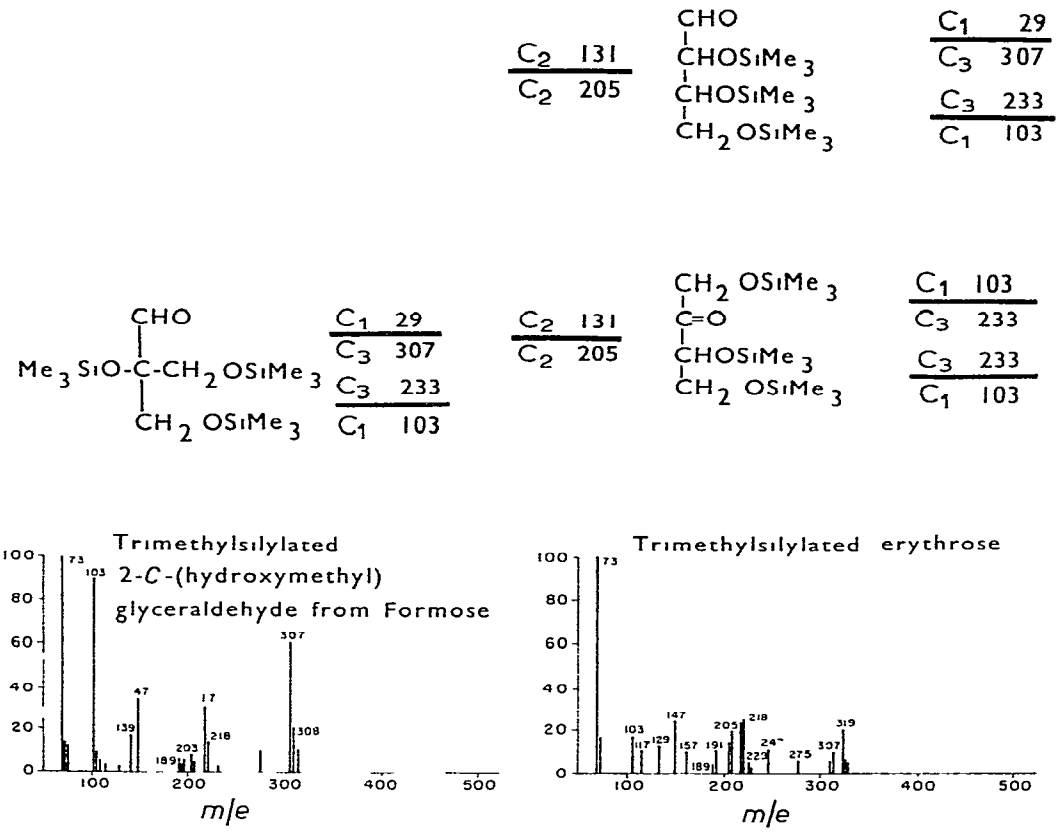


Fig 8 Identification by g l c –m s of branched-chain tetrose in trimethylsilylated formose

however, shows much greater relative abundance of *m/e* 204 and 205, indicating the expected cleavage at C-2 The low relative abundance of m/*e* 307 and the appearance of *m/e* 319 is characteristic for erythrose, an aldose No molecular ion at *m/e* 336 was observed Therefore, it is concluded that the species is the branched tetrose, 2-*C*-(hydroxymethyl)glyceraldehyde

EXPERIMENTAL

General methods — Many of the carbohydrates and alditols used in this study were commercially available preparations 2-*C*-(Hydroxymethyl)tetritol was prepared by borohydride reduction of apiose obtained by hydrolysis of di-*O*-isopropylidene-apiose The Me_3Si derivative gave a single peak by g l c 2-*C*-(Hydroxymethyl)-glycerol, $(HOCH_2)_3COH$, was synthesized by deamination of tris(hydroxymethyl)-aminomethane (Tris) to a solution of 12 1 g (0 10 mole) of Tris in 17 1 ml of acetic acid and 100 ml of water at 5° was slowly added, with external cooling, a solution of 6 9 g of sodium nitrite in 25 ml of water After being kept cold for 0 5 h the solution

was warmed to room temperature and then evaporated to dryness *in vacuo* The residue was dissolved in water, passed through a column of Dowex 50W-X8 resin (H^+ form), and the eluate evaporated *in vacuo* A sample of the syrupy residue was converted into the Me_3Si derivative and subjected to g l c About 98% of the injected material emerges as one peak This result was confirmed by high-pressure liquid–liquid chromotography (Waters ALC-201 equipment)

