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BIOIDS

X. IDENTIFICATION OF FORMOSE SUGARS, PRESUMABLE PREBIOTIC METABOLITES, USING CAPILLARY GAS CHROMATOGRAPHY/GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF *n*-BUTOXIME TRIFLUOROACETATES ON OV-225

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SUMMARY

Most monosaccharides, including 3-uloses and branched-chain species formed by the autocatalytic condensation of formaldehyde, have been separated by capillary gas chromatography (GC) and isobutane chemical ionization GC–mass spectrometry (MS) on OV-225 of the trifluoroacetyl *n*-butoximes, and identified using commercial sugars, oxidation products of alditols and aldolization products of formaldehyde and/or glycolaldehyde and trioses, and by selected ion monitoring. The product pattern of a reaction of 0.156 *M* formaldehyde with 0.036 *M* calcium acetate and 0.087 *M* sodium hydroxide at 40°C, started by the addition of 0.55 m*M* glycolaldehyde is reported. In addition to the already known trioses, tetroses, pentoses and hex-2-uloses, also pent-2-uloses (20%, including 13% of 2-xylulose), 6.5% of hex-3-uloses, 5% of D,L-3-C-hydroxymethyltetrose (apiose) and 6.3% of branched hexoses were found; 90.3% of the total volatile derivatives of 3–6-carbon sugars have been identified; conspicuously, 50–80% of the reacted formaldehyde does not afford volatile sugar derivatives.

Because of its autocatalytic kinetics, in a prebiotic scenario a formol reaction could evolve into a self-organizing non-enzymatic system steadily producing sugars, including branched-chain sugars, as precursors of amino acids, of the isoprene moiety and of the branched chains of valine and leucines.

INTRODUCTION

Formose, the mixture of sugars arising in the autocatalytic condensation of formaldehyde (F) in the presence of Ca^{2+} and OH^- ions[1], has been investigated chromatographically using cellulose column chromatography[2], paper chromatography[3–5] and gas chromatography (GC) of the alditol[6] and sugar[6–8] trimethylsilyl (TMS) derivatives. Alditol GC involves a loss of information because of the reduction of the carbonyl group, and direct TMS derivatization may give up to four peaks of

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anomeric species, and derivatives of the dimers of trioses in unpredictable proportions. Therefore, and because of the complexity of the mixture, complete resolution has not been achieved so far. Indeed, formose contains all isomeric aldoses and 2-uloses containing from two up to at least six carbon atoms. Additionally, as we report here, also 3-uloses and a series of branched-chain sugars occur in considerable amounts.

The reaction deserves special interest as a non-enzymatic source of sugars in a prebiotic scenario[9–12], where F arose by atmospheric oxidation of methane. In such a system, involving continuous supply and decomposition, the formol reaction would represent a "bioid", an open system which can switch ("mutate") between different steady states[9,12]. Once switched into the reacting state, a formol bioid would represent a self-stabilizing source of sugars and the diverse reaction products thereof.

Branched-chain pentoses, formation of 2-xylulose and the hydroxymethylation of xylulose by F to give 3-hexuloses reported here are considered as precursors of the isoprene moiety, of the Calvin–Horecker cycle and of bacterial C-1 pathways[13], respectively. Like glycolysis[14] they were natural prebiotic pathways which became improved by the evolution of proteins as standardized catalysts or catalyst carriers.

For better resolution we developed an improved method, involving capillary GC on OV-225[15–19]. OV-225 has a special selectivity for structural differences of isomeric sugars and trifluoroacetylated butoxime derivatives. Oxime derivatives afford exactly two peaks from each sugar species, except for symmetrical and hindered α-branched sugars (see below) belonging to the *E* and *Z* isomers. This redundancy may save information in the case of overlapping peaks.

