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BIFUNCTIONAL CATALYSIS OF α-HYDROGEN EXCHANGE IN ISOBUTYRALDEHYDE-2-δ BY OCTAKIS-Ο-(3-AMINOPROPYL)SUCROSE

DISSertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Stephen Scott Ulrey, B. S.

* * * * *

The Ohio State University
1973

Reading Committee:

Professor Jack Hine
Professor Robert J. Ouellette
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Approved By

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INTRODUCTION

The remarkable efficiency of enzymes is a widely recognized, but poorly understood, chemical phenomenon. Enzymes are nature's catalysts and are generally much more effective than catalysts devised so far by man, despite the fact that they must operate under very mild conditions of temperature and pH. Enzymes have three features that distinguish them from man-made catalysts: (1) the enormous magnitude of their rate accelerations, (2) their specificity, and (3) in many cases, their susceptibility to controls. Rate accelerations of $10^{10}$ and more are known for enzymes, comparing the uncatalyzed rate to the rate in the presence of $\sim 10^{-7} \text{ M}$ enzyme. Of course this comparison is approximate because the uncatalyzed rate is frequently too slow to measure and because of different pH dependencies and rate laws in the two reactions. Nevertheless, it is clear that enzymes are tremendously effective catalysts. The second, and perhaps the most characteristic feature of enzymes, is their specificity. For example, when complexed to sucrose phosphorylase, glucose-1-phosphate does not transfer its glucosyl group to water (although it is surely present at the active site if fructose is not available) but only to fructose. The third characteristic of enzymes is that they frequently can be controlled (i.e., turned on and off) by other agents not participating directly in the reaction.
Enzymes function by complexing the reactant(s), transforming the complexed reactant(s) into complexed product(s), and decomplexing the product(s). Complexing, which must be reversible, may be accomplished by covalent, hydrogen, electrostatic, or hydrophobic bonding, or some combination thereof. The combination of many effects is responsible for the dramatic rate increases; among them are approximation (bringing the reactants together), orientation (holding the substrate so its reactive site is turned toward the attacking agent), activation (enhancing the substrate's reactivity through complexing, e.g. electronically or conformationally), and distortion (bending or stretching bonds to make the reactant begin to resemble the transition state). Since many functional groups are required to effect all these contributions, enzyme catalysis may be described as polyfunctional catalysis. The simplest form of polyfunctional catalysis is bifunctional catalysis, which is the subject of this dissertation.

The literature contains many examples of intramolecular reactions proceeding much faster than the corresponding intermolecular reactions. For example, the first order rate constant for loss of phenolate ion from phenyl-γ-(4-imidazolyl) butyrate

\[ \text{Ph}-\gamma-(4\text{-imidazolyl})\text{butyrate} \rightarrow \text{Ph} + \gamma-(4\text{-imidazolyl})\text{carboxylic acid} \]

\[ k = 2.58 \text{ min}^{-1} \]
is 24 times as large as the pseudo-first-order rate constant for loss of phenolate ion from phenyl acetate in the presence of 1 M imidazole.  

\[ \text{CH}_3\text{-C-O} + \text{N} \rightarrow \text{CH}_3\text{-C-N} + \text{OH} \]

Equivalently, the two rates may be compared as the quotient of the first and second order rate constants. The quotient (24 M) is the concentration of imidazole that would be required around phenyl acetate to obtain the rate of the intramolecular reaction. Another example is the dehydration rates of succinic and acetic acids. Succinic anhydride forms $3 \times 10^5$ times as fast as acetic anhydride forms from 1 M acetic acid. Hence, to accelerate the acetic acid rate to equal the succinic acid rate, the obviously impossible acetic acid concentration of $3 \times 10^5$ M would be required. It can be seen that the term, "effective concentration" must not always be taken literally, since other effects plainly contribute in this case.

---


In the foregoing examples, the orientation and proximity factors have been built into the system by chemical synthesis. On the other hand, an enzyme must perform these functions by itself using those (weaker) binding forces that are available. Thus, for lack of complexing and decomplexing capabilities, these examples are not good models of enzymes. An early example of enzyme-like catalysis in smaller molecules is Franzen's work on the transformation of phenylglyoxal to mandelic acid. The reaction is bifunctionally catalyzed by β-diethylaminomethyl mercaptan by the mechanism shown.

Another example is described in the series of papers by Hine and coworkers on the monofunctional and bifunctional catalysis of α-hydrogen exchange. The exchange of α-hydrogen in carbonyl compounds is a reaction of significance (it is the first step of epimerizations, racemizations, halogenations, and eliminations) and could be studied conveniently, using acetone-d₆ and isobutyraldehyde-2-d as substrates. Acetone-d₆ is subject to most effective bifunctional catalysis of exchange by 2-(dimethylaminomethyl)cyclopentylamine. The mechanism includes enzyme-like

---


features of complexation by imine formation, activation by protonation on nitrogen, and intramolecular removal of the deuteron.

Then, after the protonated amine has exchanged with the solvent, the enamine is reprotonated by the reverse of the steps shown. In the presence of the bifunctional catalyst, the rate is about 100 times faster than the rate predicted for monofunctional amine catalysis alone.

Bifunctional catalysis of exchange in isobutyraldehyde-2-\textsubscript{d} is theoretically a more difficult problem because a much larger ring in the transition state is required. This is because (1) the imine is usually formed trans.
greatly increasing the number of atoms required to reach the reactive site and (2) the small amount of cis-imine that presumably is formed is less reactive for conformational reasons. Whereas exchange in acetone-$d_6$ was catalyzed effectively through an eight-atom transition state ring, models indicate that for isobutyraldehyde about a 15-atom transition state ring would be required. Also, 3-dimethylaminopropylamine, a bifunctional catalyst for exchange in acetone, acted monofunctionally with isobutyraldehyde, largely because it could not reach the deuterium atom from the trans imine. In spite of the large transition state ring requirement, polyethylenimines catalyzed the exchange bifunctionally with a rate of 12-36 times the rate of monofunctional model compounds. The experiments on polyethylenimine confirmed the predicted size of the transition state ring; it was concluded that the most favorable transition states contain 13-22 atoms. On the other hand, even the simplest cis-imine is predicted to be unreactive because the isopropyl group cannot rotate into the reactive conformer. In the reactive conformer the C-D bond must be perpendicular to the plane of the double bond, since


as deuterium is removed it becomes a π-cloud in the enamine. Formation of this reactive conformer, shown for cis-N-methylisobutyraldimine,

\[ \text{H}_3\text{C} \quad \equiv \quad \text{C} \quad \equiv \quad \text{N} \]
\[ \text{H} \quad \text{C} \quad \equiv \quad \text{H} \quad \text{H} \]
\[ \text{H} \quad \text{H} \]

requires that the two methyl groups occupy some of the same volume. Thus, little or no reaction through cis imine is expected for isobutyraldehyde. Experimentally this is supported by the fact that 3-dimethylaminopropylamine effected no detectable bifunctional catalysis in isobutyraldehyde-2-d.\(^6\)

Except for the polyethylenimines, only one catalyst has been shown to act bifunctionally in the α-hydrogen exchange of isobutyraldehyde, 1-dimethylamino-8-amino-2-octyne.\(^9\) It caused the exchange to proceed about three times as fast as was predicted from model compounds for monofunctional amine catalysis alone.

From the knowledge gained from previous catalysts, it was felt a better catalyst could be designed. However, a fully rigid catalyst

---

seemed like a formidable synthetic task and there was no guarantee that it would be a good bifunctional catalyst once it was prepared. As a compromise between projected catalytic activity and ease of synthesis, octakis-\(\beta\)-(3-aminopropyl)sucrose was chosen. This was still somewhat a "shotgun approach" to achieving bifunctional catalysis (i.e., a large number of amine groups in a small volume so that probably some base would be in the right place to deuterate the protonated imine) but not as much so as was polyethylenimine. The octaamine would have several other advantages over the polymer. First, it would not share the polymer's disadvantage of tying up large amounts of substrate as unreactive imidazolidines. Second, its structure would be simpler and known in considerably more detail, enabling good estimates of its basicity and imine formation constants. Third, adequate model compounds were available for estimating the monofunctional component of the catalysis.
EXPERIMENTAL SECTION

Chemicals

Chemicals that are widely available in high quality and were used without purification are not listed.

Benzyl Chloroformate, 95%, was purchased from the Aldrich Chemical Co. It was purified by low temperature (-78°C) crystallization from pentane.

2-Cyanoethyl Ether, tech., was purchased from the Aldrich Chemical Co. It was purified by distillation, bp 95-98°C (0.005 mm) [lit. bp 161-163°C (5.5 mm)].

Octakis-2-(2-cyanoethyl)sucrose, labeled Cyanoethyl Sucrose, was purchased from the Eastman Chemical Co.

Diglyme (Dimethyl Ether of Diethylene Glycol) was purchased from Matheson, Coleman, and Bell. Before use it was dried overnight with calcium hydride and distilled in vacuo from lithium aluminum hydride, bp 75°C (30 mm) [lit. bp 62-63°C (15 mm)].

Deuterium Oxide, 100.0 atom per cent, was purchased from Diaprep, Inc. It was checked by nmr and found to have less than 0.1% protium.

Hydrochloric Acid Solutions were prepared and standardized by The Ohio State University Reagent Laboratory.

Isobutyraldehyde-2-d was prepared by the heavy water hydrolysis of the enol acetate of isobutyraldehyde as described before, usually with isotopic purity of at least 95%. Immediately before use it was purified by preparative glpc (10 ft x $\frac{1}{4}$ in diethylene glycol succinate at 50°) and stored at -78° until use.

2-Methoxyethylamine was purchased from Eastman Organic Chemicals. Before use it was purified by distillation through a 90 cm x 1 cm glass-helix column using a 5:1 reflux ratio, bp 90° [lit. 13 bp 95°]. It was checked by glpc (6 ft x $\frac{1}{8}$ in carbowax-KOH at 100°) and found to be at least 99.9% pure.

3-Methoxypropylamine was purchased from the Aldrich Chemical Co. Before use it was purified by distillation under nitrogen through a 90 cm x 1 cm glass-helix column using a 2:1 reflux ratio, bp 116-117° [lit. 14 bp 117-118° (733 mm)].

Propylamine was purchased from Eastman Organic Chemicals. Before use it was distilled under nitrogen through a 90 cm x 1 cm glass-helix column using a 2:1 reflux ratio, bp 48-49° [lit. 15 bp 47.8°].

Raney Nickel, Activated was purchased from W. R. Grace and Co., Raney Catalyst Division, Chattanooga, Tennessee. It was obtained as a 50% aqueous slurry.

Sephadex, grades G-10, G-15, and G-25, was purchased from Pharmacia Fine Chemicals.

Sodium Hydroxide Solutions were prepared and standardized by The Ohio State University Reagent Laboratory.

Sodium Nitrite, purified, was purchased from the Fisher Scientific Co. Before use in Van Slyke Analysis, it was recrystallized from water.

Water, double distilled, was purchased from The Ohio State University Reagent Laboratory. Carbon dioxide was excluded from the air space in the carboy by Ascarite and the water was degassed before use.

Instrumental

Boiling Point Determinations

Boiling points were taken as the distillation temperature of the fraction. All bp's are uncorrected.

Constant Temperature Baths

For pH measurements and kinetic runs, a Sargent Heater-Circulator was used with a Sargent Thermonitor control unit, maintaining the temperature at 35.00 ± 0.05°.

In automatic titrations, the sample was held at 35.0 ± 0.5° by a Precision Scientific Co. Lo-Temptrol 154.

During uv measurements, the temperature was maintained at 35.0 ± 0.5° by a Haake Constant Temperature Circulator, model FE.
Elemental Analyses

Carbon, hydrogen, and nitrogen analyses were carried out in duplicate by the Scandinavian Microanalytical Laboratory, Herlev, Denmark.

Gas Liquid Partition Chromatography

An F and M Model 720 dual column temperature programmed gas chromatograph was used for analyses. A Varian Aerograph model A-700 'Auto-prep' with thermal conductivity detector was used for preparative work.

Gel Permeation Chromatography

Preparative gpc was done on Sephadex G-15 in a Sephadex column, model K50/100, 5.0 x 100 cm. The effluent was separated into fractions by a Buchler Fraction Collector, model 3-4002.

High Pressure Liquid Chromatography

The apparatus used consisted of a Varian constant-pressure pump, a Varian 3/8 in x 50 cm column, and a Varian differential refractive index detector. The column was dry-packed with Porosil C (a silica gel), 35-72 µ, obtained from Waters Associates.

Hydrogenation Reactions

A Parr Hydrogenation Apparatus, model 3911, was used in low pressure reductions with hydrogen.

Infrared Spectra

A Perkin-Elmer 337 grating infrared spectrometer was used.
Molecular Distillation

A Nester-Faust molecular still, model NFMS-75, was used with a Consolidated Vacuum Corp. model VMF-10 oil diffusion pump. Pressure was indicated by a Televac thermocouple vacuum gauge, model 2A.

