24 h at 50 °C to ensure an equilibrium distribution of guest molecules in the zeolite.

C. **Diffuse Reflectance Spectra.** Diffuse reflectance spectra of the zeolite solid samples were measured in 2-mm-path-length quartz cells using a Varian 2400 spectrometer equipped with either an integrating sphere (Varian) or a "praying mantis" all reflective light collection system (Harrick Scientific), in both cases using barium sulfate (Kodak, white reflectance standard) as the reference. Sample packing densities were not determined nor were they specifically controlled. Spectra were recorded between 220 and 800 nm. For comparison, spectra of the empty zeolites were also recorded. Data were recorded digitally, and appropriate background corrections were carried out using the computer program SpectraCalc (Galactic Industries).

D. Emission Spectra. Emission spectra were recorded at room temperature and at 77 K in Supracil quartz EPR tubes under degassed conditions with a Spex Fluorolog 212 or 222 spectrofluorimeter. Spectra were corrected for detector sensitivity. Near-IR emission spectra from long-chain polyenes were recorded using a Spex 212 fluorimeter (Spex Industries) modified to accept a high-sensitivity germanium detector (North Coast Model EO-817L, useful wavelength range ≈750–1750 nm). Spectra were corrected for detector and optical system response using curves determined with an NBS-traceable, calibrated tungsten lamp (Optronics Laboratories). The signal to noise ratio was improved by lock-in detection (Stanford Instruments) with the chopper located at the entrance slit of the emission monochromator. Scattered light was removed by use of either short-wave-pass or narrow-band-pass interference filters (Corion Corp.) placed between the excitation monochromator and the sample and by use of long-wave-pass interference filters (Corion Corp.) placed between the sample and the emission monochromator. Background scans of both as prepared and activated zeolite samples showed no detectable emission in this wavelength region.

E. Lifetimes. Fluorescence lifetimes were measured at room temperature using a PRA single photon counting apparatus, deconvolution was performed by nonlinear least-squares routines minimizing χ^2, and goodness of fit was determined with plots of residuals, autocorrelation function, and reference to the Durbin–Watson statistic.[26] All decays except that for Cs X could be fitted to a single-exponential function. Triplet lifetimes were measured by conventional flash photolysis techniques using a xenon flash lamp as the excitation source. Emission intensities were monitored at the maximum of the 0–0 vibronic band, and data was fit to simple mono- or biexponential decay laws using an unweighted least-squares analysis.

F. Oxygen Quenching Studies. Dry zeolite complex was taken in an ESR tube fitted with a Teflon stopcock and degassed thoroughly (10^{-5} mm) on a vacuum line fitted with double-valved stopcocks for oxygen and nitrogen leaks. The degassed sample was filled with oxygen at high pressures. The degassing and oxygen-filling cycle was repeated three times. The spectrum of the sample thus filled with oxygen was recorded. In all cases, after the emission spectrum was recorded, the sample was either filled with nitrogen or degassed thoroughly. The intensities of the fluorescence and phosphorescence reached the original levels before oxygen filling. The oxygen and nitrogen cycle was carried out at least three times, and to our great surprise the intensities of the emissions were highly reproducible.

Acknowledgment. It is a pleasure to thank D. R. Sanderson, A. Pittman, J. Lockhart, and P. Hollins for expert technical assistance. We are thankful to Professors R. S. H. Liu and J. Saltiel and Dr. A. Asato for samples of olefins and useful discussions.

Registry No. (*E*)-4-MeOC$_6$H$_4$CH=CHC$_6$H$_4$-4-OMe, 15638-14-9; (*E*)-4-CNC$_6$H$_4$CH=CHC$_6$H$_4$-4-CN, 5216-37-5; (*E*)-4-MeC$_6$H$_4$CH=CHPh, 1860-17-9; (*E*)-4-MeOC$_6$H$_4$CH=CHPh, 1694-19-5; (*E*)-4-MeOC(O)C$_6$H$_4$CH=CHPh, 1149-18-4; (*E*)-4-ClC$_6$H$_4$CH=CHPh, 1657-50-7; (*E,E*)-PhCH=CHCH=CHPh, 538-81-8; (*E,E*)-4-ClC$_6$H$_4$CH=CHCH=CHPh, 37985-13-0; (*E,E*)-4-MeC$_6$H$_4$CH=CHCH=CHPh, 37985-11-8; (*E,E*)-4-MeOC$_6$H$_4$CH=CHCH=CHPh, 22145-08-0; (*E,E*)-4-MeOC(O)C$_6$H$_4$CH=CHCH=CHPh, 89510-60-1; (*E,E*)-4-CNC$_6$H$_4$CH=CHCH=CHC$_6$H$_4$-4-CN, 89510-54-3; (*E*)-PhCH=CHPh, 103-30-0; (*E,E,E*)-Ph(CH=CH)$_3$Ph, 17329-15-6; (*E,E,E,E*)-Ph(CH=CH)$_4$Ph, 22828-29-1; Li, 7439-93-2; Na, 7440-23-5; K, 7440-09-7; Rb, 7440-17-7; Cs, 7440-46-2; Tl, 7440-28-0; phenanthrene, 85-01-8; pyrene, 129-00-0; hexahydropyrene, 20330-24-9; acenaphthene, 83-32-9; chrysene, 218-01-9; 2-phenylindene, 4505-48-0; indenoindene, 6543-29-9; 1-phenylcyclopentene, 825-54-7; 1-phenylcyclohexene, 771-98-2; indene, 95-13-6; 1,2-dihydronaphthalene, 447-53-0; naphthalene, 91-20-3.