Additionally, selected ion monitoring affords undisturbed chromatograms of isomeric sugar classes, as all peaks of different constitution and/or carbon number are suppressed. These techniques allowed for the first time an almost complete identification (Fig. 1) and quantitation of all C3–6 sugar species present in formose and may help to elucidate its so far obscure[8] reaction mechanism.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 5830A gas chromatograph was used, allowing internal standard or peak area calculations. It was equipped with a flame-ionization detector and a 50-m capillary column wall-coated with OV-225 (WGA, Griesheim, G.F.R.); the split liner was filled to about 2 cm with 3% OV-225 on Chromosorb W HP (80–100 mesh) (Varian, Walnut Creek, CA, U.S.A.), held on both sides by quartz-wool.

The MAT 44S quadrupole gas chromatograph–mass spectrometer (MAT, Bremen, G.F.R.), also with a 50-m capillary column wall-coated with OV-225 (WGA), included equipment for chemical ionization (using isobutane) and selected ion monitoring.

Materials

Formaldehyde solution (35%) (a), glycolaldehyde (b), D-glyceraldehyde (b), dihydroxyacetone (b), D-erythrose (b), D-arabinose (b), D-ribose (c), D-xylose (d), D-lyxose (d), D-2-ribulose (b), D-glucose, D-galactose and D-mannose (a), D-talose (b), the remaining aldohexoses (e), O-*n*-butylhydroxylammonium chloride (f), sodium

borohydride (a) and chromotropic acid sodium salt were commercial samples from (a) Merck (Darmstadt, G.F.R.), (b) Serva (Heidelberg, G.F.R.), (c) Roth (Karlsruhe, G.F.R.), (d) Fluka (Buchs, Switzerland), (e) Sigma (Munich, G.F.R.) and (f) Applied Science (Oud-Beijerland, The Netherlands). Threose was a gift from Dr. Morgenlie, Agricultural University, Ås-NLH, Norway.

Samples containing mixtures of keto sugars allowing identification of all 2- and 3-pentuloses and -hexuloses were obtained using bromine oxidation of the respective alditols[17,19].

Derivatization and gas chromatography

Derivatives were prepared[15] by incubation at 60°C of a sample containing *ca.* 1 mg of sugars with 5 mg of O-*n*-butylhydroxylammonium chloride and 6 mg of sodium acetate in 0.1 ml of water, evaporating in an air flow at 60°C, deposition of salts by evaporation with methanol and repeated evaporation with benzene, closing the vial using a screw-cap with septum and reacting with 30 μl of trifluoroacetic anhydride and 15 μl of ethyl acetate at room temperature for *ca.* 2 h, or overnight in a refrigerator. Corresponding alditol mixtures were prepared using sodium borohydride reduction[20]. Erythritol (retention time 17.61 min under conditions of Fig. 1) was used as an internal standard.

"Portability" of retention times

As retention times (t_R) depend strongly on GC conditions, only the relative positions of the peaks represent truly "portable" information. Better "portability" is exhibited by the t_R values with linear temperature programming. Like R_M values in paper and thin-layer chromatography according to Martin's law t_R is a linear function of the constitution of a compound (and of the stationary phase)[21]. Therefore, they may be manipulated like R_M values[22] by linear transformation from system A into system B using appropriate coefficients:

$$B_i = aA_i + b$$

For the transformation of a published t_R series, A_i, into t_R values B_i in one's own laboratory, one can chose two compounds contained in both series, say X and Y, and calculate the coefficients for transformation of A_i into B_i[23]:

$$a = \frac{B_X - B_Y}{A_X - A_Y}$$

$$b = B_X - aA_X$$

Unfortunately, because of the temperature limits of OV-225 on our glass capillary columns we had to switch from temperature programming to isothermal GC at 180°C, *i.e.*, at 18 min, in order to prolong the column lifetime (*ca.* 1000 runs; a quartz capillary column allowing higher temperature had performed better). Therefore, for the comparison of the t_R values reported here with values elsewhere, one must consider empirically the spreading of the peaks in the isothermal part above 180°C.