Nuclear Magnetic Resonance Spectra

NMR Spectra were determined on Varian A-60 and A-60A instruments.

Optical Rotation Measurements

A Perkin-Elmer model 141 polarimeter, with digital readout to the nearest 0.001°, was used.

pH Measurements

pH measurements for kinetic runs were made at 35° using a Radiometer model 26 pH meter with a Radiometer GK 2301 C combination electrode. The instrument was standardized on pH 4.02 and pH 9.102 buffers.

pH Titrations

Automatic titrations were carried out using a Radiometer model 26 pH meter, model 11 Titrator, model ABU16 Auto-Burette, model SER2c Titragraph recorder, TTA3 Titration Assembly with thermostatted cell holder, and G202B glass and K401 reference electrodes. Micro-titrations used the same equipment with smaller electrodes and vessel.

UV Measurements

Absorbances in the uv region were measured on a Cary Model 1605 Recording Spectrophotometer at 35° using 1 cm quartz cells.
All accurate measurements of weight were made on a Mettler model 35H26 balance, readable to the nearest 0.1 mg.

Reduction of Cyanoethylsucrose

Cyanoethylsucrose (10.0 g) was dissolved in 250 ml of methanol saturated with ammonia at 10°. Activated Raney Nickel (3 spatulas full or 35-40 g of a 50% aqueous slurry) was added and the vessel was flushed with ammonia to exclude air until it was pressurized with hydrogen to 60 lb. Shaking was started and the electrical heater set on 45 v, giving a temperature of 54°.

When the reaction was judged complete by hydrogen uptake, it was continued approximately one additional hour. Pressure was then released cautiously with gentle shaking, since the supersaturated mixture can bump badly. Next, the mixture was filtered (twice through paper, once through Millipore Polyvic, 2.0 μ) and concentrated under vacuum, giving a viscous green syrup. The cloudiness that formed on dissolving in water was then removed by centrifugation and filtration through Millipore Polyvic, 2.0 μ. Water was removed by freeze-drying, giving 8.8 g (85%) of pale green semi-solid octakis-O-(3-aminopropyl)sucrose (OAPS): infrared (neat) 3360 and 3300 cm⁻¹ (N-H), 2925 and 2865 cm⁻¹ (C-H), and 1090 cm⁻¹ (C-O); nmr (D₂O) τ 8.08 (quintet, 16.6, J = 6.5 Hz, C-CH₂-C), τ 7.12 (triplet, 14.8, J = 7 Hz, -CH₂-N), τ 6.12, 6.22 (complex absorption, 29.6, all O-CH and O-CH₂), and τ 5.25 ppm (singlet, HOD). The theoretical integrals are 16, 16, and 29, respectively.
The material used in kinetic runs was purified by gel permeation chromatography on Sephadex G-15. It was obtained as a hygroscopic, amorphous, off-white solid. The yield from the chromatography purification averaged 4% for 50 preparative separations. The column seemed to deteriorate with use since both the quality of the separation and the yield gradually decreased over the course of the work.

Preparation of OAPS Hydrochloride

Octakis-O-(3-aminopropyl)sucrose (3.0 g), dissolved in the minimum amount of water, was quickly and accurately neutralized with 1.0 N HCl, consulting the titration curve for the amount of acid required. The solution was filtered and added to ~100 ml of dioxane, precipitating the amine hydrochloride. After cooling in the refrigerator for a few hours to assure complete separation of the layers, the lower layer (about 8 cc) was withdrawn. The water was removed on the Rotovap at \( \sim 30^\circ \) and methanol was added. The methanol was then removed at 30 mm pressure (water aspirator). When a syrup of the proper viscosity was obtained, heat and greater vacuum were applied together, causing the syrup to foam up and fill the entire 100-ml flask. Most of the remaining solvent was removed from the sponge-like material by a mechanical pump at 0.005 mm overnight, giving 2.90 g (71%) of the amine hydrochloride: ir (KBr pellet) 3600-3500 cm\(^{-1}\) (N-H), 3000-2900 cm\(^{-1}\) (C-H), 1095 cm\(^{-1}\) (C-O), and 1020 cm\(^{-1}\) (C-O); nmr (D\(_2\)O) \( \tau \) 7.85 (quintet, 15.9, \( J = 7 \) Hz, C-CH\(_2\)-C), \( \tau \) 6.68 (triplet, 14.6, \( J = 6.5 \) Hz -CH\(_2\)-N), \( \tau \) 6.03 6.13 (complex absorption, 30.2, all O-CH and O-CH\(_2\)), and \( \tau \) 5.25 ppm (singlet, HOD). The theoretical integrals are 16, 16, and 29, respectively.
Preparation of n-Propylamine Hydrochloride

One mole of n-propylamine (59 g) was cooled in an ice bath and neutralized by slowly adding one equivalent of concentrated hydrochloric acid. The salt was precipitated by adding benzene with enough ethanol to render the mixture homogeneous. The hydrochloride was then recrystallized three times from benzene-ethanol mixtures and dried under vacuum at 80° and 20 mm pressure.

Preparation of 3-Methoxypropylamine Hydrochloride

One mole of 3-methoxypropylamine (96 g) was cooled in an ice-water bath and neutralized by slowly adding one equivalent of concentrated hydrochloric acid. The salt was precipitated by adding diethyl ether and enough ethanol to render all components miscible. The hydrochloride was then recrystallized twice from diethyl ether--ethanol mixtures and dried in vacuo at 80° and 20 mm pressure. Desiccator storage was found desirable.

Preparation of Bis-γ-Aminopropyl Ether

The diamine was prepared according to the procedure of Freifelder. Bis-β-cyanoethyl ether (2.6 g, 0.0209 mol) was dissolved in 200 ml of methanol which had been saturated with ammonia at room temperature. Then 0.52 g of 5% rhodium on alumina was added under a blanket of ammonia and the mixture was pressurized to 60 lb with hydrogen. The calculated hydro-

gen uptake was observed after 4 hr. The mixture was filtered, concentrated, and distilled, giving 1.47 g (55%) of the diamine: bp 69-70° (1.3 mm) [lit. bp 117-120° (6 mm)], nrmr (25% soln in 100.0 atom % D₂O) τ 8.13 (quintet, 4, J = 6.4 Hz, C-CH₂-C), τ 7.15 (triplet, 4, J = 6.7 Hz, CH₂N), τ 6.18 (triplet, 4, J = 6.2 Hz, OCH₃), and τ 5.25 ppm (singlet, 4, DOH from NH₂); ir 3360 and 3280 cm⁻¹ (N-H), 2940 and 2860 cm⁻¹ (C-H), and 1113 cm⁻¹ (C-O). Glpc analysis (6 ft x 1/8 in carbowax--KOH, 150°) showed the sample to be 98% pure. Titration analysis indicated 94% purity.

Bis-Y-aminopropyl ether was obtained in pure form by preparative glpc (10 ft x 1/4 in carbowax--KOH, oven 155°, detector 190°). Column pre-treatment by injecting five 0.5-ml portions of triethylamine was found essential to the purification. Analysis of the sample gave 99.3% by glpc and 98.7% by titration.

Preparation of the Carbobenzoxy Derivative of OAPS

A 1.00-g sample of crude OAPS was dissolved in 50 ml of 85:15 methanol-water by volume. Sodium bicarbonate (1.94 g) was dissolved as much as possible and 2.22 g of purified benzyl chloroformate was added with magnetic stirring over a one hour period...Stirring was continued overnight, after which the mixture consisted of three phases: (1) the solvent (containing most of the product), (2) a white syrup (more of the product), and (3) white crystals (sodium salts). The solvent-phase product was recovered

by precipitation with 50 ml of water and centrifugation. The syrup-phase product was washed with water and removed from the reaction flask with methanol. The products were combined and precipitated from methanol with water three times and dried by vacuum overnight giving 2.20 g (94%) of the carbobenzoxy derivative: ir (neat) 3340 cm\(^{-1}\) (N-H), 3075 and 3040 cm\(^{-1}\) (aromatic C-H), 2950 and 2880 cm\(^{-1}\) (aliphatic C-H), 1700 cm\(^{-1}\) (C=O), 1135 and 1080 cm\(^{-1}\) (C-O); nmr \(\tau\) 8.5 (unresolved quintet, 17.2, C-CH\(_2\)-C), \(\tau\) 7.0 and 6.6 (unresolved triplet and complex absorption, 44.0, C-CH\(_2\)-N and all O-CH\(_2\)-), \(\tau\) 5.1 (singlet, 15.8, Ph-CH\(_2\)-0), and \(\tau\) 2.9 ppm (singlet, 39.3, Ph). The theoretical integrals are 16, 45, 16, and 40, respectively.

The material thus obtained was fractionated by precipitation from methanol at -78\(^\circ\) C, where the material was a solid. Typically, 0.5 g was dissolved in 20 ml of methanol and slowly cooled to -78\(^\circ\) C with agitation. After 10 min, the solvent was filtered from the solid at -78\(^\circ\), giving two roughly equal portions of carbobenzoxy OAPS.

**Hydrogenolysis of Carbobenzoxy OAPS**

A 3.28-g sample of unfractionated carbobenzoxy OAPS was dissolved in 90 ml of methanol and added to a 100-ml cylinder containing 200 mg of 5% palladium on charcoal (adding the catalyst to the methanol instead ignited the methanol vapor). Hydrogen was introduced through a glass frit and the catalyst was kept suspended by magnetic stirring. The reaction was complete after 4 hr. The mixture was filtered and concentrated under vacuum giving 1.28 g (92%) of OAPS with the same spectral properties as
OAPS prepared by direct reduction of the nitrile. The same procedure was also carried out separately on the two fractions obtained by low temperature precipitation.

**Gel Permeation Chromatography on Sephadex**

Sephadex was prepared and packed according to procedures well described by the manufacturer. Prior to use, newly-packed columns were checked for homogeneity by running a solution of Blue Dextran 2000. This experiment also measured the void volume since the blue dye of mw 2,000,000 is fully excluded from all Sephadex gels.

For analytical chromatography, ordinary glass columns were used. Samples were applied by preparing a 25% aqueous solution and layering it onto the Sephadex bed beneath the eluant. The effluent was led through 1.5 mm i.d. polyethylene tubing to a level slightly lower than the eluant head to obtain proper flow rates. There it entered the fraction collector, which was set to give 2.5-ml fractions. The optical rotation of each sample was measured and a plot of rotation vs. effluent volume was constructed.

For preparative chromatography, a Sephadex model K 50/100 column, 5.0 x 100 cm was used with Sephadex G-15. Since the Sephadex column included a sample applicator, the samples were applied semi-automatically by means of a sample reservoir connected between the eluant reservoir and the column by a three-way valve. The fraction cutter was set to deliver 12.7-

ml fractions and the effluent was analyzed in the same way as on the analytical columns.

**Kinetic Runs**

The exchange of isobutyraldehyde-2-d was followed by periodically quenching samples and determining their nmr spectra.

The amine solution was adjusted to the desired pH by the addition of standardized hydrochloric acid. Aliquots of 10.00 ml were pipetted into 15-ml ground glass stoppered centrifuge tubes and placed in the 35° bath. Final pH measurements were made in the centrifuge tubes at 35°.

To start the exchange reaction, 50 µl of isobutyraldehyde-2-d (purified by preparative glpc immediately before use) was injected under the surface of the catalyst solution. The mixture was shaken and replaced in the 35° bath. Since the pH is changed by the introduction of aldehyde, it was measured again.

After the desired length of time, the reaction was stopped by protonating all amine groups with a 50-100% excess of acetic acid. The isobutyraldehyde/isobutyraldehyde-2-d mixture was then extracted from aqueous solution by shaking vigorously with 0.6 ml of chloroform. After settling, the chloroform layer was removed by a long-needled syringe and placed in a nitrogen purged nmr tube. Normally, a kinetic run consisted of at least six samples.

The nmr spectrum was observed in the τ 8.90 ppm region where the two equivalent methyl groups absorb. The methyl groups themselves do not
change during the reaction, but their splitting by the isotope on the adjacent carbon does change. In isobutyaldehyde, the methyl absorption is split into a doublet by the adjacent hydrogen atom, \( J = 6.9 \) Hz. In \textit{isobutyaldehyde-2-\textsuperscript{d}}, the methyl absorption is split into a triplet by the adjacent deuterium, \( J = 1.05 \) Hz. Thus, as the reaction proceeds, the wide doublet is seen growing outside the shrinking, narrow-spaced triplet.

Fortuitously, it was found that the composition of the \textit{isobutyaldehyde/isobutyaldehyde-2-\textsuperscript{d}} mixture is adequately determined by measurement of peak heights; thus area measurements were not required. This was shown by the linearity and zero-intercept (within experimental error) of a plot of \( d/(d+h) \) vs fraction isobutyaldehyde-2\textsuperscript{d} for a series of known \textit{IBA/IBA-2d} mixtures, where \( d \) denotes the height of the center member of the triplet and \( h \) denotes the height of the downfield peak of the doublet.

Since the reaction is first order in any given run, the following rate equation was used:

\[
\text{rate} = \frac{dc}{dt} = k_p c
\]

where \( c = [\text{IBA - 2d}] \) and \( k_p \) is the observed first-order rate constant.