(26) Eaton, D. F. *Pure Appl. Chem.* **1990**, *62*, 1631.

Formation of Carbohydrates in the Reaction of Atomic Carbon with Water

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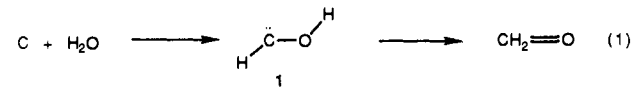
Abstract: The cocondensation of atomic carbon with water at 77 K generates a mixture of straight chain aldoses with up to five carbons. A proposed mechanism involves initial reaction of C with H_2O to give hydroxymethylene which rearranges to formaldehyde. Subsequent nucleophilic addition of hydroxymethylene to formaldehyde with hydrogen transfer generates glycolaldehyde which reacts with additional hydroxymethylenes to build up the higher carbohydrates. Confirmation of this mechanism is provided by deuterium labeling studies.

Atomic carbon has been identified as an interstellar species[1] and in association with cometary emissions.[2] In attempts to determine the reactions which would occur when carbon atoms are condensed on cold surfaces with some of the more abundant extraterrestrial molecules, we have reported that cocondensation of carbon with water and ammonia at 77 K generates amino acids.[3,4] We have now found that low-temperature condensation of carbon with water alone yields carbohydrates.

The initial reaction of atomic carbon with water gives deoxygenation to hydrogen and carbon monoxide along with the divalent carbon intermediate hydroxymethylene, **1**, which can rearrange to formaldehyde as shown in eq 1.[5] We have previously calculated



(1)

(1) Keene, J.; Blake, G. A.; Phillips, T. G.; Huggins, P. J.; Beichman, C. A. *Astrophys. J.* **1985**, *299* (2, pt. 1), 967.

(2) (a) Balsiger, H. et al. *Nature* **1986**, *321*, 330. (b) Woods, T. N.; Feldman, P. D.; Dymond, K. F. *Astron. Astrophys.* **1987**, *187*, 380, and references cited therein.

(3) Shevlin, P. B.; McPherson, D. W.; Melius, P. *J. Am. Chem. Soc.* **1983**, *105*, 488.

(4) Shevlin, P. N.; McPherson, D. W.; Melius, P. *J. Am. Chem. Soc.* **1981**, *103*, 7006.

(5) Ahmed, S. N.; McKee, M. L.; Shevlin, P. B. *J. Am. Chem. Soc.* **1983**, *105*, 3942.

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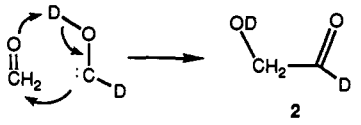
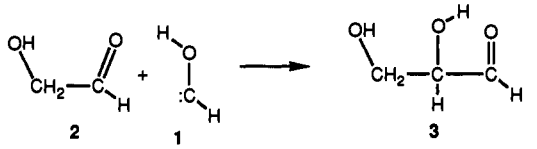
(MP2/6-31G***/6-31G) and demonstrated experimentally that the barrier to hydrogen migration in carbene **1** is high enough to permit intermolecular trapping of **1** to compete with rearrangement. An interesting intermolecular reaction of **1** is with formaldehyde to generate glycolaldehyde, **2**.[6] Although **2** is formally the result of a C–H insertion by **1** on formaldehyde, we have used deuterium labeling to demonstrate that it is instead formed by the novel nucleophilic addition–hydrogen transfer mechanism in eq 2.[6] This nucleophilic addition–H transfer



(2)

2

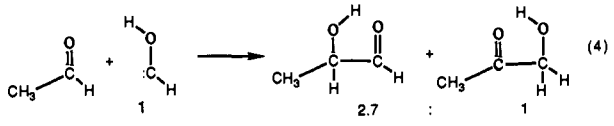
mechanism appears to predominate in the reaction of **1** with other aldehydes as well. For example, addition to **2** results in glyceraldehyde, **3**, as the major product (eq 3) and reaction of **1** with