Abbreviations

Where desirable, instead of full names or compound numbers in Table I we use the following self-explanatory mnemonics: A*x* and *y*K*x* for aldoses (A) and ketoses (K) which *x* carbon atoms in the straight chain and a keto group at carbon atom No. *y*, and *z*H- as a prefix for a branching C-hydroxymethyl group in position *z*; and 2-DHE for 2-(C-1,2-dihydroxyethyl)-. Hyphenation, except after the H and redundant *y* and *z*, is omitted.

Aldolization

Most remaining "unknowns" were identified by comparison with products of appropriate aldolization experiments. Mixtures of A2, glyceraldehyde, dihydroxyacetone and F were reacted at 20 or 40°C at pH 12 (adjusted with sodium hydroxide). Ca^{2+} was added as calcium acetate[9] when required. Mixtures containing 3-hexuloses were obtained by a two-step aldolization procedure; we first aldolized a mixture of 50 mg of A2 and 50 mg of K3 in 5 ml of water containing 9 mg of calcium hydroxide for 30 min at 20°C, obtaining a mixture containing mainly 2-xylulose (*threo*-2K5), and continuing incubation for 1 h after addition of 100 mg of F as a 35% solution.

Formol reaction

For better reproducibility the reaction was performed in a homogeneous medium. A typical reaction mixture contained 0.156 mol/l of F, 1.56 mmol/l of A2 as autocatalyst, 0.036 mol/l of calcium acetate and 0.087 mol/l of sodium hydroxide. The autocatalysis was started at 40°C by addition of the A2 or the sodium hydroxide. Unreacted F was determined by adding an appropriately diluted sample (0.2 ml with 2–20 μg of F) to 2.0 ml of a reagent prepared from 2 g of chromotropic acid sodium salt in 15 ml of water and 400 ml of concentrated sulphuric acid, heating at 100°C for 15 min, dilution with 10 ml of water and spectrophotometry at 578 nm.

RESULTS AND DISCUSSION

Kinetic course and products

The consumption of F under our relatively mild conditions follows a characteristic course distinct from the S-shaped course of a simple autocatalysis of type A + B = 2B[12]. With A2 amounts of 1% of F or less there is a lag time that depends on the logarithm of glycolaldehyde concentration and then an almost linear slope where *ca.* 0.3 mg/ml · min of F is constantly consumed extending to nearly total exhaustion, soon followed by the appearance of a yellow colour (Fig. 2). Without initiator there was a lag time of *ca.* 2 h. Fig. 3 shows the results of selected ion monitoring of tetroses, pentoses and hexoses.

Higher temperature and prolonged reaction at lower pH and/or Ca^{2+} concentrations additionally afforded reduced products of cross-Cannizaro reactions such as glycerol or trihydroxymethylcarbinol, the latter arising from the branched tetrose as described by Shigemasa *et al.*[8].