Rearranging and integrating gives

\[
\ln(c_0/c) = k_p t
\]

However \( c_0/c \) equals \( (d + h)/d \), thus

\[
\ln\left(\frac{d + h}{d}\right) = k_p t
\]
Rate constants were obtained from the slopes of plots of $\ln\left(\frac{d+h}{d}\right)$ vs. $t$. The best straight line through the points was determined by a least squares procedure, minimizing the sum of the squares of the absolute deviations.

**Van Slyke Analysis**

The apparatus shown in Figure 1 was used for geometric primary amine analysis. It consisted of a reaction section, a gas delivery section, a gas measurement section, and a gas washing section. The apparatus was prepared for use by half-filling the Orsat bulb (d) with fresh 5% KMnO$_4$--2.5% KOH solution and thoroughly purging the entire apparatus with wet nitrogen. Then a magnetic stirring bar, 9.00 ml of 0.060 $\pm$ 0.004 M amine solution, and 1.00 ml of glacial acetic acid were placed in the flask (a), which was also purged with nitrogen. The solution in the Orsat bulb was then pushed up to a mark just below the joint and closed off from the rest of the apparatus by stopcock (b). The flask was connected to the apparatus and the buret was zeroed by turning stopcock (c) to connect the buret to the atmosphere and moving the water level to zero; then the same stopcock was turned to connect the reaction and delivery sections to the atmosphere, and finally, the reaction and delivery sections were connected to the buret. (The water level did not change since both sections were at atmospheric pressure.)

The analysis was begun by injecting 2.00 ml of 11.6 M sodium nitrite solution through the septum into the reaction solution and beginning the
(a.) Reaction flask with septum
(b.) Stopcock
(c.) Stopcock
(d.) Orsat bulb (left chamber filled with glass tubes)
(e.) 25-ml buret
(f.) Overflow vessel
(g.) Leveling bulb
(h.) Rubber bag

Figure 1. Apparatus for Van Slyke Analysis
stirring. The water level in the buret was followed with the leveling bulb, keeping the inside pressure at atmospheric pressure. After 20-30 min, the water level was near the bottom of vessel (e) and nitrogen evolution was complete. With the buret precisely leveled, stopcock (b) was turned to connect the Orsat bulb to the gas measurement section and the leveling bulb was raised to force the gas into the Orsat bulb. The gas was moved back and forth, and when constant volume was obtained, the permanganate--hydroxide solution was returned to its position at the mark. The buret reading was taken.

All aqueous solutions used were prepared from nitrogen-saturated water. The Orsat bulb was cleaned with concentrated hydrochloric acid after each determination.

Before calculating the results it was necessary to know the dead volume. The dead volume exists because the section from the liquid surface in the flask to the first stopcock initially contained nitrogen, which was measured with the sample. Since all the nitrogen was flushed into the measuring section by the nitrogen oxides (generated by decomposition of the nitrous acid), the dead volume contained no nitrogen at the end of the analysis. Consequently, the dead volume must be subtracted from the observed volume to obtain the volume generated by the sample.

The absence of nitrogen in the dead volume at the end of the analysis is reasonable in view of the facts that (1) nitrogen oxides comprise over three-fourths of the evolved gas, and (2) nitrogen evolution is essentially complete after 1-2 minutes, while the evolution
of nitrogen oxides continues steadily throughout the 20-30 minute analy-
sis. Thus, it can be estimated that the void volume is flushed with
at least six void volumes of nitrogen oxides. For most analyses, the
magnitude of the void volume was determined by direct measurement
(filling the appropriate section of the apparatus with water and weigh-
ing) and by running a carefully purified standard. The observed agree-
ment of the void volumes obtained by these two methods shows that there
was no nitrogen in the void volume at the end of the analyses. Other-
wise, the standard solution would have required a larger void volume to
compensate for some nitrogen not reaching the measurement section of
the apparatus. For the last analyses, the void volume was determined
by running zero normal amine solutions (just water).

The nitrogen volume evolved by the sample \( (V_g) \) was calculated by
subtracting the dead volume \( (V_D) \) from the observed volume \( (V_{obs}) \). Then
the number of millimoles represented by \( V_g \) was calculated and compared
to the number of millimoles of amine initially pipetted into the flask.
A sample calculation follows; using the data from the first run in
Table 6, page 66.

\[
\begin{align*}
N_{\text{soln}} &= 0.06372 \\
V_{\text{soln}} &= 9.00 \text{ ml} \\
T &= 27.4^\circ \text{C} \\
v_p (H_2O) &= 27.37 \text{ mm} \\
p &= 744.6 \text{ mm} \\
V_D &= 9.75 \text{ ml} \\
V_{obs} &= 24.60 \text{ ml}
\end{align*}
\]
\[ V_S = V_{\text{obs}} - V_D = 14.85 \text{ ml} \]

\[
\text{observed } n = \frac{PV}{RT} = \frac{(744.6 - 27.4)(14.85)}{(0.08205)(760)(300.5)} = 0.5684
\]

\[
\text{theoretical } n = (0.06372)(9.00) = 0.5735
\]

\[
\text{purity} = \frac{0.5684}{0.5735} = 99.1\%
\]

The pressure inside the apparatus (atmospheric pressure) must be reduced by the vapor pressure of water to obtain the pressure due to nitrogen.

**Measurement of Carbon Dioxide in OAPS**

The carbon dioxide in aqueous solutions of OAPS was measured directly by gas chromatography. A 6 ft x 1/4 in Poropak QS column at 70° cleanly separated air, carbon dioxide, and water. The analysis was carried out by injecting acidified aqueous samples of OAPS into the chromatograph and measuring the areas of the carbon dioxide peaks. The areas thus obtained were converted to concentration by use of a calibration curve prepared from analysis of standard solutions.

Standard solutions were prepared from an accurately weighed amount of sodium bicarbonate and a buret-measured volume of standardized hydrochloric acid. To avoid loss of carbon dioxide during the preparation of solutions, the bicarbonate and the acid were kept separate by a frozen matrix isolation technique. In this procedure, the sodium bicarbonate powder was tapped to one side of the 50-ml volumetric flask, covered with
5 ml of water (by buret), and frozen on the side in a Dry-Ice bath. Very little solid dissolved during this time. Then, while cooling the flask in a methanol--ice bath at -15°, the required amount of 0.1000 N hydrochloric acid was run down the other side of the flask and frozen there. Finally with cooling, the flask was filled (by buret) with water, and capped with a serum stopper. No noticeable melting took place during the addition of water. The sum of the delivered volumes of acid and water was taken as the total solution volume. The space above the liquid which was produced by the contraction of the melting mixture was filled with the carbon dioxide--air mixture that would be in equilibrium with that solution, as calculated from the solubility of carbon dioxide in water and Raoult's Law. Strict compliance with Raoult's Law would not be required since the amount of carbon dioxide added was only about 1% of the amount already in solution.

All injections were made from a 50-μl microsyringe fitted with a Chaney adaptor. The first stop on the Chaney was set on 5 μl, the second on 30 μl. In order to minimize vaporization from the needle during injection, a 5 μl plug of air was put behind the 25 μl sample, using the Chaney stops. The peak areas were measured with a K. and E. Compensating Circular Planimeter. Each sample and standard was analyzed at least 5 times to demonstrate reproducibility and to assure precise data.

Measurement of Unreduced Nitrile Groups in OAPS

The completion of cyanoethylsucrose reduction was checked by infrared measurements in 0.1 mm Irtran cells. First a 30% (by weight)
OAPS solution in 50/50 (v/v) diglyme--water was scanned differentially (vs the solvent) in the 2500-2000 cm\(^{-1}\) region. No detectable 2250 cm\(^{-1}\) nitrile absorption was present, although perhaps an absorbance of 0.01 or less could have been obscured by the interference pattern whose peak-to-valley amplitude was 0.03 absorbance units. Then a small amount of bis-\(\beta\)-cyanoethyl ether (1.66% by weight) was added and the solution was rescanned. A 2250 cm\(^{-1}\) absorbance of 0.10 was obtained. From the OAPS equivalent weight of 115.5 g/eq (from the titration, p 57) it was calculated that 2.6 meq/g of solution was present. For bis-\(\beta\)-cyanoethyl ether, 0.27 meq/g of solution was calculated to be present, which corresponds to about 9% unreduced nitrile in the catalyst. Since this amount gave an absorbance of 0.10 and since the maximum possible absorbance from OAPS nitrile groups was 0.01, the amount of unreduced nitrile groups in OAPS can be calculated to be less than 0.9%.

**Measurement of Bis-\(\beta\)-cyanoethyl Ether in Cyanoethylsucrose**

The amount of bis-\(\beta\)-cyanoethyl ether in cyanoethylsucrose was determined by high pressure liquid chromatography. This was done by comparing peak areas from a standard bis-\(\beta\)-cyanoethyl ether solution to areas of the bis-\(\beta\)-cyanoethyl ether component present in a cyanoethylsucrose solution. Eluting with ethyl acetate at ca. 50 lb gave a flow rate of 2.9 ml/min, which cleanly separated the bis-\(\beta\)-cyanoethyl ether component from the other components of cyanoethylsucrose. Six 50-\(\mu\)l injections of 0.1092 M bis-\(\beta\)-cyanoethyl ether in ethyl acetate gave
peak areas averaging 63.7 (areas measured with a planimeter). Four 50-μl injections of an ethyl acetate solution of untreated cyanoethyl-
sucrose (2.6924 g/10 ml soln) gave an average peak area of 47.5. Hence, the cyanoethylsucrose solution must have contained 0.0184 M bis-β-
cyanoethyl ether, or 0.101 g/10 ml. Thus, of the untreated cyano-
ethylsucrose that was weighed out, 0.101 g must have been bis-β-cyano-
ethyl ether and 2.591 g must have been cyanoethylsucrose.
RESULTS

Attempted Preparations and Purifications

The first OAPS preparation was attempted by catalytic hydrogenation with Raney nickel in acetic anhydride, followed by hydrolysis of the amide to give the amine. Acetic anhydride was recommended to suppress the side reaction leading to secondary amine products. Secondary amines can arise in nitrile reductions by addition of an already-formed primary amine to an intermediate aldimine, loss of ammonia, and addition of hydrogen. Thus, the function of the acetic anhydride

\[
\begin{align*}
R-C≡N & \xrightarrow{1\text{ H}_2} R-CH=\text{NH} \\
R-C≡N & \xrightarrow{2\text{ H}_2} R-\text{CH}_2\text{NH}_2 \\
\text{RCH}_2-\text{NH}-\text{CH}_2\text{R} & \xrightarrow{\text{H}_2} R-\text{CH} \backslash \n\text{N} \backslash \text{CH}_2\text{R}
\end{align*}
\]


was to acetylate the primary amines as fast as they were formed. Cyanoethylsucrose was reduced and acetylated at 50 pounds hydrogen pressure and 60-80°, giving a viscous green syrup. It took up the theoretical amount of hydrogen, the 2250 cm⁻¹ nitrile absorption disappeared while the 1650 cm⁻¹ amide carbonyl absorption appeared in the ir spectrum, and the nmr spectrum changed from that of the starting material. However, the amide could not be hydrolyzed successfully. Since the acetal linkage in sucrose is acid sensitive, hydrolysis was attempted in basic solutions. Treatment with KOH—water—methanol gave no reaction after 15 hours at 50-60° and aqueous sodium hydroxide at 60° for 40 hours gave a dark brown syrup of dubious character.

Then low-pressure hydrogenation directly to the amine was attempted in methanolic ammonia with Raney nickel, using a procedure similar to that of Huber. Activated Raney nickel from Research Organic-Inorganic Chemical Corporation gave slow reactions (2 days) and poor results (~40% of theoretical titratable amine groups). However, when activated Raney nickel from W. R. Grace was substituted, the reduction went faster (4 hr) and gave a much better product (~70% of theoretical titratable amine groups). This procedure evolved into the procedure that was used to prepare the amine catalyst for kinetic studies.

A rhodium catalyst was tried according to the procedure of Frei-felder, who reported that facile reduction of several nitriles of the type R-O-CH₂CH₂CN where R is an alkyl group. Electronically, these

groups are virtually identical to cyanoethylsucrose, yet cyanoethyl-
sucrose gave no reaction under the same conditions in twice the time
required for reduction of the β-alkoxypropionitriles.

Raney nickel reductions were also attempted at much higher pressures.
For reasons unknown, much more severe conditions were required; 4 hours
at 3400 lb and 60° (although the temperature was unruly and reached
100° for a short time) gave essentially complete reduction. However,
analysis on Sephadex G-15 showed no advantage for high-pressure reduc-
tion since the product was just as highly hydrolyzed as in the low-
pressure reductions with Raney nickel.