(3)

2 **1** **3**

acetaldehyde gives 2-hydroxyproanal and hydroxy acetone in a 2.7:1 ratio (eq 4).[6]



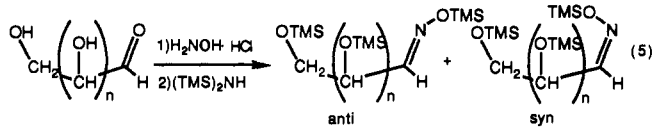
(4)

1 2.7 : 1

The fact that **1** adds to carbonyl carbons by nucleophilic addition raises the possibility that reaction product **3** will react further with additional molecules of **1** to progressively generate a mixture of higher carbohydrates. The products of the reactions in eqs 3 and 4, in which addition of **1** to aldehydic carbons generates a new aldehydic carbon, indicates that straight chain aldoses will predominate in this reaction.[7] In order to test this possibility, we have cocondensed carbon atoms[8] with water at 77 K and analyzed the reaction products for the presence of straight chain aldoses.

Results and Discussion

Aldose Analysis. Product analysis involved the standard derivatization of carbohydrates to oximes which are converted to trimethylsilyl (TMS) derivatives and analyzed by gas chromatography/mass spectrometry (GC/MS).[9,10] This common derivatization procedure, shown in eq 5, yields both the syn and anti oxime derivatives which are volatile and separable by GC.



(5)

anti syn

In order to identify the aldoses generated in the carbon atom reaction, standard solutions of all the straight chain D-aldoses through C_5 as well as those of glucose, mannose, and galactose were converted to their TMS–oxime derivatives and their retention times and mass spectra measured. In all cases, peaks corresponding to the syn and anti oxime derivatives were observed. Both syn and anti derivatives showed parent molecular ions in their mass spectrum with one of them having a tendency to lose a methyl group. The mass spectral fragmentation pattern of these oxime derivatives is straightforward with all having common fragments of m/e = 73 $(OTMS)^+$, 103 $(CH_2OTMS)^+$, and 116 $(CH{=}NOTMS)^+$. Individual aldose derivatives show fragments corresponding to loss of CH_2OTMS, $CH{=}NOTMS$, $TMSOHC_2(CHOTMS)_n$, and $TMSON{=}CH(CHOTMS)_n$. Although the mass spectra are quite characteristic for each C_n aldose, the diastereomeric aldoses have similar mass spectra and it is difficult to distinguish diastereomers on this basis. However, the retention times of the diastereomeric aldose derivatives up to C_4 are different and these compounds may be identified by a combination of their mass spectra and retention times. The situation is more complex for the aldopentoses which are not completely separated under our GC/MS conditions. Although eight GC peaks are expected for a standard mixture of the TMS–oxime derivatives of the four diastereomeric aldopentoses, our mass chromatograms generally showed only five peaks (dilute samples sometimes gave six or seven peaks). The five peaks in order of elution were (1) the first peak of lyxose, (2) the first peak of xylose, (3) a mixture of the second peaks of lyxose and xylose and the first peak of arabinose, (4) the second peak of arabinose, and (5) both peaks of ribose. The criteria adopted for the positive identification of aldoses in these reactions are that they show peaks for both syn and anti derivatives and that these peaks exhibit retention times and mass spectra similar to those of standard samples.

Aldoses in the Reaction of Carbon with Water. The reactions of atomic carbon with water were carried out by vaporizing carbon in an arc under high vacuum and cocondensing it with water at 77 K in a standard apparatus.[11] At the conclusion of the reaction, the aqueous product mixture was converted to TMS oxime derivatives by standard procedures and analyzed by GC/MS. This analysis resulted in the conclusive identification and quantitation of glycolaldehyde, glyceraldehyde, erythrose, and threose. Aldoses through C_4 all show peaks for both syn and anti derivatives. The analysis also revealed peaks whose retention times and mass spectra indicated that they were derivatives of the aldopentoses. The low yields of the C_5 aldoses make identification of all diastereomers highly problematical. In some reactions, peaks corresponding to all the diastereomeric aldopentoses were observed while in other cases they were not. However, there is always a peak corresponding in retention time and mass spectra to xylose and this peak predominates when other diastereomers are present. We thus feel that xylose, which is formed in the highest yield, has been conclusively identified. Mass spectral data from a number of runs indicates that the other aldopentoses are present as well. In any case, incomplete separation of peaks and low yields do not permit reliable quantitation of the C_5 sugars. In this and in all reactions in which arc-generated carbon is condensed with substrate at 77 K, the variable amount and rate of carbon evaporation make it difficult to obtain reproducible product yields. For this reason, we have chosen to measure the yields relative to glycolaldehyde, **2**. Table I lists these yields and their standard deviations under various sets of conditions along with the masses of the molecular ions observed in the mass spectra. In all cases, GC retention times and mass spectra of the product aldoses are similar to those of authentic samples. Since our proposed mechanism of aldose formation dictates formation of the higher sugars by progressive addition of **1** to a reaction product, the yields of the aldoses decrease with increasing number of carbon atoms in the product. As expected for such a reaction, the absolute yields are quite low (0.08%) for glycoaldehyde. However, these yields are based on the weight of carbon lost from the graphite rods and should be considered lower limits, as much carbon is lost in macroscopic pieces during arcing.