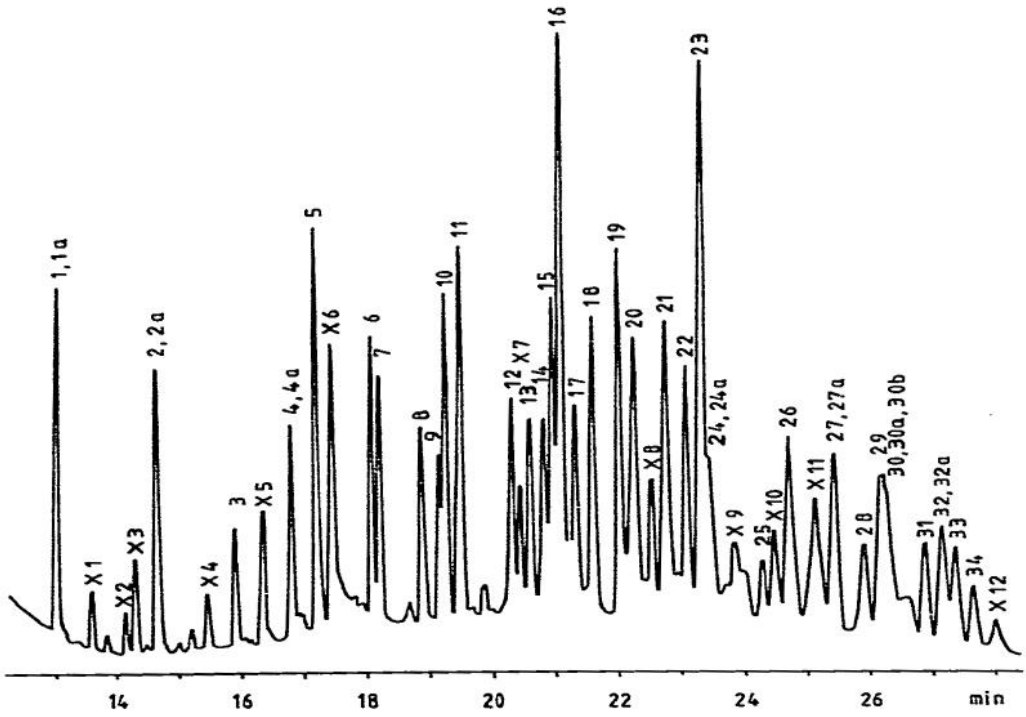

Fig. 1. Gas chromatogram of *n*-butoxime trifluoroacetyl derivatives of carbohydrates arising in the condensation of formaldehyde. Temperatures: column, 100°C for 2 min, then increased from 100 to 180°C at 5°C/min, final temperature 180°C; injection and detector, 250°C. Gas flow-rates: nitrogen carrier gas, 2 ml/min; hydrogen, 20 ml/min; air, 200 ml/min. Sample volume: 1 μl. Splitting ratio: 1:12. Peak identities: see Table I(A).

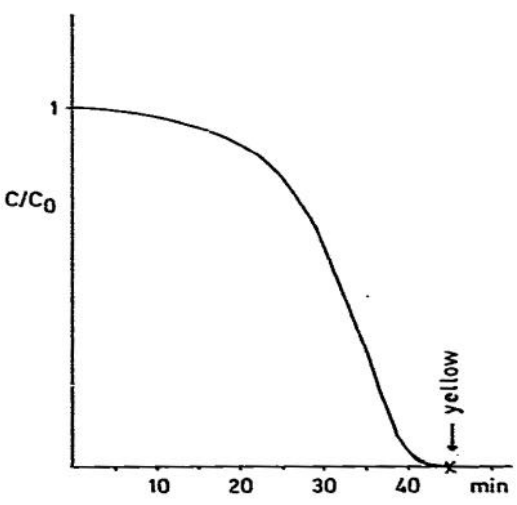

Fig. 2. Autocatalytic consumption of 0.156 mol/l formaldehyde at 40°C in the presence of 0.036 mol/l calcium acetate and 0.087 mol/l NaOH, started by addition of 0.55 mmol/l glycolaldehyde. × = Sample shown in Fig. 1 and Table I.