Reduction with diborane was also attempted. Brown reported the
reduction of adiponitrile to hexamethylenediamine in good yield and
purity. This was of interest not only as another way to reduce ni-
triles, but also because no cyclized secondary amine was reported.
Diborane was generated externally and bubbled into a diglyme solution
of cyanoethylsucrose at room temperature. As water was added to decom-
pose excess diborane, a white precipitate formed; the precipitate must
have been boric acid since OAPS is soluble in both diglyme and water.
A white solid was obtained by evaporating solvent from the liquid phase
at low temperature, but its nmr spectrum showed little, if any, of the
desired product.

A lithium aluminum hydride reduction was excluded for the lack of
a suitable solvent and anticipated difficulties in the work-up. Of

(1960).
the common solvents used in LAH reductions, only diglyme dissolves the nitrile. But dissolving the nitrile is only the first requirement; the solvent must dissolve the polar intermediates produced by the addition of $\text{AlH}_4^-$ to the $\text{C}≡\text{N}$ bond. This is not the case in the reduction of mono-nitriles, since the ionic adduct could precipitate from solution and redissolve when alcohol or water is added at the end of the reaction. However, the analogous process should not work for the octa-nitrile; if the molecule precipitates after partial reduction, then the nitrile groups still unreduced should not get reduced. It was considered unlikely that diglyme would dissolve a species bearing 16 positive charges.

The behavior of OAPS in adsorption chromatography may be simply described as non-mobile. In tlc experiments, $R_f$ values of 0.0 were consistently observed for OAPS, even on development with methanol. $R_f$ values of zero are perhaps reasonable for a molecule with eight amino groups. If only one group is adsorbed, then the molecule cannot move. A model compound, 3-methoxypropylamine, was observed to have an $R_f$ of 0.3 on basic alumina developed with methanol; i.e. the molecule spends about 30% of its time in the moving phase. But a molecule with eight amine groups, each spending 30% of its time in the moving phase, would be expected to have an $R_f$ of \((0.3)^8 \approx 7 \times 10^{-5}\).

Column adsorption chromatography was run on basic alumina, Florisil, and activated charcoal, all with unsatisfactory results. The 3 cm x 30 cm alumina column was eluted with 10% of methanol and methanol--water,
but only a small fraction of the material put on the column was recovered, although all fractions in the last 90% of the effluent volume gave positive Molisch tests. The Florisil column gave a small amount of material, but its nmr spectrum suggested decomposition. The charcoal column gave back no material.

Partition chromatography gave more useful results. Analytical chromatograms on paper showed measurable mobility in partition chromatography, although some adsorption took place at the same time. Butanol-ethanol-water (4:1:5 and 3:1:1) was used in descending paper chromatography. In the former system, the spots moved less than 2% as fast as the solvent front and tailed badly. Development with just water also gave very slow migration, so there must have been some adsorption. Spots were visualized with ninhydrin by a standard method. Partition chromatography on Sephadex was used successfully for analysis and purification and showed no evidence of adsorption effects.

The feasibility of purifying cyanoethylsucrose by molecular distillation was investigated by boiling point numbers. Boiling point numbers provide an empirical correlation between structure and boiling point. Each atom in the carbon skeleton and each functional group

23. Water would be both the moving and stationary phase. Thus, the distribution coefficient is unity and the migration rate should be equal to the migration rate of the solvent front times the ratio of the volumes of the two phases, if no adsorption takes place.


is assigned a number, representing its contribution to the boiling point. The numbers for all the components of the molecule are then summed and converted to the boiling point by the equation

\[ \text{bp} = 230.14 - \sqrt[2]{\text{bpn}} - 543 \]

where \( \text{bp} \) is the boiling point in degrees centigrade and \( \text{bpn} \) is the molecular boiling point number. For many molecules, good agreement is obtained between calculated and observed boiling points. Kinney reported in 1940 that the average deviation was 4.06°C for all aliphatic hydrocarbons whose atmospheric-pressure boiling points were in the literature (762 compounds). For the six normal ethers from dimethyl to dipropyl, the average deviation is 6°. For n-alkyl nitriles from hydrogen cyanide to butyl cyanide, the average deviation is 6°. Unfortunately, for poly-ols and cyanoethylated alcohols, the deviations are larger. For ethylene glycol, glycerol, 1,2,3-butanetriol, 1,2,4-butanetriol, and 1,2,3,4-butanetriol (erythritol) the calculated boiling points were an average of 53° lower than the experimental values. For cyanoethylated methanol, ethylene glycol and glycerol the calculated boiling points were an average of 88° higher than observed. Although the deviations became larger for the more complicated compounds, it was believed the method would have some qualitative value for cyanoethylsucrose. For a \( \text{bpn} \) of 203 for octacyanoethylsucrose, an atmospheric pressure \( \text{bp} \) of 810°C was calculated. Then the boiling point was extrapolated to lower pressure using the Clausius-Clapeyron equation.
Thereby a bp of 213°C was calculated for 0.001 mm pressure, which is well within the capability of the molecular still. When cyanoethylosucrose was passed through the molecular still at 150°C (≤ 0.001 mm) a clear liquid distilled, which was identified as bis-β-cyanoethyl ether by nmr and glpc. The temperature was raised to 200°C (≤ 0.001 mm) and nothing distilled. At 250°C still no material distilled. Finally at 300°C decomposition began to take place as the pressure could not be reduced below 0.010-0.015 mm.

Since OAPS would not crystallize, purification by fractional crystallization was not possible. However, two derivatives were prepared that were believed to have a reasonable chance of crystallizing and could be converted back to the amine. The amine hydrochloride was prepared and characterized by nmr, ir, pH titration, and chloride analysis. The spectral data were entirely consistent with the octakis-O-(3-aminopropyl)-sucrose structure, but the titration and chloride analysis both indicated about 70% of theoretical, the same value as was obtained by titration of the amine from which the hydrochloride was made. (Theoretical values were calculated assuming pure material with eight amino groups

\[ \ln \frac{P_2}{P_1} = \frac{\Delta H_V}{R} \frac{(T_2 - T_1)}{T_2 T_1} \]

and Trouton's rule

\[ \Delta H_V = 22T \]
per molecule.) However, the hydrochloride could not be induced to crys-
tallize, even from dry solvents in the dry box.

The other derivative prepared for fractional crystallization was
the carbobenzoxyl derivative. Carbobenzoxyl chloride was used success-
fully for the purification of the amino-sugar mucosamine by Wintersteiner
and coworkers. They isolated crude mucosamine hydrochloride from
nystatin, but were unable to induce crystallization; however, both
the carbobenzoxyl derivative and the mucosamine obtained from it were
readily crystallized. The fully carbobenzoxylated OAPS was prepared in
aqueous methanol, with enough methanol to render heptacarbobenzoxylated
OAPS sufficiently soluble that it was converted to octacarbobenzoxylated
OAPS, but at the same time with enough water to dissolve sufficient
sodium bicarbonate to neutralize the hydrochloric acid produced by the
carbobenzoxylation. The nmr spectrum indicated that all amino groups
had been carbobenzoxylated and the material gave a negative ninhydrin
test. The ir spectrum showed a strong carbonyl band at 1700 cm\(^{-1}\), free
from any shoulder from the carbobenzoxyl chloride carbonyl group (1760
\(\text{cm}^{-1}\)). Crystallization was attempted from methanol--ether solutions
without success. Then low-temperature crystallization (ultimately \(-78^\circ\))
from methanol gave solid flakes of apparently amorphous material with
some fractionation, as shown by the nmr spectra and the gel filtration
chromatograms of the decarbobenzoxylated products. However, both frac-
tions still contained large amounts of the disaccharide component.

27. J. D. Dutcher, D. R. Walters, and O. Wintersteiner, J. Org. Chem.,
Analysis of Cyanoethylsucrose

Cyanoethylsucrose was obtained as a very viscous, colorless, odorless syrup. The evidence presented in the following paragraphs suggests that the material fell short of bona fide octakis-O-(2-cyanoethyl)sucrose in three major ways: (1) it contained minor amounts of bis-β-cyanoethyl ether, (2) it was partially hydrolyzed to its monosaccharide components, and (3) it was not completely cyanoethylated.

Bis-β-cyanoethyl ether was detected on three occasions. It was collected in molecular distillation and identified by nmr and glpc by comparison with an authentic sample. Also bis-β-cyanoethyl ether condensed on the upper surface of the flask during low-pressure degassing and was identified by ir and glpc by comparison with an authentic sample. The impurity was also revealed by tlc and identified by adding an authentic sample and observing homogeneity. Bis-β-cyanoethyl ether is apparently a by-product of the cyanoethylation, arising from the cyanoethylation of hydrated acrylonitrile.

The 60 MHz nmr spectrum in chloroform-d is generally consistent with the octakis-O-(2-cyanoethyl)sucrose structure with τ 7.35 (triplet, 16.1, J = 6 Hz, -CH₂-CN), τ 6.27 (complex absorption, 29.1, O-CH₂-C and O-CH₂), and τ 4.40 ppm (doublet, 0.9, J = 3 Hz, O₂CH-C) absorptions.

The theoretical integrals are 16, 29, and 1, respectively. However, on closer examination of the $\tau$ 4-6 section, an impurity in the sample became apparent. The $\tau$ 4.40 doublet was assigned to the lone proton on the C-1 carbon of the glucose residue (the only proton on a carbon atom with two adjacent oxygens). However, at $\tau$ 4.68 another doublet

\[
\text{octakis-}\beta-(2\text{-cyanoethyl})\text{sucrose}
\]

\((J = 3 \text{ Hz})\) was normally concealed in the baseline noise and was revealed only in a highly amplified spectrum (see Figure 2). It was assigned to the analogous C-1 proton in cyanoethylated $\alpha$-glucose,

\[
\text{2,3,4,6-tetrakis-}\beta-(2\text{-cyanoethyl})-\alpha\text{-glucose}
\]
since the magnitude of the coupling constant for splitting by the proton on C-2 is correct for coupling between axial and equatorial protons on adjacent carbons. The resonance for the C-1 proton on cyanoethylated β-glucose was present at τ 5.42 ppm in chloroform-d (doublet, \( J = 6.4 \) Hz), with a coupling constant of the correct magnitude for axial-axial proton coupling.

These assignments were confirmed by subjecting the sucrose to hydrolysis conditions (hydrochloric acid with methanol co-solvent) and observing the low field spectrum of samples from the hydrolyzing mixture. The nmr spectra in chloroform-d of partially hydrolyzed samples are shown in Figure 2. Methanolysis probably competed with hydrolysis, since the solvent contained 28 mole % water and 72 mole % methanol. However, the same kind of spectrum would be expected from the methyl acetal of the glucose. Hence, from the spectra, it seems that the amount of methyl acetal is negligible or its peaks coincide with those of the glucose. Peaks with identical chemical shifts and splitting were obtained by hydrolyzing in deuterated hydrochloric acid.

The assignments were further confirmed by the deuterium oxide spectra of sucrose and glucose. The sucrose spectrum shows a low field doublet, τ 4.53 (relative to HOD at τ 5.25 ppm), \( J = 3 \) Hz, which is assigned to the C-1 proton of the glucose ring. The glucose spectrum (actually the equilibrium mixture of α- and β-glucose) shows two low-field doublets, τ 4.70 and τ 5.30 ppm, \( J = 3 \) Hz and \( J = 7 \) Hz, for α-glucose and β-

---

Figure 2. 60 MHz nmr Spectra of Cyanoethylsucrose at Various Stages of Hydrolysis.
glucose, respectively. The chemical shift of the β-glucose C-1 proton was determined by adding a small amount of deuterated hydrochloric acid to shift the DOH resonance downfield and assuming the sucrose resonance did not shift. These observations and assignments are in agreement with the literature spectra, although some uncertainty arises from the fact that the literature spectra were run at different temperatures, causing the DOH resonance to appear at different positions.

With the ratio of α-glucose to β-glucose (obtained best from the second spectrum of Figure 2, assuming the equilibrium between α-glucose and β-glucose had been established) it was possible to calculate the composition of the initial mixture. If $K_{\alpha-\beta}$ is defined to equal $[\alpha\text{-glucose}]/[\beta\text{-glucose}]$, then $K_{\alpha-\beta} = 1.4$, the ratio of the integrals of the two C-1 glucose proton resonances. Knowledge of this equilibrium constant enables calculation of the amount of the β-glucose (whose C-1 proton resonance is too small to see in the spectrum). Thus, from cut-and-weigh integrals of 508 mg and 90 mg for the low-field protons of the sucrose and the α-glucose, respectively, 64 mg was calculated for the β-glucose. From this, it was calculated that 77% of the glucose rings was present in the sucrose and 23% was present as glucose, 13.5% α- and 9.5% β-glucose.

The fact that cyanoethylation was incomplete was shown best by the elemental analysis results.