(6) Ahmed, S. N.; McKee, M. L.; Shevlin, P. B. *J. Am. Chem. Soc.* **1985**, *105*, 1320.

(7) Although it is well-known that the aldoses exist as monomeric and dimeric cyclic hemiacetals, they would be formed here on low-temperature surfaces and lack the energy to cyclize until the matrix was warmed.

(8) For reviews of the chemistry of atomic carbon see (a) Skell, P. S.; Havel, J.; McGlinchey, M. J. *Acc. Chem. Res.* **1973**, *6*, 97. (b) MacKay, C. In *Carbenes*; Moss, R. A., Jones, M., Jr., Eds.; Wiley-Interscience: New York, 1975; Vol. II, pp 1–42. (c) Shevlin, P. N. In *Reactive Intermediates*; Abramovitch, R. A., Ed.; Plenum Press: New York, 1980; Vol. I, pp 1–36.

(9) Yamaguchi, H.; Ikenaka, T.; Matsushima, Y. *J. Biochem.* **1968**, *63*, 553.

(10) Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. *J. Am. Chem. Soc.* **1963**, *85*, 2497.

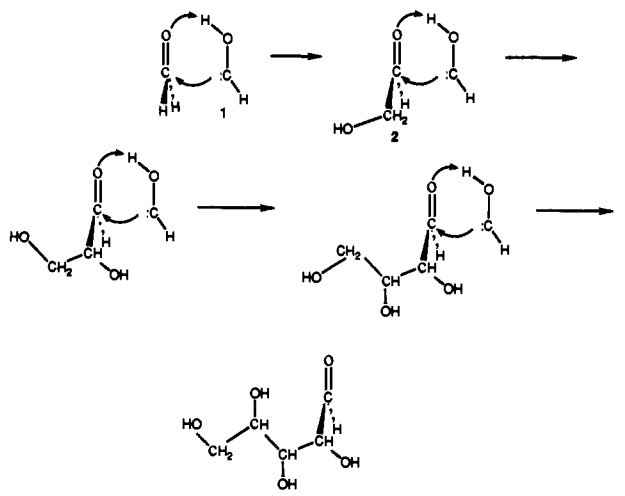
(11) Skell, P. S.; Wescott, L. D.; Goldstein, J. P.; Engel, R. R. *J. Am. Chem. Soc.* **1965**, *87*, 2829.

Table I. Aldose Yields and Mass Spectral Data for the Reaction of Atomic Carbon with Water

	reactants						
	C + H_2O		C + D_2O		C + D_2O + CH_2O		
product aldoses	yield[a,b]	*m/e* of parent	yield[c]	*m/e* of parent	yield[d]	*m/e* of parent	*m/e* 103/105
glycolaldehyde	1	219	1	222	1	220	4.0
glycolaldehyde	0.25 ± 0.11	321	0.27 ± 0.08	325	0.38 ± 0.35	323	2.1
erythrose	0.11 ± 0.08	423	0.09 ± 0.05	428	0.18 ± 0.23	426	2.4
threose	0.15 ± 0.14	423	0.08 ± 0.03	428	0.13 ± 0.13	426	1.8
aldopentoses	*e*	525	*e*	531	*e*	529	2.0

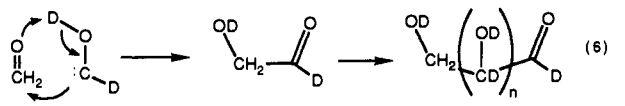
[a] Yields are reported relative to glycolaldehyde with appropriate standard deviations. The absolute yield of glycolaldehyde in a typical C + H_2O experiment in which 54 mmol of C was reacted with 277 mmol of H_2O was 0.045 mmol. [b] Average of six runs. [c] Average of seven runs. [d] Average of four runs. [e] Low yields and overlapping peaks made quantitation difficult.