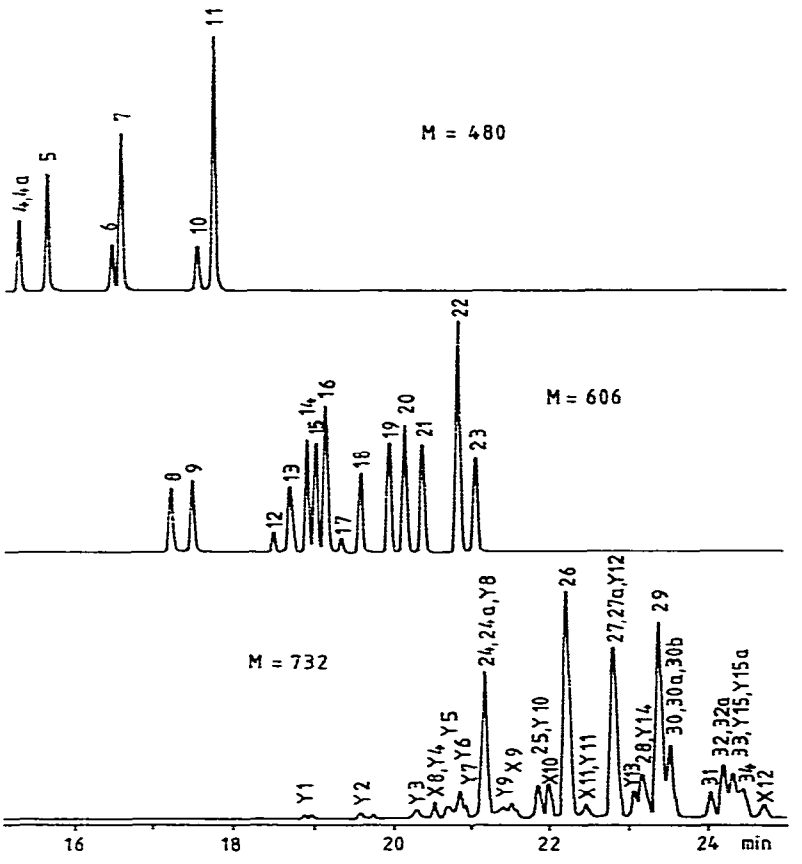

Fig. 3. Gas chromatogram of *n*-butoxime trifluoroacetates of condensation products of formaldehyde using selected ion monitoring at $m/e = M + 1$ (tetroses, $M + 1 = 480$; pentoses, $M + 1 = 606$; hexoses, $M + 1 = 732$). Temperatures: column as in Fig. 1; injection port, 250°C; GC separator, GC line of sight and source, 220°C. Carrier gas helium, flow-rate 1.5 ml/min. Sample volume: 1 μl. Splitting ratio: 1:10. Pressures: in CI box, 390 μbar; of the forepump, 37 μbar. Electron energy: 150 eV. Emission current: 0.7 mA. Voltage on the secondary electron multiplier: 1800 V. Peak identities: see Table I(B).

Products, general

We preferred the *n*-butoxime derivatives because the higher t_R values ensure quantitation of A3 and K3 (although the first peak of A3 coincides with glycerol and the second with K3). Moreover, although A2 cannot be obtained quantitatively because of its volatility during derivative preparation, at least qualitatively it can be seen at $t_R = 6.2$ min between solvent peaks. With methoximes there is less overlapping between the tetrose, pentose and hexose series, but as selected ion monitoring always allows one to determine the carbon number, this drawback is outweighed by the wider t_R spreading of the *n*-butoximes.

Aldolization products

The comparison with products of aldolization and/or A2, A3 and K3, the main intermediary substrates of the reaction network, gave useful information on certain

constituents and pathways[2,5]. Such aldolizations proceed at 20°C, where the feedbacks of the formol autocatalysis play no role, and may help to elucidate the feedback mechanisms of the reaction[24]. Here we shall mention only results relevant to the identification of formose products. Retention times and the peak numbers refer to Table I, conditions A, and Fig. 1; the yields are given as a percentage of the total area of the C3–6 region, sampled at 45 min (*cf.* Fig. 2).

TABLE I

RETENTION TIMES (t_R) OF FORMOSE PRODUCTS AS *n*-BUTOXIME TRIFLUOROACETATES (CARBOHYDRATES) AND TRIFLUOROACETATES (ALDITOLS)

50-m capillary column. Conditions: (A) as in Fig. 1 and (B) as in Fig. 3.