32. Ibid., No. 6245.
Table 1. Elemental Analysis of Cyanoethylsucrose

<table>
<thead>
<tr>
<th></th>
<th>C (%)</th>
<th>H (%)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated for C₃₆H₇₀₀₁₁N₈</td>
<td>56.39</td>
<td>6.05</td>
<td>14.61</td>
</tr>
<tr>
<td>Found (average of four analyses)</td>
<td>55.34 ± 0.09</td>
<td>6.18 ± 0.04</td>
<td>14.06 ± 0.10</td>
</tr>
</tbody>
</table>

The observed deviations are too large to attribute to random experimental uncertainty (for example, the calculated and found values for carbon differed by almost 12 standard deviations). However, the observed deviations can be explained by incomplete cyanoethylation and by the presence of water and bis-β-cyanoethyl ether. Three parameters were defined as follows: N, the number of cyanoethyl groups per cyanoethylsucrose molecule; W, the number of water molecules per cyanoethyl sucrose molecule; and B, the number of bis-β-cyanoethyl ether molecules per cyanoethyl sucrose molecule. However, since two cyanoethyl groups plus one water are indistinguishable from one bis-β-cyanoethyl ether to the elemental analysis, the analyses were fit only in terms of the apparent N and W. Then, after an independent determination of B, the true values of N and W were calculated by the equations,

\[ N = N_{\text{app}} - 2B \]
\[ W = W_{\text{app}} - B \]

33. The tolerances given are the standard deviations.
Figure 3 shows the dependence of the combustion analyses on $N_{\text{app}}$ and $W_{\text{app}}$. The calculated carbon and nitrogen contents were affected considerably by changes in $N_{\text{app}}$ and $W_{\text{app}}$, but the hydrogen content was insensitive to both. The calculated analyses were obtained by means of the following equations, each of the general form (weight of the element in 1 mole)/(molecular weight).

$$\% C = \frac{(12.011)(12 + 3N_{\text{app}})}{m_w} \times 100$$

$$\% H = \frac{(1.008)(22 + 3W_{\text{app}} + 2W_{\text{app}})}{m_w} \times 100$$

$$\% N = \frac{(14.007)N_{\text{app}}}{m_w} \times 100$$

where $m_w = 342.30 + 53.064N_{\text{app}} + 18.015W_{\text{app}}$. The calculated analyses were fit to the experimental analyses by varying the parameters ($N_{\text{app}}$ and $W_{\text{app}}$) such that the sum of the squares of the deviations in the C,H,N analyses was minimized. One parameter was held constant while the other was optimized, and vice versa, until both parameters had reached their optimum values. With both parameters free to assume any value, this procedure gave values of $N_{\text{app}} = 7.55$ and $W_{\text{app}} = 0.51$, precise to the nearest hundredth. The following analysis was calculated from the optimized parameters: C, 55.34; H, 6.12; and N, 14.06 (mean deviation from the experimental value was 0.02). Of course, since the precision of the fit exceeds the precision of the data, the parameters should not be taken as accurate to the number of figures given.
Figure 3. The Dependence of the Elemental Composition on the Parameters $N_{app}$ and $W_{app}$,

$N_{app}$, the number of cyanoethyls per sucrose.
However, $W_{app}$ could be determined independently, also. $W_{app}$ consisted of free water, water in monosaccharide hydrolysis products, and water in bis-$\beta$-cyanoethyl ether. Free water was determined by Karl Fischer titration to be 0.8% by weight or 0.33 waters per sucrose molecule. The NMR spectrum indicated that the sucrose was 23% hydrolyzed, which contributed 0.23 to the value of $W_{app}$. The parameter $B$ was calculated to 0.23, as described two paragraphs hence. Thus, independent measurements gave a $W_{app}$ of $0.33 + 0.23 + 0.23 = 0.79$, which is probably a more reliable value than the 0.55 previously calculated from the elemental analysis.

Since a $W_{app}$ of 0.79 is believed to be more reliable than the other value, the value of $N_{app}$ was recalculated from the analyses. The calculated analyses were fit to the experimental values as before, except $W_{app}$ was held at its preferred value. This gave a $N_{app}$ of 7.82 cyanoethyls per sucrose molecule. With the preferred values of the parameters, the following analysis was calculated: C, 55.21; H, 6.15; N, 14.20 (mean deviation from the experimental values was 0.10). Although not as good as before, the fit was judged acceptable.

The parameter $B$ could then be calculated. The high pressure liquid chromatography experiment concluded that there was 0.101 g of bis-$\beta$-cyanoethyl ether with each 2.591 g of cyanoethylsucrose. Thus $B$, the molar ratio, is given by

$$B = \frac{(0.101)/mw}{(2.591)/mw} = \frac{0.000814}{2.591/mw}$$
The mw for cyanoethylsucrose was calculated from the equation

\[ \text{mw} = 342.30 + 53.064 (7.82 - 2B) + 18.015 (0.79 - B) \]

Substituting the mw relationship and solving for B gave B = 0.23. Thus, the values of N and W were

\[ N = 7.36 \text{ cyanoethylys per sucrose} \]
\[ W = 0.56 \text{ waters per sucrose} \]

More evidence for incomplete cyanoethylation came from the ir spectrum of cyanoethylsucrose. There was a large band (about half the C-H intensity) in the O-H region (3480 cm\(^{-1}\)) that did not change detectably after degassing at 0.005 mm for 26 hours. Thus, most of the band was probably due to OH groups on sugar molecules. Some of the absorption was probably due to glucose C-1 and fructose C-2 hydroxyl groups from hydrolysis, since the sample was known by nmr to be partly hydrolyzed (unless the hydrolysis occurred early enough that the hydroxyl groups were cyanoethylated). It does seem likely that some of the absorption was due to uncyanoeethylated sucrose hydroxyls, since the band was rather large. It was not possible to evaluate the contribution from all possible hydroxyls quantitatively because the extinction coefficients were not known.

The nmr spectrum also suggested incomplete cyanoethylation after correction for the bis-β-cyanoethyl ether present in the sample (uncorrected integrals from upfield to downfield were 16.1, 29.1, and 0.9; corrected integrals 15.8, 29.3, and 0.9; theoretical integrals 16, 29, and 1). Using the ratio of the first two absorptions, an N of 7.6 was calculated.
Assignment of low-field nmr absorptions. The low field resonances were identified by the same kind of experiment that was used to identify the analogous resonances in cyanoethylsucrose. Two peaks (presumably unresolved doublets) were present in the τ 4.5 ppm region at τ 4.31 and τ 4.50 ppm, relative to DOH at τ 5.25 ppm. In crude samples the τ 4.50 ppm peak was always the larger. When deuterium chloride in heavy water was added to OAPS hydrochloride in heavy water, the DOH peak shifted downfield, revealing the resonance for the β-glucose C-1 proton at τ 5.20 ppm (relative to the original DOH absorption at τ 5.25 ppm). After 55 minutes, the τ 4.31 ppm absorption had vanished; hence it was due to the C-1 proton on the glucose ring of the sucrose. Although it was not well resolved, the β-glucose absorption was consistent with a doublet with J = 7 Hz. Three spectra provided estimates of K_{α-β} (K_{α-β} = [α-glucose]/[β-glucose]) for OAPS hydrochloride, with an average value of 0.84. Due to baseline and other uncertainties, this is not a reliable value; however, since none of the ensuing arguments require accurate knowledge of K_{α-β}, 0.84 will be used.

Gel Permeation Chromatography. Purification by gel permeation chromatography (gpc) was studied before it was scaled up to preparative amounts. First gpc on Sephadex G-10 was attempted (see Table 2 for characteristics of the Sephadex gels used). With Sephadex G-10, the optical rotation vs
Table 2 - Characteristics of Sephadex Gels

<table>
<thead>
<tr>
<th>Sephadex type</th>
<th>Dry particle diameter (μ)</th>
<th>Fractionation range (mw)</th>
<th>Bed volume (ml/g of dry Sephadex)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-10</td>
<td>40-120</td>
<td>-700</td>
<td>2-3</td>
</tr>
<tr>
<td>G-15</td>
<td>40-120</td>
<td>-1500</td>
<td>2.5-3.5</td>
</tr>
<tr>
<td>G-25 fine</td>
<td>20-80</td>
<td>100-5000</td>
<td>4-6</td>
</tr>
</tbody>
</table>

effluent volume curve was bell-shaped, indicating no separation whatsoever (Figure 4a). Little or no separation was further indicated by the fact that the fraction was eluted with a volume only slightly greater than the void volume (V₀). Furthermore, the nmr spectrum of the first fractions was essentially indistinguishable from that of the last fractions, confirming that no fractionation took place. Next, Sephadex G-25 was tried (Figure 4b). No new peaks were resolved completely, but there was an unmistakable shoulder on the leading side (the high molecular weight side) of the peak. The nmr spectra of the early and last fractions were significantly different in the region of the C-1 proton on the glucose ring. Using \( K_{eq} = 0.84 \) (determined for the amine hydrochloride in aqueous solution) it was calculated that the early and late fractions contained 24% and 11% sucrose, respectively, by nmr. The value of the equilibrium constant need not be known in order to tell which sample contains more sucrose. Thus, considerable enrichment, but no pure components, were obtained.
Figure 4. Analytical Chromatography of OAPS.

(a.) Sephadex G-10
1.9 x 60 cm
1.0 g sample

(b.) Sephadex G-25
1.9 x 67 cm
0.8 g sample

(c.) Sephadex G-15
2.75 x 57 cm
0.7 g sample
Considerably cleaner separations were obtained on Sephadex G-15 columns (see Figure 4c). Nmr analysis of the first component eluted (the high molecular weight component) indicated about 65% sucrose (assuming the same α-glucose/β-glucose distribution as before), with recovery of about 15% of the sample put on the column. The sucrose content differs from 100% for at least two reasons: (1) inefficiency of the column, and (2) the presence of secondary amine products formed from two monosaccharide fragments. Molecular weights differing by only a factor of 2 is about as difficult a preparative separation by gpc as is ordinarily worth trying, so there is undoubtedly some overlap. Secondary amines (a common nitrile reduction side product), formed from two monosaccharide fragments, would have essentially the same molecular weight as the sucrose product and, thus, probably be indistinguishable to the column. Of course, this kind of product would have no sucrose nmr resonance. When rechromatographed, the first component showed some tailing into the region of the second component, but no detectable shoulder for the second component. Tailing of the first peak into the second is consistent with the observation that the nmr spectrum of the second component indicated about 20% sucrose.

The preparative column used to purify the material for kinetic runs was 80% longer and gave a comparable separation of the sucrose from the monosaccharides. In some preparative columns, a third component was observed with an elution volume smaller than that of the sucrose component (see Figure 5a). The component of elution volume 780 ml was present to various extents in different preparations of OAPS; the Figure 5a chroma-
Figure 5. Preparative Chromatography of OAPS.
togram shows about the most ever obtained. The nmr spectrum of the 780-
ml component indicated about 60% sucrose. This component is believed to
be molecules consisting of three monosaccharide units, joined in part
through their side chains by secondary amine formation.

OAPS was purified for kinetics by double chromatography. In the
initial separation, crude OAPS samples of 4-5 g were chromatographed and
a rather wide cut was taken from the sucrose (V_e = 860 ml) peak. Then
sucrose components from five or six initial columns were combined and
rechromatographed, producing the chromatogram shown in Figure 5b. To
eliminate suspected problems from differences in the different batches,
one large homogeneous sample of highest purity, doubly-chromatographed
OAPS was prepared. All analyses and kinetic runs were done on portions of
this 8.91-g sample. The nmr spectrum of highest purity OAPS showed α-
glucose components at the threshold of detection; thus the sample con­tained > 85% by weight sucrose. The detection threshold was higher than
might be expected for three reasons. Looking at one hydrogen divided
among three peaks in a 78-hydrogen molecule requires a highly amplified
spectrum. Therefore, (1) the spectrum is noisy, and (2) small spinning
side bonds and trace impurities become significant. Also, (3) only the
α-glucose can be seen (β-glucose obscured by DOH).

In addition to the nmr spectra, retention volume correlations were
used to identify fractions. Figure 6 shows retention volume data for
telomers of ethylene glycol on Sephadex G-10 and G-15 in 1.27 x 102 cm
and 1.4 x 102 cm columns. Columns of different dimensions may be com-

34. J. A. Dean, "Chemical Separation Methods," Van Nostrand Reinhold
Figure 6. Elution Volume vs Molecular Weight for Polyethylene Glycols on Sephadex G-10 and G-15.
pared by their relative elution volumes, \( V_e/V_0 \), where \( V_e \) is the elution volume and \( V_0 \) is the void volume. The observed relative elution volumes in Table 3 identify the components in agreement with their nmr spectra. The monosaccharide component (theoretical \( \text{mw} \ 408 \)) had the same relative elution volume as a \( \text{mw} \ 400 \) ethylene glycol telomer and the disaccharide component (theoretical \( \text{mw} \ 799 \)) had the same relative elution volume as the \( \text{mw} \ 800 \) ethylene glycol telomer, within experimental error.

The relative elution volume data was of some value in elucidating the nature of the first peak from the preparative columns. As shown in Table 3, the relative elution volume corresponds to an ethylene glycol.