Scheme I



Mechanism of Aldose Formation. In view of the fact that carbene **1** reacts with aldehydes by nucleophilic addition to the aldehydic carbon (eqs 1–3), it seems reasonable to postulate that formation of aldoses in this system occurs by this mechanism which is outlined in Scheme I. In order to test the mechanism in Scheme I and further confirm the identity of the aldoses, atomic carbon was condensed with D_2O to generate the deuterated sugars. Since the deuteriums on the oxygens are removed by the derivatization process, the mass spectra of the deuterated TMS–oxime derivatives are expected to show parent molecular ions which have increased by 3, 4, 5, and 6 mass units for the C_2, C_3, C_4, and C_5 aldoses as compared to their undeuterated counterparts. Data in Table I confirm this expectation. The other fragments in the mass spectra also show the expected deuteration pattern. For example, the large *m/e* 103 peak in each spectrum becomes a *m/e* 105 peak upon deuteration.

The mechanism in Scheme I postulates an initial reaction of **1** with formaldehyde in which the methylene carbon of formaldehyde eventually becomes the terminal hydroxymethyl carbon of the aldoses. Thus, inclusion of protiated formaldehyde with the C + D_2O cocondensate should generate aldose derivatives with molecular ions 1, 2, 3, and 4 mass units higher than the corresponding C_2, C_3, C_4, and C_5 nondeuterated aldoses (eq 6). Although this experiment is complicated by the fact that deuterated formaldehyde results from the carbon + D_2O reaction, Table I shows that there are distinct peaks for molecular ions corresponding to the aldoses in which the terminal methylene is protiated and the remaining carbons are deuterated.



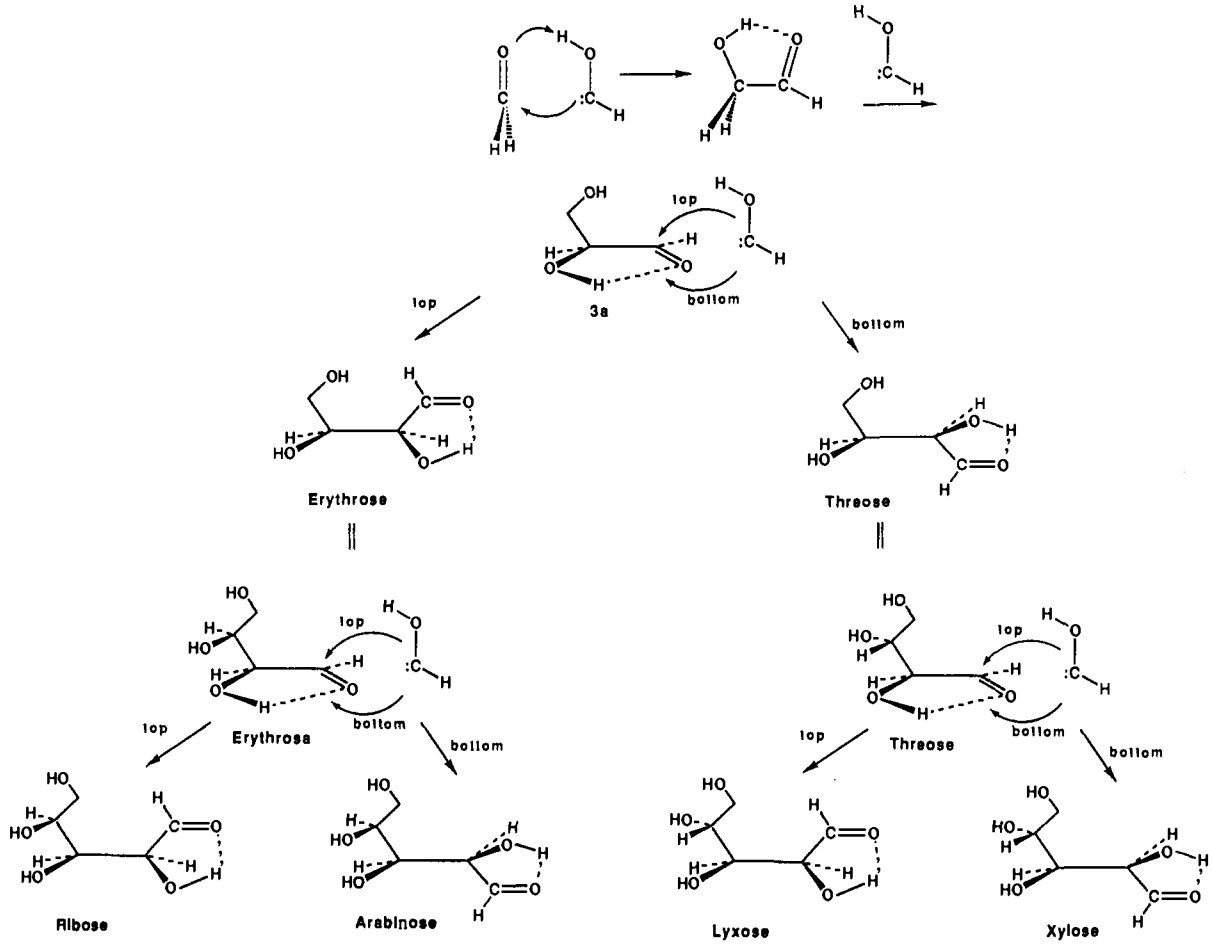
(6)