Peak	*Product*	t_R *(min)*		*Peak*	*Product*	t_R *(min)*	
		A	*B*			*A*	*B*
1	Glyceraldehyde	13.02	11.67	30	Tagatose	26.30	23.52
1a	Glycerol			30a	*ribo*-Hex-3-ulose		
2	Glyceraldehyde	14.65	13.18	30b	*xylo*-Hex-3-ulose		
2a	Dihydroxyacetone			31	*lyxo*-Hex-ulose	26.85	24.02
3	2-C-Hydroxymethylglycerol	15.91		32	Tagatose	27.12	24.17
4	Erythrose	16.78	15.26	32a	*arabino*-Hex-3-ulose		
4a	2-C-Hydroxymethylglyceraldehyde			33	*lyxo*-Hex-3-ulose	27.34	24.28
5	Threose	17.17	15.62	34	*xylo*-Hex-3-ulose	27.63	24.42
6	Erythrulose	18.05	16.49	*x*1		13.59	
7	Erythrose	18.17	16.61	*x*2		14.13	
8	Ribose	18.85	17.24	*x*3		14.29	
9	Arabinose	19.14	17.51	*x*4		15.47	
10	Erythrulose	19.25	17.57	*x*5		16.35	
11	Threose	19.47	17.77	*x*6		17.43	
12	3-C-Hydroxymethyltetrose	20.29	18.52	*x*7		20.42	
13	Lyxose	20.57	18.72	*x*8	Branched hexose	22.51	20.50
14	Ribose	20.80	18.94	*x*9	Branched hexose	23.81	21.50
15	Xylose	20.94	19.05	*x*10	Branched hexose	24.43	21.98
16	*threo*-Pent-2-ulose	21.06	19.18	*x*11	Branched hexose	25.08	22.44
16a	2-C-Hydroxymethyltetrose			*x*12	Branched hexose	27.97	24.67
17	3-C-Hydroxymethyltetrose	21.31	19.38	*y*1	Allose		18.87
18	*erythro*-Pent-2-ulose	21.59	19.61	*y*2	Altrose		19.55
18a	3-C-Hydroxymethyltetr-2-ulose			*y*3	Mannose		20.27
19	*erythro*-Pent-2-ulose	22.00	20.16	*y*4	Gulose		20.50
20	Arabinose	22.23	20.38	*y*5	Talose		20.67
21	Lyxose	22.73	20.63	*y*6	Galactose		20.84
22	Xylose	23.05	20.82	*y*7	Glucose		20.92
23	*threo*-Pent-2-ulose	23.29	21.04	*y*8	Allose		21.17
24	Fructose	23.81	21.17	*y*9	Idose		21.40
24a	Psicose			*y*10	Altrose		21.86
25	Psicose	24.23	21.86	*y*11	Mannose		22.44
26	Sorbose	24.67	22.11	*y*12	Talose		22.78
27	Fructose	25.37	22.78	*y*13	Gulose		23.06
27a	*arabino*-Hex-3-ulose			*y*14	Glucose		23.17
28	*ribo*-Hex-3-ulose	25.87	23.17	*y*15	Idose		24.28
29	Sorbose	26.14	23.37	*y*15a	Galactose		

Tetroses

A test mixture of all straight-chain tetroses was obtained by incubation for 10 min at 20°C of 100 mg of A2 in 5 ml of water made alkaline with 1 drop of 2 *N* sodium hydroxide solution. It contained 24% of erythrose, 45% of threose, 4.7% of erythrulose and *ca.* 20% of hexoses. In formose mixtures more erythrulose was found, probably because it also arises from K3 and F.

Aldoses

In addition to normal pentoses, we found 2-pentuloses and branched pentoses in considerable amounts. In accord with earlier observations, aldohexoses occurred only in trace amounts, as seen in the mass scan (Fig. 3).

2-Uloses

In the presence of sufficient Ca^{2+} and OH^-, 2-xylulose (16 and 23) is the main single product, accounting for as much as 12% of the total area in Fig. 1. Its peaks are only 0.12 and 0.24 min from the ribose peaks 15 and 22, respectively, requiring capillary columns for separation; 2-ribulose (18 and 19) accounts only for half as much because of predominant *threo* aldolization. Similarly among 2-ketohexoses[25], fructose and sorbose represent about 80% of the total 2K6, as can be seen by comparing 25 (psicose II) and 26 (sorbose I) (other peaks overlap with the 3K6 peaks and with each other).

3-Uloses

Erythro- and *threo*-pent-3-ulose, which have been identified among the bromine oxidation products of the respective pentitols[17,19], should arise from K4 and F in formose; however, only small peaks arising transiently at the beginning of the reaction emerge at the respective t_R values of 22.54 and 22.84 min.