<table>
<thead>
<tr>
<th>( \text{mw} )</th>
<th>Ethylene Glycols(^a)</th>
<th>Cyanoethylsuccrose Reduction Products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analytical Column(^b)</td>
<td>Preparative Column(^c)</td>
</tr>
<tr>
<td>400</td>
<td>1.45</td>
<td>1.45</td>
</tr>
<tr>
<td>800</td>
<td>1.22</td>
<td>1.23</td>
</tr>
<tr>
<td>~1100</td>
<td>1.15</td>
<td>--</td>
</tr>
</tbody>
</table>

\(^a\)\( V_0 \) taken as \( V_e \) of polyethylene glycol of \( \text{mw} \ 4000 \).

\(^b\)\( V_e \) was the average of at least seven values. \( V_0 \) was determined with Blue Dextran 2000.

\(^c\)\( V_e \) was the average of at least seven values. \( V_0 \) was determined in newly packed columns with Blue Dextran 2000, but decreased as the bed compressed with use. For the calculation of relative elution volumes, \( V_0 \) was taken as the maximum of the first product detected (680 ml).
of mw about 1100. This evidence suggests that the material is created by secondary amine formation between one mono- and one disaccharide. This is consistent with the nmr observation of ~60% sucrose since random reaction of this type would call for a sucrose in every molecule and a glucose in every other molecule (or 67% sucrose).

Also Figure 6 affords a correct a priori prediction on which column will give the better separation. The G-10 curve predicts only a 3-ml separation between mw 400 and mw 800, whereas the G-15 curve predicts a separation of 12 ml.

Titration. Doubly chromatographed OAPS was also analyzed by titration with standard acid. First, a 0.1 N solution was prepared by dissolving 2.5930 g in 250 ml. Since the Sephadex columns were eluted with 0.0100 M sodium chloride solution and the number and volume of fractions collected to make the sample were known, it was calculated that 2.4991 g of the amount dissolved was OAPS. Assuming the theoretical molecular weight of 799.1, the solution should be 0.1001 N. However, according to the titrations of 2.00-ml aliquots, the solution was only 0.0865 N (this corresponds to an equivalent weight of 115.5 g/eq). The discrepancy will be explained in terms of impurities (carbon dioxide and water) and less than the theoretical number of amines per molecule (from incomplete cyanooethylation and intramolecular secondary amine formation). The curve obtained (Figure 7) shows several minor inflections along the way to complete protonation, as expected for a complex amine. Complete protonation is achieved at pH 4.07 since the final end point is sharp.
Table 4. Data from the Titration of 1.988 ml of 0.0865 N OAPS at 35° with 1.000 N HCl.

<table>
<thead>
<tr>
<th>Volume added (ml)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000</td>
<td>10.190</td>
</tr>
<tr>
<td>0.0056</td>
<td>10.068</td>
</tr>
<tr>
<td>0.0106</td>
<td>9.986</td>
</tr>
<tr>
<td>0.0156</td>
<td>9.894</td>
</tr>
<tr>
<td>0.0206</td>
<td>9.803</td>
</tr>
<tr>
<td>0.0256</td>
<td>9.722</td>
</tr>
<tr>
<td>0.0306</td>
<td>9.508</td>
</tr>
<tr>
<td>0.0356</td>
<td>9.294</td>
</tr>
<tr>
<td>0.0406</td>
<td>9.081</td>
</tr>
<tr>
<td>0.0757</td>
<td>8.847</td>
</tr>
<tr>
<td>0.0883</td>
<td>8.562</td>
</tr>
<tr>
<td>0.1008</td>
<td>8.186</td>
</tr>
<tr>
<td>0.1133</td>
<td>7.697</td>
</tr>
<tr>
<td>0.1258</td>
<td>7.320</td>
</tr>
<tr>
<td>0.1383</td>
<td>6.883</td>
</tr>
<tr>
<td>0.1509</td>
<td>6.282</td>
</tr>
<tr>
<td>0.1634</td>
<td>5.601</td>
</tr>
<tr>
<td>0.1705</td>
<td>4.07 (end point)</td>
</tr>
</tbody>
</table>
Figure 7. Titration Curve for OAPS.
and the curve after the end point is about the same as for the acidification of water.

**Carbon dioxide analysis.** Octakis-\(Q-(3\)-aminopropyl)sucrose was analyzed for carbon dioxide as described in the Experimental Section. Three known carbon dioxide solutions, distilled degassed water, and one OAPS solution were run, with results tabulated in Table 5. Reproducible peak areas were obtained. The calibration curve is shown in Figure 8.

The 0.1409 \(N\) (by titration) OAPS hydrochloride gave carbon dioxide peaks with an average area of 224.5, corresponding to 0.02705 \(M\) carbon dioxide after correcting by a factor of 1.992 for different attenuator settings on the chromatograph.

The most convenient way to express the amount of carbon dioxide (which exists as a mixture of carbonate and bicarbonate in the amine sample) is as a per cent of the amount required to protonate all amine groups, with the number of amine groups determined by titration. The molecular weight need not be known, so errors introduced by uncertainty therein are avoided. Thus, counting carbon dioxide as a dibasic acid, there is enough present to protonate \(\frac{0.02705 \times 2}{0.1409} \times 100 = 36\%\) of the amine groups present.

It should be added that hydrogen chloride and carbon dioxide had identical retention times on the Poropak QS column. However, the possibility of hydrogen chloride interference can be largely excluded for two reasons: (1) The amine solutions analyzed were neutralized with exactly one equivalent of hydrochloric acid; no excess was present.
Table 5. Data for Carbon Dioxide Calibration and Analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Area</th>
<th>Average area</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01402 M CO₂</td>
<td>227</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01402 M CO₂</td>
<td>222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01402 M CO₂</td>
<td>221</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01402 M CO₂</td>
<td>227</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01402 M CO₂</td>
<td>227</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01402 M CO₂</td>
<td>227</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01402 M CO₂</td>
<td>225</td>
<td>225.1</td>
<td>1.2%</td>
</tr>
<tr>
<td>0.01655 M CO₂</td>
<td>283</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01655 M CO₂</td>
<td>277</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01655 M CO₂</td>
<td>279</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01655 M CO₂</td>
<td>267</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01655 M CO₂</td>
<td>272</td>
<td>275.6</td>
<td>2.3%</td>
</tr>
<tr>
<td>0.01899 M CO₂</td>
<td>315</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01899 M CO₂</td>
<td>310</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01899 M CO₂</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0.01899 M CO₂</td>
<td>302</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01899 M CO₂</td>
<td>305</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01899 M CO₂</td>
<td>316</td>
<td>310.3</td>
<td>1.9%</td>
</tr>
<tr>
<td>distilled water</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>distilled water</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1409 M OAPS</td>
<td>224</td>
<td>12.5</td>
<td>--</td>
</tr>
<tr>
<td>0.1409 M OAPS</td>
<td>225</td>
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</tr>
<tr>
<td>0.1409 M OAPS</td>
<td>225</td>
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<td>0.1409 M OAPS</td>
<td>226</td>
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<tr>
<td>0.1409 M OAPS</td>
<td>224</td>
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<tr>
<td>0.1409 M OAPS</td>
<td>223</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 8. Calibration Curve for Carbon Dioxide Analysis.
Standard solutions of 3-methoxypropylamine hydrochloride and carbon dioxide were analyzed and gave carbon dioxide values about 15% less than theoretical. Since these solutions were prepared by ordinary methods, it was expected that a little carbon dioxide would be lost. If hydrogen chloride had interfered, then the results would have been higher than expected, likely much higher in view of the 7-fold excess of hydrogen chloride over carbon dioxide.

Van Slyke Analysis. Before using the Van Slyke apparatus to analyze OAPS, it was necessary to check the method by analyzing compounds of known structure. The following three amines were selected: n-propylamine was chosen for its availability, 3-methoxypropylamine for its availability and similarity to OAPS, and bis-γ-aminopropyl ether for similarity to OAPS with the added possibility of participation by an intramolecular amine group. The mechanism for primary amine diazotization is

\[
\begin{align*}
\text{HNO}_2 & \rightleftharpoons \text{H}^+ + \text{NO}_2^- \\
\text{H}^+ + \text{HNO}_2 & \rightleftharpoons \text{H}_2\text{NO}_2^+ \\
\text{H}_2\text{NO}_2^+ + \text{NO}_2^- & \rightleftharpoons \text{N}_2\text{O}_3 + \text{H}_2\text{O}
\end{align*}
\]

and it was possible that the intermediate could undergo an internal displacement reaction, shown below for bis-γ-aminopropyl ether.

The occurrence of this process would lower the volume of nitrogen evolved.

The measurements on propylamine proved it an unsatisfactory standard. After thorough washing in the Orsat bulb, the evolved gas was
examined by ir and found to have a spectrum (nitrogen is transparent
to ir radiation) that agreed satisfactorily with the literature spec-
trum of propene. The gas also showed a non-water component by vpc,
whose 5 minute retention time on Poropak QS at 70° was believed rea-
sonable for propene.

With 3-methoxypropylamine hydrochloride good results were obtained,
as shown in Table 6. The dead volume was first determined by the weight
difference before and after filling the appropriate section (i.e., from
the liquid surface in the flask containing 12 ml of water to the first
stopcock) with distilled water. The value obtained was 9.60 ± 0.1 ml.
In the calculations the slightly different value of 9.75 ml was used
because it fit the data better. The dead volume, measured by weight
difference, would be expected to be a little low due to the mixing volume
of acetic acid and water. It can be calculated from the density of a 10%
acetic acid solution that the 1-ml volume of acetic acid makes only a
0.90-ml contribution to the solution volume. Hence the 9.60 ± 0.10 ml
becomes 9.70 ± 0.10 ml, which is in good agreement with the best-fit
value of 9.75 ml.

The diamine, bis-γ-aminopropyl ether, also gave good results once
samples of sufficient purity were obtained. Table 7 shows the results
of three analyses that were obtained on the diamine in three states of
purity.

36. 'Sadler Standard Prism Spectra,' The Sadler Research Labora-
Table 6. Van Slyke Data for Calibration and Bis-γ-aminopropyl ether Analysis using 9.00-ml Aliquots of amine solutions.

<table>
<thead>
<tr>
<th>Amine</th>
<th>meq soln</th>
<th>T (°C)</th>
<th>vp of water (mm)</th>
<th>P(mm)</th>
<th>Vobs (ml)</th>
<th>Vg (ml)</th>
<th>mmoles gas</th>
<th>purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Methoxy-propylamine, 0.06372 N</td>
<td>0.5735</td>
<td>27.4</td>
<td>27.4</td>
<td>744.6</td>
<td>24.60</td>
<td>14.85</td>
<td>0.5684</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td>0.5735</td>
<td>27.7</td>
<td>28.1</td>
<td>744.0</td>
<td>24.85</td>
<td>15.10</td>
<td>0.5763</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>0.5735</td>
<td>27.0</td>
<td>26.7</td>
<td>742.4</td>
<td>24.73</td>
<td>14.98</td>
<td>0.5729</td>
<td>99.9</td>
</tr>
<tr>
<td></td>
<td>0.5735</td>
<td>26.2</td>
<td>25.5</td>
<td>749.4</td>
<td>24.64</td>
<td>14.89</td>
<td>0.5775</td>
<td>100.7</td>
</tr>
<tr>
<td>Bis-γ-Aminopropyl ether, 0.04425 N</td>
<td>0.3982</td>
<td>23.8</td>
<td>22.1</td>
<td>740.1</td>
<td>20.04</td>
<td>10.29</td>
<td>0.3991</td>
<td>100.2</td>
</tr>
<tr>
<td></td>
<td>0.3982</td>
<td>24.0</td>
<td>22.4</td>
<td>739.4</td>
<td>19.92</td>
<td>10.17</td>
<td>0.3936</td>
<td>98.8</td>
</tr>
</tbody>
</table>

Table 7. Percent Purity of Bis-Y-APE Samples by Three Methods

<table>
<thead>
<tr>
<th>Stage of Purification</th>
<th>Van Slyke</th>
<th>Titration</th>
<th>GLPC^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>distilled</td>
<td>92.4^a</td>
<td>94.3^a</td>
<td>97.8</td>
</tr>
<tr>
<td>prep. glpc</td>
<td>96.6</td>
<td>95.1^a</td>
<td>94-95</td>
</tr>
<tr>
<td>modified prep. glpc</td>
<td>99.5^a</td>
<td>98.7^a</td>
<td>99.3</td>
</tr>
</tbody>
</table>

^aAverage of 2 or more determinations.
^b6 ft x 1/4 in Carbowax-KOH column at 160°. Area integrations by planimeter.

Thus, after distillation only, the purity was not sufficient for drawing conclusions on the diamine's behavior on treatment with nitrous acid (Table 7, line 1). At first, preparative glpc on Carbowax-KOH at 155° did not help much (Table 7, line 2), but after the chromatograph was treated with five ½-ml portions of triethylamine the method worked well (Table 7, line 3). This treatment apparently neutralized acidic sites in the chromatograph. The data on the material of highest purity clearly shows that the diamine evolves two equivalents of nitrogen on nitrous acid treatment.

37. The author is indebted to Dr. A. W. Klueppel of the Universidad de Simon Bolivar for this suggestion.
Table 8. Data for Calibration and OAPS Analysis, using 9.00-ml aliquots of amine solution.