The mass spectrum of the aldoses generated in the C + D_2O + CH_2=O reaction clearly demonstrates that these aldoses contain a terminal CH_2. In the mass spectra of the protiated carbohydrate derivatives, the *m/e* = 103 peak corresponds to $[CH_2OTMS]^+$ in which the CH_2 is derived from the terminal hydroxymethyl group in the aldose. In the mass spectrum of the products of the C + D_2O reaction, this peak has shifted to *m/e* = 105 for all aldoses. However, the mass spectra of the products from the C + D_2O + CH_2=O reaction exhibits peak at both *m/e* = 103 and *m/e* = 105. The 103 peak results from the reaction of D—C—OD with CH_2=O while the 105 peak corresponds to reaction of D—C—OD with CD_2=O produced in the course of the reaction. These results indicate that the carbon in the added CH_2=O becomes the terminal hydroxymethyl carbon in the product aldoses. The last column of Table I gives the ratio of *m/e* 103 to *m/e* 105 for each aldose resulting from this last reaction.

Selectivity of Carbohydrate Formation. The main thrust of this investigation has been to determine if straight chain aldoses can be generated in the reaction of atomic carbon with water and to establish the mechanism of their formation. While the GC/MS data establish the formation of straight chain aldoses and the labeling studies implicate the mechanism in Scheme I, questions concerning the formation of other carbohydrates are still open. With regard to the question of selectivity for straight chain aldoses, we are hampered by the fact that authentic samples of branched aldoses were generally not available and standard GC/MS samples could not be prepared. However, we do observe that the predominate peaks in the GC retention time range for a given C_n carbohydrate correspond to those of the straight chain aldose. For example, erythrose and threose exhibit peaks in the retention time range of 1420–1495 s under our conditions and they are the predominate peaks in the retention time domain from 1200 to 1600 s. Although the xylose predominates among the C_5 aldoses, there are smaller peaks whose spectra show the *m/e* 103 peak which shifts to 105 upon deuteration. These compounds have slightly shorter retention times than the TMS–oxime derivatives of any of the our aldose standards and may correspond to other isomeric pentoses.

Another interesting question in these investigations concerns the diastereomeric selectivity among the aldoses which are formed. A certain amount of such selectivity seems to be indicated by the fact that xylose always predominates among the C_5 aldoses. It is clear that, if the mechanism in Scheme I is correct, threose must be the precursor of lyxose and xylose while erythrose leads to arabinose and ribose. Thus, a preference for threose over erythrose would lead to a preponderance of lyxose and xylose over arabinose and ribose. It is tempting to ascribe the preference for xylose formation to a cumulative steric effect on attack of **1** on the aldehydic carbon. Scheme II, in which attack of **1** on **2** with hydrogen transfer generates an initial conformer of glyceraldehyde, **3a**, with an O_1–C_1–C_2–O_2 dihedral angle of 0°, depicts the way in which such an effect could operate. If the glyceraldehyde is held in this initial conformation by a combination of the low reaction temperature and intramolecular hydrogen bonding, the least hindered approach for subsequent attack by another molecule of **1** will be from the side opposite the CH_2OH group (the bottom in Scheme II) leading to threose rather than erythrose. Likewise attack of **1** on threose will have a least hindered side (the bottom in Scheme II) which will lead to xylose over lyxose. Such considerations rationalize the observed preference for xylose formation in this reaction. Although the threose:erythrose ratio (1.5) for the C + H_2O reaction in Table I is not statistically different from 1, the effect would be amplified in the second addition leading

Scheme II



to a predominance of xylose. Alternately, erythrose and threose may be formed in equal amounts with the latter simply reacting faster.

The formation of carbohydrates in reactions involving formaldehyde is well-known to occur in the formose reaction in which the polymerization of formaldehyde to a complex mixture of carbohydrates is catalyzed by divalent metal ions.[12] This reaction has been postulated to have been involved in prebiotic carbohydrate formation on the primitive earth.[13] However, the reaction reported here proceeds by a quite different mechanism from that of the formose reaction and should lead to carbohydrates which are the products of kinetic rather than thermodynamic control, as would be expected under the high temperature conditions of the formose reaction.[14] While it is difficult to imagine the reactions discussed here to have played a role in terrestrial prebiotic chemistry, these investigations raise the possibility that condensation of extraterrestrial carbon on an ice surface, such as that of a comet, may generate simple carbohydrates.