Unexpectedly, 6.1% of hex-3-uloses, which we earlier obtained by hexitol oxidation, were found in formose, *i.e.*, as much as half the amount of the long known hex-2-uloses (12.4%). They can arise along two pathways, first by

$$2\,A2 = A4;\ A4 = K4;\ K4 + A2 = 3K6$$

and accordingly arise by prolonged aldolization of A2 together with all aldohexoses (from A4 + A2) and a peak in the position of sorbose (26) which may represent 2-DHE-tetrose. Alternatively, the same 3K6-series (*cf.*, Fig. 1) is obtained by the incubation of pre-formed 2K5 with F, as described under Experimental.

Branched-chain sugars

The following observations gave indications of the occurrence and probable constitution of branched-chain species: (i) retention times incompatible with known sugars; (ii) only one isomer peak because of steric hindrance in α-branched species (symmetrical uloses such as K3 and 3K5[17] also give a single peak); (iii) an MS fragmentation pattern in α-branched sugars involving the loss of the branching carbon atom such as $CF_3 \cdot CO \cdot CH_2 \cdot CO \cdot CF_3$ (mol. wt. 240); (iv) the preferential formation from defined substrates in aldolization mixtures, often revealing species masked by normal peaks in the formose mixture; (v) the formation of branched-chain alditols by reduction with sodium borohydride.

Several branched species have been described by Weiss *et al.*[6] and Shigemasa *et al.*[26], *e.g.*, C-hydroxymethylglyceraldehyde (4a), which in our system coincides with the first erythrose peak. At low pH, in the absence of Ca^{2+}, and by aldolization of A3 or A2 with a surplus of F at 20°C, it can accumulate to up to 40% of the total area. At 40°C it is slowly reduced to trihydroxymethylcarbinol (3) by cross-Cannizaro reactions.

Branched pentoses

There exist three branched pentoses, 3H-A4, 2H-A4 and 3H-K4. The latter two are α-branched and therefore exhibit single peaks (18a and 16a) and the characteristic loss of M − 240 mass units in GC–MS. In formose they are masked by the xylulose and ribulose peaks (16 and 18), respectively, as they do not arise in great amounts. In aldolization experiments a large peak of 3H-K4 is obtained from K3 + F. Similarly, 2H-A4 is formed by slow addition of A2 to F at 20°C, probably from intermediary A3-enol and A2. Presumably it is the *threo* form; a corresponding *erythro* isomer so far has not been seen. Racemic *apiose*, 3H-A4[27], affording peaks 12 and 17, regularly occurs in formose. It is a transient species, disappearing after the end of the reaction but in the middle of the reaction it may exceed 2-xylulose.

Branched hexoses

There exist eighteen *meso* and DL species of branched-chain hexoses: ten H-aldopentoses (=H-A5), three 2-DHE-tetroses, four H-pentuloses (=H-γK5) and one 2,3-di-H-A4, almost all of which appear accessible by aldolizations within the formose reaction network.

2H-A5 (*hamamelose*) is a natural product which might arise from 2 A3; 3H-A5 from K4 and A2; 4H-A5 by condensation of H-A3 (4a) with A2; 2-DHE-tetroses from A4 and A2; 4H-3K5 from K4 and A2; 4H-3K5 from 3H-K4 (16a) and F; 3H-2K5 from K4 and A2; 4H-2K5 (*dendroketose*) from 2 K3[25] and 2,3-di-H-A4 from K3 and A3.

Several unknown peaks in the hexose region (6.3% of the total area and 24% of the total hexose area) show that such branched-chain hexoses do occur. Several more might be concealed in known peaks. The identification is not yet complete. Both dendroketose and 2-DHE-tetrose emerge in the position of sorbose I (26). 2-DHE-tetrose arises together with all aldohexoses and 3-hexuloses on prolonged incubation of A2 in alkaline solution.