<table>
<thead>
<tr>
<th>Amine</th>
<th>meq soln.</th>
<th>T (°C)</th>
<th>vp of water (mm)</th>
<th>P(mm)</th>
<th>V_{obs} (ml)</th>
<th>V_{s} (ml)</th>
<th>mmoles gas</th>
<th>purity (%)</th>
<th>Calculated N soln.</th>
<th>orig. soln.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (0.000 N)</td>
<td>0.000</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>9.90</td>
<td>0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>9.87</td>
<td>0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>9.88</td>
<td>0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>3-Methoxy-</td>
<td>0.5671</td>
<td>24.6</td>
<td>23.2</td>
<td>743.9</td>
<td>24.47</td>
<td>14.59</td>
<td>0.5664</td>
<td>99.9</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>propyl-</td>
<td>0.5671</td>
<td>24.8</td>
<td>23.5</td>
<td>743.9</td>
<td>24.30</td>
<td>14.42</td>
<td>0.5592</td>
<td>98.6</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>amine, 0.06301 N</td>
<td>0.5671</td>
<td>23.0</td>
<td>21.1</td>
<td>749.6</td>
<td>24.44</td>
<td>14.56</td>
<td>0.5745</td>
<td>101.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>0.5671</td>
<td>23.4</td>
<td>21.6</td>
<td>748.8</td>
<td>24.43</td>
<td>14.55</td>
<td>0.5723</td>
<td>100.9</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>OAPS</td>
<td>--</td>
<td>24.6</td>
<td>23.2</td>
<td>747.1</td>
<td>27.40</td>
<td>17.52</td>
<td>0.6832</td>
<td>--</td>
<td>0.07591</td>
<td>0.0849</td>
</tr>
<tr>
<td></td>
<td>--</td>
<td>24.4</td>
<td>22.9</td>
<td>748.8</td>
<td>24.36</td>
<td>14.48</td>
<td>0.5666</td>
<td>--</td>
<td>0.06295</td>
<td>0.0860</td>
</tr>
<tr>
<td></td>
<td>--</td>
<td>25.0</td>
<td>23.8</td>
<td>747.3</td>
<td>24.10</td>
<td>14.22</td>
<td>0.5535</td>
<td>--</td>
<td>0.06150</td>
<td>0.0835</td>
</tr>
<tr>
<td></td>
<td>--</td>
<td>24.6</td>
<td>23.2</td>
<td>747.1</td>
<td>24.33</td>
<td>14.45</td>
<td>0.5635</td>
<td>--</td>
<td>0.06261</td>
<td>0.0848</td>
</tr>
</tbody>
</table>

---

This quantity was obtained from the previous column by dividing by the factor by which the original solution was diluted for analysis. Dilution factors were 17/19, 17/19 x 9/11, 14/19, and 14/19, respectively. Average value 0.0848 ± 0.0010 N.
The same OAPS used for kinetics was analyzed in the Van Slyke apparatus. However, the apparatus had a different reaction flask, and hence, had to be recalibrated. Calibration (measuring the dead volume) was done by an improved method. The analysis was run on zero normal amine solutions; thus errors due to the mixing volume of water and acetic acid and to isopiestic migration of water vapor from the buret to the Orsat solution were eliminated. The results are shown in Table 8, lines 1-3. The dead volume thus determined was confirmed by running solutions of 3-methoxypropylamine hydrochloride (Table 8, lines 4-9). Finally OAPS was run, using samples from the solution that was analyzed by titration. To bring the volume within the range of the buret some dilution was necessary. This was done by mixing pipet-measured volumes of the solution and of water. Then the experimental normalities were corrected by the factor by which they were diluted to give the Van Slyke normality of the original solution. The average of four determinations was 0.0848 ± 0.0010 N where the tolerance given is the standard deviation.

Elemental Analysis. Elemental analysis of highest purity OAPS gave the results shown in Table 9.

**Table 9. Elemental Analysis of OAPS**

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>H</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated</td>
<td>54.11</td>
<td>9.84</td>
<td>14.02</td>
</tr>
<tr>
<td>Found</td>
<td>48.12</td>
<td>8.54</td>
<td>11.46</td>
</tr>
<tr>
<td></td>
<td>48.29</td>
<td>8.55</td>
<td>11.30</td>
</tr>
</tbody>
</table>
As in the previous case of cyanoethylsucrose, the calculated elemental composition was modified by parameters describing possible defects in the product. Five parameters were defined as follows:

- \(N\), the number of alkylated hydroxyl groups per sucrose molecule
- \(W\), the number of water molecules per sucrose molecule
- \(D\), the number of carbon dioxide molecules per sucrose molecule
- \(A\), the number of ammonia molecules lost by secondary amine formation per sucrose molecule
- \(S\), the amount of sodium chloride present, as a weight fraction.

One equation for each element analyzed may be written, where each equation is of the general form, \((\text{weight of the element present in one mole} \times 100)/\text{molecular weight}\). Complete reduction is assumed in the \(\% H\) equation.

\[
\% C = \frac{(12.011)(12 + 3N + D)(1 - S)}{\text{mw}} \times 100
\]

\[
\% H = \frac{(1.008)(22 + 7N + 2W - 3A)(1 - S)}{\text{mw}} \times 100
\]

\[
\% N = \frac{(14.007)(N - A)(1 - S)}{\text{mw}} \times 100.
\]

where \(\text{mw} = 342.30 + 57.096N + 18.015W + 44.013D - 17.031A\).

With only three equations, a unique solution for three parameters was possible. However, the parameters \(D\), \(A\), and \(S\) have been determined independently, each probably with greater certainty than they could be
determined from the elemental analyses. Hence, these three parameters were fixed at their known values and the calculated C, H, N values were fit to the experimental C, H, N values by varying N and W.

The parameter A, describing the loss of ammonia by secondary amine formation, was obtained from the results of the titration and Van Slyke analyses. All amine groups were titratable, but only primary amine groups would be detected in the Van Slyke analysis. Thus, the titration value of \(0.0865 \, \text{N}\) was the concentration of all amine groups and the Van Slyke value of \(0.0848 \, \text{N}\) was the concentration of primary amine groups. The difference, \(0.0017 \, \text{N}\), should equal the concentration of secondary amine groups or the concentration of ammonia that was lost. A is the concentration of ammonia that was lost divided by the OAPS concentration, or

\[
A = \frac{0.0017}{[\text{OAPS}]}
\]

Alternatively, the OAPS concentration can be written as \(0.0865/(N - A)\), where \((N - A)\) is the total number of amine groups per sucrose molecule. Then,

\[
A = \frac{0.0017}{0.0865/(N - A)}
\]

or

\[
A = 0.0193 \, N
\]

For carbon dioxide, a value of 38% (enough dibasic carbonic acid to protonate 38% of the amine groups) was determined previously. This value is related to D by the equation
\[
\frac{2D}{N - A} \times 100 = 38
\]

or

\[
D = 0.19 (N - A)
\]

Substitution of the A value obtained above gives

\[
D = 0.1863 N
\]

The parameter, S, for sodium chloride was calculated to be 0.0362 from the total volume of effluent collected to make the sample and the concentration of sodium chloride in the eluant.

After substituting these values for D, A, and S, the three equations become

\[
\% C = \frac{13891 + 3688.1 N}{mw}
\]

\[
\% H = \frac{2137.3 + 674.42 N + 194.30 W}{mw}
\]

\[
\% N = \frac{1323.9 N}{mw}
\]

where \( mw = 342.30 + 64.967 N + 18.015 W \).

The calculated values were fit to the experimental values by varying \( N \) and \( W \) such that the sum of the squares of the deviations in the C, H, N analyses was minimized. One parameter was held constant while the other was optimized, and vice versa, until both parameters had reached their optimum values. Thus, the values,
were obtained. The following analysis was calculated from the optimized parameters: C, 48.23; H, 8.70; and N, 11.33 (the mean deviation from the experimental values was 0.11). With the value of N determined, the values of D and A could be calculated. Using the equations given before,

\[ D = 1.33 \]
\[ A = 0.14 \]

The possibility of monosaccharide formation by hydrogenolysis instead of hydrolysis during the reduction of cyanoethylsucrose can be largely excluded for the following three reasons: (1) If the ether linkage between the rings were cleaved by hydrogenolysis, then some of the ether units in the side chains should have suffered a similar fate. However, the calculated values of N for cyanoethylsucrose and OAPS (7.36 and 7.14, respectively) indicated no significant amount of side chain cleavage. (2) In the nmr spectrum of OAPS, the low-field proton integral was 0.9. If much hydrogenolysis took place, it should be lower because in the product resulting from cleavage of the bond between the glucose ring and linking oxygen, the C-1 glucose protons would have a different chemical shift. (3) If hydrogenolysis took place, then the calculated hydrogen content would be higher. However, the calculated hydrogen analysis (8.70%) is already higher than the experimental value (8.54%).
**Kinetic Studies**

Two types of kinetic studies were carried out: rate constant as a function of pH and rate constant as a function of catalyst concentration. Kinetic runs were done as described in the Experimental Section; data from a typical run is shown in Table 10.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Start</th>
<th>Stop</th>
<th>time(min)</th>
<th>( \frac{d+h}{d} )</th>
<th>( \ln \frac{d+h}{d} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>--</td>
<td>0</td>
<td>1.10</td>
<td>0.098</td>
</tr>
<tr>
<td>2</td>
<td>1:54</td>
<td>2:11</td>
<td>17</td>
<td>1.35</td>
<td>0.299</td>
</tr>
<tr>
<td>3</td>
<td>1:53</td>
<td>2:26</td>
<td>33</td>
<td>1.67</td>
<td>0.512</td>
</tr>
<tr>
<td>4</td>
<td>1:52</td>
<td>2:39</td>
<td>47</td>
<td>1.92</td>
<td>0.653</td>
</tr>
<tr>
<td>5</td>
<td>1:30</td>
<td>2:33</td>
<td>63</td>
<td>2.42</td>
<td>0.885</td>
</tr>
<tr>
<td>6</td>
<td>1:29</td>
<td>2:47</td>
<td>78</td>
<td>2.82</td>
<td>1.038</td>
</tr>
<tr>
<td>7</td>
<td>1:28</td>
<td>3:01</td>
<td>93</td>
<td>3.36</td>
<td>1.211</td>
</tr>
</tbody>
</table>

To reduce the error from the nmr measurement, each sample was analyzed twice, optimizing the Y-gradient setting independently for each scan. Duplicate scanning was apparently worthwhile, since the members of the
pairs differed. The kinetic plot for the same data is shown in Figure 9. The rate constant is equal to the slope of the line, 1.201 x 10^{-2} \text{ min}^{-1} or 20.02 x 10^{-5} \text{ sec}^{-1}.

The nine runs used to determine the pH-rate curve are detailed in Table 11 (runs 1-9). One discrepancy should be noted between runs 4 and 9. They had essentially the same pH, but considerably different rate constants. This is believed to be caused by a loss in catalyst activity on standing in aqueous solution, since the solution used in runs 1-4 was three weeks old at the time of the kinetic runs. Thereafter fresh solutions were used. To allow roughly for the loss of activity, the rate constants for runs 1-4 were adjusted upward by 9.2%, the difference between runs 4 and 9. In the pH-rate curve, shown in Figure 10, the solid circles indicate observed points and the dotted circles indicate corrected points. Differences in catalyst concentration, ranging over only 1%, were ignored.

The effect of catalyst concentration of the rate constant is shown in Figure 11. The curve shown contains four points from Table 11 (runs 10-13), one point read from the Figure 10 graph \((k_p = 20.0 \times 10^{-5} \text{ sec}^{-1}\) for 0.086 N OAPS at pH 8.5), and a calculated zero point. The rate constant at zero catalyst concentration was calculated for an aqueous sodium hydroxide solution of pH 8.5. The observed pseudo-first order rate constant, \(k_p\), would be

\[
k_p = k_h[OH^-] + k_w[\text{H}_2\text{O}]
\]
where $k_h$ is the catalytic constant for hydroxide ion and $k_w$ is the catalytic constant for water. From the $k_h$ and $k_w$ values of $3.1 \times 10^{-2}$ $M^{-1} \text{sec}^{-1}$ and $3.3 \times 10^{-10}$ $M^{-1} \text{sec}^{-1}$, respectively, $k_p$ for zero catalyst concentration was calculated to be $0.021 \times 10^{-5}$ $\text{sec}^{-1}$, which is indistinguishable from zero on the graph.
Figure 9. Typical Kinetic Plot.
Table 11. Kinetic Data

<table>
<thead>
<tr>
<th>Run</th>
<th>Catalyst</th>
<th>pH</th>
<th>conc, N</th>
<th>$k \times 10^5$(sec$^{-1}$)</th>
<th>number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OAPS</td>
<td>9.82</td>
<td>0.0865$^a$</td>
<td>8.55</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>OAPS</td>
<td>9.33</td>
<td>0.0863$^a$</td>
<td>14.20</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>OAPS</td>
<td>9.04</td>
<td>0.0862$^a$</td>
<td>16.22</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>OAPS</td>
<td>8.71</td>
<td>0.0861$^a$</td>
<td>17.60</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>OAPS</td>
<td>8.39</td>
<td>0.0858</td>
<td>20.02</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>OAPS</td>
<td>7.98</td>
<td>0.0856</td>
<td>17.50</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>OAPS</td>
<td>7.64</td>
<td>0.0855</td>
<td>13.01</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>OAPS</td>
<td>7.25</td>
<td>0.0854</td>
<td>7.11</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>OAPS</td>
<td>8.68</td>
<td>0.0859</td>
<td>19.22</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>OAPS</td>
<td>8.53</td>
<td>0.0959</td>
<td>21.5</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>OAPS</td>
<td>8.62</td>
<td>0.0482</td>
<td>15.8</td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td>OAPS</td>
<td>8.42</td>
<td>0.0241</td>
<td>7.38</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>OAPS</td>
<td>8.54</td>
<td>0.189</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>MeOCH$_2$-CH$_2$NH$_2$</td>
<td>9.09</td>
<td>0.086</td>
<td>1.60</td>
<td>6</td>
</tr>
</tbody>
</table>

$^a$Solution was three weeks old at time of kinetic run.
Figure 10. pH-Rate Profile.
Figure 11. The Effect of Catalyst Concentration on the Rate Constant.
DISCUSSION

Characterization of the Catalyst

The Structure and Purity. The values of the parameters describing the structure and purity were determined previously to be

\[ N = 7.14 \]
\[ W = 1.55 \]
\[ D = 1.33 \]
\[ A = 0.14 \]
\[ s = 0.0362 \]

Two of these parameters, D and N, merit additional discussion.