Experimental Section

Materials. The following chemicals were purchased from standard suppliers and used without further purification: glycoaldehyde, glyceraldehyde, erythrose, ribose, lyxose, arabinose, xylose, glucose, galactose, mannose, hydroxylamine hydrochloride, pyridine, trimethylchlorosilane, trifluoroacetic acid, 1,1,1,3,3,3-hexamethyldisilazane, 1,3-dihydroxyacetone, paraldehyde. Erythrose and threose were prepared according to literature procedures.[16]

Apparatus. GC/MS data were obtained with a Varian 3700 gas chromatograph interfaced to either a Finnigan MAT-700 ion trap system or Vacuum Generators (VG) Analytical 7000E mass spectrometer. The gas chromatographic separation utilized a J and W 30 m × 0.25 mm DB-5 fused silica capillary column. Temperature program was 50 °C for 1 min and 5 °C per minute heatup rate to 280 °C. Infrared measurements were performed on Perkin-Elmer 580 and 983 spectrophotometers.

Preparation of TMS–Oxime Derivatives of Standard Sugars.[9,10] Individual standard solutions of glycoaldehyde, glyceraldehyde, erythrose, threose, arabinose, lyxose, ribose, xylose, mannose, galactose, and glucose were prepared by dissolving 100 mg of each in 100 mL of distilled water. Various combinations of the standard sugars were converted to oxime derivatives by dissolving 200 mg of hydroxylamine hydrochloride in a known volume of the sugar solution. The solution was frozen, placed under vacuum, allowed to thaw, and heated at 80 °C in an oil bath while being magnetically stirred for 1–1.5 h. Dried oxime derivatives were obtained by removing the solvent under vacuum on a rotary evaporator. The oxime derivative was dissolved in 1.0 mL of pyridine, the solution was placed under vacuum, and hexamethyldisilizane (0.5 mL) and chlorotrimethylsilane (0.25 mL) were added. The solution was then shaken for 1 min and placed under N_2 overpressure. After being allowed to stand for at least 15 min the solutions were analyzed by GC/MS using the DB-5 column.

Reaction of Arc Generated Carbon Vapor with Water. The reactor design has been reported by Skell, Wescott, Goldstein, and Engel.[11] Carbon was vaporized by striking an intermittent arc between two graphite rods attached to water-cooled brass electrodes and condensed on the walls of the reactor at 77 K with reactant under vacuum. In a typical reaction, distilled water (222.0 mmol) was cocondensed with carbon vapor (30.2 mmol). At the conclusion of the reaction, volatile products were pumped from the reactor through traps at 195 and 77 K. Analysis of the 77 K trap by IR revealed formaldehyde (0.98 mmol). The reactor bottom and the contents of the 195 K trap were dissolved in distilled water (250–300 mL). The solution was filtered to remove graphite and

(12) Mizuno, T.; Weiss, A. H. *Adv. Carbohyd. Chem. Biochem.* **1974**, *29*, 173.

(13) Miller, S.; Orgel, L. E. *The Origin of Life on Earth*; Prentice Hall: Englewood Cliffs, NJ, 1974.

(14) The fact that substantial amounts of deuterium are incorporated indicates that our products do not arise by polymerization of formaldehyde. The primary reaction between atomic carbon and formaldehyde is deoxygenation to methylene and carbon monoxide.[15]

(15) Ahmed, S. N.; Shevlin, P. B. *J. Am. Chem. Soc.* **1983**, *104*, 6488.

(16) Serianni, A. S.; Nunez, H. A.; Barker, R. *Carbohydr. Res.* **1979**, *72*, 71.

the filtrate converted to TMS-oxime derivatives by the method above. The yields in Table I were obtained from the mass chromatograms using the TMS-oxime derivative of galactose, which was not a product, as an internal standard and are the average of six runs. In some runs, a GC peak corresponding in retention time to the TMS derivative of glucose was observed; no MS data were obtained.

Reaction of Arc Generated Carbon Vapor with D_2O. In a typical reaction, deuterium oxide (250 mmol) was reacted with carbon vapor using the method described above. GC/MS analysis of the TMS-oxime derivatives demonstrated that they contained the appropriate amount of deuterium and gave the yields in Table I which are the average of seven runs.

Reaction of Arc Generated Carbon Vapor with D_2O and Formaldehyde. In a typical reaction, deuterium oxide (250 mmol) and gaseous formaldehyde (27.6 mmol, from paraformaldehyde) were introduced separately into the reactor and condensed with carbon vapor. GC/MS analysis of the TMS-oxime derivatives demonstrated that the original carbohydrates contained both a CH_2O and a CD_2O and that the remaining carbons were deuterated (table). The yields in Table I are the average of four runs.