Heptoses

Albeit arising in minimal amounts in the formose mixtures studied, heptoses are clearly seen in the respective mass scans at M + 1 = 858. A large peak with t_R = 27.38 min in a product obtained by slow addition of K3 to excess of F probably represents 2,4-C-dihydroxymethyl-3-pentulose, recently isolated by Shigemasa *et al.*[28] from formose obtained using barium hydroxide.

Total composition

Whereas we could identify or assign with fair confidence almost all constituents of the mixture in Table I, it must be noted that under special conditions dramatically different product patterns may occur[24]. Using, for example, low or zero calcium levels and/or low pH at elevated temperature, the reaction runs slower and charac-

teristic reduction products of branched-chain sugars accumulate[29]; an entirely different pattern also arises after prolonged incubation.

In summary, in our example (Table I), (i) erythrulose (33% of total tetroses) is more predominant than in mixtures from A2 aldolization (5%), thus probably also arising from F and K3; (ii) aldopentoses (19.4%) and 2-pentuloses (19.5%) occur in about equal amounts, 2-xylulose (12.7%) representing the main single compound in the mixture; (iii) apiose (4.3%) accounts for as much as 10% of the pentose fraction, arising at the beginning of the reaction and disappearing later (*cf.*, Figs. 1 and 3); (iv) 3-hexuloses and so far unspecified branched-chain hexoses account for one quarter each of the hexose fraction, the other half being represented by 2-hexuloses; (v) the branched-chain tetrose 4a did not accumulate under the conditions used and only 0.85% of its reduction product (3) is seen; (vi) 5% of trioses, 15.2% of tetroses (including 4a), 45.2% of pentoses and 25.9% of hexoses (including unknown branched-chain species) account for as much as 91.3% of the total area between C3 and C6; (vii) another 7.5% represent unknown peaks in the C3–5 region, which apparently do not belong to regular sugar derivatives and deserve further study. Thus 98.8% of the fraction has been resolved into individual peaks.

A major problem: the yield

An important gap in our knowledge of the formol reaction must be emphasized: in the experiment discussed (Figs. 1 and 3, Table I), where all F was consumed at 38°C at pH 12 in less than 30 min, only 20% of the formaldehyde (as determined using erythritol as the internal standard) was converted into sugars producing volatile derivatives. Under most conditions we and other workers[6–8] obtained yields of less than 50%. Apparently the remainder is fixed in some high-molecular-weight form that deserves further elucidation.

Prebiotic implications

Many modern enzymatic pathways can be considered as spontaneous or as metal ion or organically catalysed reactions[30], which were improved by the *standardization* (and refinement) *of catalysts* through the evolution of template-directed polypeptide synthesis. A logical implication of the concept of standardization is the preexistence of a non-enzymatic precursor, *i.e., metabolism must have preceded enzymes.*

In our Hannover programme, searching for the first steps of chemical self-organization in the origin of life[31], the present work adds new support to our conjecture[9] that the formose reaction may represent a non-enzymatic precursor network of modern pathways. In contrast to prebiotic syntheses requiring boiling or even electrical discharges, these pathways represent fast reactions occurring under mild conditions in dilute solution at room temperature which would fit into a primeval carbon cycle involving methane and formaldehyde. As has long been known, the decomposition of fructose and glucose in dilute solutions is very reminiscent of the glycolysis pathway[14] affording trioses, methylglyoxal and lactic acid (and in the presence of ammonia also alanine); the aldolization network in the formol reaction resulting in 2-xylulose as a main product may well be considered as a non-enzymatic version of the Calvin–Horecker cycle; the latter aspect is further strengthened by the conversion of 2-xylulose into 3-hexuloses. This reaction directly corresponds to the C-1 pathways in microorganisms[13] which metabolize methane, methanol and F. Fi-

nally, branched-chain pentoses contain the *isoprene* skeleton. Conspicuously, the most ancient bacterial pylum, the methanogens, also contain isoprenoids, phytol ethers of glycerine, instead of fatty acid glycerides in their cell wall[32]. Similarly, branched-chain pentoses and hexoses, in the same way that trioses afford alanine, may afford precursors of *valine*, *leucine* and *isoleucine*.

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