The Me_3Si derivatives were prepared according to the method of Sweeley, *et al*,[19] by using Tri Sil (Pierce Chemical Company) premixed reagent The sample (10–20 mg) was dissolved in 1 ml of the reagent, with slight warming if necessary, and allowed to react overnight at room temperature Prior to gas chromatography, the sample was extracted into chloroform[20]

The trifluoroacetates of the alditols were prepared and analyzed accordingly to the method of Shapira[10] by using a 200 1 mixture of trifluoroacetic anhydride and pyridine Generally, 1 ml of reagent was used for a 10 mg sample However, much more sample could be tolerated

Reduction of the formose sugars was accomplished by adding approximately 10 mg of sodium borohydride in 1 ml of water to a neutralized solution of formose (1–2 ml) containing the equivalent of 10–20 mg of sugar This mixture was then shaken and allowed to react overnight at room temperature Acetone (1 ml) was then added and the acidity of the solution adjusted to pH 3–4 with HCl (about 10 drops) The solution was then evaporated to near dryness by a stream of dry nitrogen, treated with 10 ml of methanol, boiled to about 5 ml, and then evaporated to dryness under nitrogen The methanol treatment was repeated twice and the derivatizing reagents added

A Perkin–Elmer Model 900 gas chromatograph having a capillary inlet-system and dual flame-ionization detectors was interfaced to a DuPont (CEC) 21–491 double-focusing, mass spectrometer by means of a heated capillary line and a jet separator

G l c of the trifluoroacetates was accomplished on a 50 ft × 0 020 in I D FS 1265 SCOT column (Perkin–Elmer) operated from 100° to 225° at 4°/min, with a helium flow of 4 0 ml/min The injector and detector temperatures were maintained at 225° and 250°, respectively

Combined gas chromatography–mass spectrometry of the Me_3Si and CF_3CO derivatives was accomplished with the SCOT columns under the same operating conditions Helium make-up gas (10 ml/min) was added to the effluent of the SCOT columns to permit optimum interfaced operation

An electron energy of 82 eV was employed for all mass spectra The source temperature was maintained at 180° for the alditol trifluoroacetates and from 200° to 250° for the Me_3Si derivatives The scanning time over the mass range *m/e* 20 to 600 was varied from about 3 to 7 seconds, depending on the peak width and resolution required The interface lines and separator temperatures were maintained at 180° to 200° for the alditol trifluoroacetates and from 225° to 250° for the Me_3Si derivatives Too high a temperature in the case of the trifluoroacetates resulted in decompos-

ition of the sample, giving decreased resolution and poor, or in some cases useless, spectra

Identifications were made on the basis of a comparison of retention time and mass-spectral data with that of known sugar and alditol derivatives Retention data were obtained by noting an increase in a peak height when the sample was mixed with some known reference material

The formose sugar preparation* was prepared as follows A plug-flow unit capable of producing 100 g/h of deionized concentrated formose has been described previously[21] The unit consisted of a stirred feed tank containing a mixture of 8% formaldehyde (prepared from 37%) CP formaldehyde solution, 0 1 moles of $Ca(OH)_2$ per mole of formaldehyde and 0 01 moles of CaO per mole of formaldehyde, which was metered at a rate of 2000 ml/h through a coil of 1/4 in Eastman Polyflow tubing with a total volume of 100 ml When the reactor was kept at 82° in a water bath, conversion into a pale-yellow product occurred in the last few turns of the coil The effluent was cooled, neutralized with gaseous carbon dioxide, filtered to remove precipitated calcium carbonate, and deionized with a 12 × 45 cm column of mixed anion- and cation exchange resins The almost colorless product was concentrated in a rotary evaporator at 50° and 30 torr to a thick syrup of variable color

CONCLUSIONS

Although tests of the product of condensation of formaldehyde catalyzed by calcium hydroxide show the presence of small proportions of many known sugars, the present study indicates that the major C_4, C_5, and C_6 products are branched-chain aldoses and ketoses Significant proportions of higher molecular-weight sugars are also suspected to be branched-chain The mass spectral fragmentation pattern of trimethylsilylated 2-*C*-(hydroxymethyl)glyceraldehyde provides conclusive evidence of the formation of 2-*C*-(hydroxymethyl)glyceraldehyde in the formose reaction, and spectra of previously unreported, branched-chain alditols derived from formose permit inference of the skeletal branched-chain structure of the parent aldoses and ketoses

The formose reaction can be considered as a unique method for producing branched-chain carbohydrates

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*The formose sugar used was prepared by J Shapira NASA Ames Research Center, Moffett Field, Calif 94035

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