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Supplementary Material Available: Retention times and mass spectral data for TMS-oxime derivatives of standard carbohydrates and those which are the products of the reactions described (9 pages). Order information is given on any current masthead.

Simple General Acid-Base Catalysis of Physiological Acetylcholinesterase Reactions

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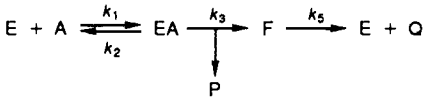
Abstract: Elements of transition-state stabilization by proton bridging have been characterized by measuring solvent isotope effects and proton inventories for hydrolyses of (acetylthio)choline (ATCh), (propionylthio)choline (PrTCh), and (butyrylthio)choline (BuTCh) catalyzed by acetylcholinesterases (AChEs) from *Electrophorus electricus*, fetal bovine serum, human erythrocytes, and *Torpedo californica*. For the *Electrophorus* enzyme, the acylation rate constant, k_{cat}/K_m {$= (V/K)/[E]_T$}, decreases in the order ATCh > PrTCh ≫ BuTCh. Solvent isotope effects for V/K of ATCh hydrolysis are usually within experimental error of unity, which is consistent with rate determination of the acylation stage of catalysis by a physical step, such as substrate diffusion. However, as substrate reactivity decreases the isotope effect increases, which indicates that the transition state of a chemical step is increasingly rate determining. A linear proton inventory for V/K of BuTCh hydrolysis indicates that this chemical transition state is stabilized by single proton transfer. Solvent isotope effects for V are ~2. and the corresponding proton inventories are invariably linear, irrespective of the source of AChE, the choice of substrate, the ionic strength of the medium, or the presence of the detergent TX100. The consistency of the results strongly suggests that AChE stabilizes chemical transition states of physiological reactions by one-proton, simple general acid-base catalysis. Therefore, elaborate themes in transition-state stabilization by proton transfer, such as multifunctional or charge-relay catalyses, do not appear to operate in the physiological functioning of AChE.

The physiological function of acetylcholinesterase (AChE[1]) is the hydrolysis of the neurotransmitter acetylcholine (ACh) at nerve-nerve synapses and neuromuscular junctions.[2-4] This reaction proceeds via an acylenzyme mechanism, outlined in Scheme I, that is similar to that of the serine proteases.[5-8] Several observations in recent years have firmly entrenched this mechanism. Froede and Wilson trapped the acetyl-AChE intermediate formed during EE-AChE-catalyzed turnover of [^{3}H]acetyl ACh and (acetylthio)choline (ATCh),[9] thereby providing the first direct evidence for the acylenzyme mechanism. Their experiments showed that V ($= k_{cat}[E]_T$) is 57-68% rate limited by the deacetylation stage of the mechanism. Comparative sequences of cholinesterases[10,11] and site-directed mutagenesis experiments[10] indicate that S200 and H440 (TC-AChE numbering) are constituents of the active site. Perhaps the most significant report is that of the crystal structure of TC-AChE.[12] This structure shows that the active site sits at the bottom of a 15-Å depression

Scheme I



in the enzyme and contains the side chains of S200, H440, and E327 in a stereochemical relationship that is similar to that of

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(1) Abbreviations: AChE, acetylcholinesterase; ATCh, (acetylthio)choline; BuTCh, (butyrylthio)choline; PrTCh, (propionylthio)choline; TCh, thiocholine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); ONPA, *o*-nitrophenyl acetate; EE-AChE, *Electrophorus electricus* AChE; FBS-AChE, fetal bovine serum AChE; HE-AChE, human erythrocyte AChE; TC-AChE, *Torpedo californica* AChE; TX100, Triton X100; UDP, umbelliferyl diethyl phosphate; K, the Michaelis constant K_m; V, the maximal velocity V_{max}; V/K, V_{max}/K_m; $^{D_2O}V = V^{H_2O}/V^{D_2O}$, solvent isotope effect on V; $^{D_2O}K = K^{H_2O}/K^{D_2O}$, solvent isotope effect on K; $^{D_2O}V/K$, solvent isotope effect on V/K.

(2) Rosenberry, T. L. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1975**, *43*, 103-218.

(3) Quinn, D. M. *Chem. Rev.* **1987**, *87*, 955-979.

(4) (a) Taylor, P. In *Pharmacological Basis of Therapeutics*; Gilman, A. G., Goodman, L. S., Murad, F., Eds.; MacMillan: New York, 1985; pp 110-129. (b) Froede, H. C.; Wilson, I. B. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic: New York, 1971; Vol. 5, pp 87-114.

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