First, the parameter D, for carbon dioxide, was determined with good precision by vpc. The value of the parameter, however, does not shed any light on what kinds of compounds were formed between carbon dioxide and the catalyst. Doubtless, in an aqueous solution of the catalyst, carbon dioxide existed as ammonium carbonates and ammonium

\[
\begin{align*}
R-\text{NH}_2 + \text{CO}_2 + \text{H}_2\text{O} &\rightleftharpoons R-\text{NH}_3^+ \text{HCO}_3^- \\
\text{an ammonium bicarbonate} \\
R-\text{NH}_3^+ \text{HCO}_3^- + \text{RNH}_2 &\rightleftharpoons (R\text{NH}_3^+)_2 \text{CO}_3^{--} \\
\text{an ammonium carbonate}
\end{align*}
\]

81
bicarbonates. However, in dry form, it seems likely that ammonium carbamates were present. No water is required to form an ammonium carbamate from carbon dioxide and an amine.

\[
\text{R-NH}_2 + \text{CO}_2 \rightarrow [\text{R-NH-C-OH}] \quad \text{R'NH}_2 \rightarrow \text{RNH-C-O}^- + \text{H}_3\text{NR'}
\]

Ammonium carbamates are suggested, but not required, by the values of \(W\) and \(D\), namely 1.55 and 1.33. If all carbon dioxide were as carbonate and bicarbonate salts, this would account for 1.33 of the value of \(W\); about another 0.1 is attributed to water of hydrolysis. Thus, only about 0.1 would remain for free water; this value, corresponding to 0.2% by weight, seems remarkably low for a hygroscopic compound. Although carbamic acids themselves are unstable, their salts are stable in dry form. Ammonium carbamate, for example, is a stable salt when dry, but rapidly decomposes when dissolved in water. Analogously, if present in the catalyst, ammonium carbamates should have rapidly formed carbonic acid salts when the catalyst was dissolved in water.

The parameter, \(N\), for the number of alkylated sucrose hydroxyl groups, was determined separately for cyanoethylsucrose and OAPS. The two values obtained (7.36 and 7.14, respectively) may not differ by much more than their uncertainties, but the observed difference is in the expected direction. The two values would be expected to differ for two reasons: (1) The monosaccharide components of cyanoethylsucrose

should be more highly cyanoethylated than the sucrose component, assum­ing hydrolysis took place with cyanoethylation. After reduction, these more highly alkylated components were removed by gel filtration chroma­tography. (2) The review by Butskus suggests the possibility of small amounts of decyanoethylatation during the reduction. For example, hydrogenolysis of the C-O bond is reported occurring to various extents in the Raney nickel reductions of β-alkoxypropionitriles, although all these reductions were done under substantially more severe conditions (50-250 atm and 110-150°). Another possibility is the transfer of cyanoethyl groups from sucrose to ammonia during the reduc­tion. This has been reported in high yield for β-alkoxypropionitriles at 50-150° and 20-200 atm of ammonia pressure in the presence of Raney catalysts. Hence, the observed difference between the two N's seems to be a reasonable one.

The Basicity of the Catalyst. The basicity of the catalyst is an interesting topic. Unlike the conjugate acid of a monoamine, whose acidity constant is nearly a constant, the apparent acidity constant of protonated OAPS varied over a factor of about 1000, according to the state of protonation of the molecule. Thus, the unprotonated catalyst is about as strong a base as would be expected for an alkyl amine, but

42. German Patent 1,003,740; Chem. Abstr., 52, 18867h (1959).
it becomes a weaker and weaker base as the amount of protonation increases. This is reasonable, since the positive charge(s) already on the molecule would destabilize yet another positive charge on the molecule.

The value of the apparent acidity constant can be calculated for points on the titration curve from the equation

\[ K_{\text{app}} = \frac{[\text{Am}][\text{H}^+]}{[\text{AmH}^+]} \]

where \([\text{Am}], [\text{AmH}^+],\) and \([\text{H}^+]\) are the concentrations of the free amine groups, protonated amine groups, and hydrogen ions. The hydrogen ion concentrations were calculated for points along the curve from pH measurements and the activity coefficient. The sum of the amine and protonated amine concentrations was known from the equivalence point of the titration. Between pH 10.2 and 8.6 (30% and 76% protonation) the carbon dioxide existed as carbonate and bicarbonate ions, with only negligible amounts of carbonic acid. Thus, in this section of the curve, there were three acids that protonated amine groups (carbonic acid, bicarbonate ion, and hydrochloric acid) and the concentration of protonated amine groups was calculated as

\[ [\text{RNH}_3^+] = 2[\text{CO}_3^{2-}] + [\text{HCO}_3^-] + [\text{Cl}^-] \]

The carbonate and bicarbonate ion concentrations were calculated from their sum (known from previous experiments) and their ratio (calculated from the second \(pK_a\) of carbonic acid and the observed pH). The chloride
ion concentration was simply the concentration of hydrochloric acid that had been added. The concentration of free amine groups was calculated as the difference between the total amine and protonated amine group concentrations. In this manner, the apparent $pK_a$ was calculated as a function of the state of protonation of the molecule, with results shown in Figure 12. The titration data from which the Figure 12 points were calculated was given with the titration curve on p 58. It should be noticed that the curve begins at 30% protonation; this initial protonation is due to the carbon dioxide present in the sample.

The second $pK_a$ of carbonic acid was calculated from the value of 10.25 given for $35^\circ$ at zero ionic strength. It was necessary to correct for ionic strength effects because the presence of the divalent carbonate ion introduced into the equilibrium constant expression the fourth power of the activity coefficient of a mono-charged ion, which could not be cancelled by a like power since there were no other divalent ions. The zero ionic strength $K_a$ is the thermodynamic $K_a$, or

$$K_{th} = \frac{a_{H^+} a_{CO_3^-}}{a_{HCO_3^-}}$$

where the $a$'s are the activities of the species. However, the appropriate $K$ should be written in terms of hydrogen ion activity and carbonate and bicarbonate concentrations.

---

Figure 12. The Effect of Protonation on the pK\textsubscript{app} of OAPS.
\[ K_{\text{obs}} = \frac{a_{H^+} [CO_3^{2-}]}{[HCO_3^-]} \]

Since the activity coefficient for a divalent ion equals the fourth power of that for a monovalent ion, and since the concentration equals the ion's activity divided by its activity coefficient, the observed equilibrium constant is

\[ K_{\text{obs}} = \frac{a_{H^+} a_{CO_3^{2-}}}{a_{HCO_3^-}} \frac{\gamma}{\gamma^4} = \frac{K_{\text{th}}}{\gamma^3} \]

where \( \gamma \) is the activity coefficient for a monovalent ion, calculated from the Davies equation,

\[ \log \gamma = -0.519 \left( \frac{\sqrt{I}}{1 + \sqrt{I}} - 0.2 I \right) \]

where \( I \) is the ionic strength of the solution. The ionic strengths were calculated assuming the amine groups were far enough apart to behave as if they were in separate molecules. From an initial ionic strength of 0.036, a \( \log \gamma \) of -0.079 was calculated. Then \( K_{\text{obs}} \) was straightforwardly calculated as follows:

\[ K_{\text{obs}} = \frac{K_{\text{th}}}{\gamma^3} \]

or

\[ \log K_{\text{obs}} = \log K_{\text{th}} - \log \gamma^3 \]

Thus,
\[
pK_{\text{obs}} = pK_{\text{th}} + 3 \log \gamma
\]
\[
= 10.250 + 3(-0.079)
\]
\[
= 10.012
\]

The ionic strength changed through the titration and the value of \( pK_{\text{obs}} \) was adjusted accordingly in calculating the points of Figure 12. Thus, over the first 12 points (from 30% to 78% protonation), the ionic strength changed from 0.036 to 0.064 and \( pK_{\text{obs}} \) changed from 10.012 to 9.955. The value of the ionic strength affected the value of \( K_{\text{obs}} \), which affected the calculated concentrations of the ions, which determined the ionic strength. Thus, the ionic strength was obtained by an iterative procedure; with a well-guessed starting value of \( I \), one iteration usually sufficed.

After 78% protonation, a similar procedure was used, except the carbonate ion concentration was negligible and the carbon dioxide was treated as entirely bicarbonate ion and carbonic acid. The apparent first \( pK_a \) of carbonic acid of 6.309 at 35\(^\circ\) and zero ionic strength was used to calculate the bicarbonate--carbonic acid ratio. Again, the literature \( K \) was a thermodynamic \( K \), or

\[
K_{\text{th}} = \frac{a_{H^+}a_{HCO_3^-}}{a_{H_2CO_3}}
\]

and the appropriate \( K \) should be written in terms of hydrogen ion activity and bicarbonate ion and carbonic acid concentrations,
Since the activity coefficient for a neutral species is assumed to be unity, $K_{obs}$ and $K_{th}$ differ by a factor of $\gamma$. As before, the pK_a's of carbonic acid used in the calculations were corrected for ionic strength effects. Thus, from the calculated amine and protonated amine group concentrations and from the observed hydrogen ion concentration, pK_{app}'s for the catalyst were calculated between 78% and 99% protonation. It was assumed in the calculations that no carbon dioxide escaped from solution; if some were lost, the calculated pK_{app}'s would be higher toward the end of the titration. The loss of much carbon dioxide seems unlikely because the maximum CO_2 concentration obtained was only about half of the amount soluble. The last point, at 100% protonation, was calculated from the pH at the end point from the acidity constant equation, by assuming the free amine and hydrogen ion concentrations to be equal.

The Equilibrium Constant for Imine Formation. The imine formation constant, $K_{Im}$, will be defined as

$$K_{Im} = \frac{[Im]}{[Am][Ald]}$$

where [Im], [Am], and [Ald] are the concentrations (in equivalents per liter) of imine groups, free amine groups, and aldehyde (free and
hydrated), respectively. Like the acidity constant for OAPS, $K_{Im}$ for OAPS is not a true constant because its value changes according to the basicity, i.e., the degree of protonation of the catalyst molecule. Imine formation constants for OAPS were obtained in two independent ways—-from pH measurements (where $K$ was based on the disappearance of amine) and from uv measurements (where $K$ was based on the disappearance of aldehyde). An equilibrium constant calculated from these kinds of data is really the sum of the equilibrium constants for formation of imine, carbinolamine, and probably cyclic aminals. However, the equilibrium amount of carbinolamine (hemiaminal) is only about 3% of the amount of imine and aminals are believed to be minor components also. Hence, $K_{Im}$ comprises the majority of the observed $K$ and for most purposes of the following discussion no distinction between the two $K$'s need be made.

The uv measurements were made at 285 nm, the maximum for isobutyraldehyde. The aldehyde absorbance was measured first in water and then in catalyst solution; that absorbance difference was converted into the amount of aldehyde that disappeared, after allowing for the absorbance of the catalyst. The concentrations of free and protonated amine groups, before addition of the aldehyde, were calculated from titration curve data. Since the pH dropped when aldehyde was added, some carbonate was converted to bicarbonate and thereby the average OAPS molecule had fewer positive charges. In other words, the equilibrium

was shifted toward the right by removal of amine groups through imine formation. Of course, amine groups protonated with hydrochloric acid could not be affected by the pH decrease. Correction for the change in the state of protonation brought about by the pH drop was made by recalculating the carbonate and bicarbonate concentrations from the pH at imine equilibrium and modifying the amounts of amine and protonated amine accordingly. The imine concentration was taken as equal to the aldehyde concentration decrease and the amine concentration decrease was taken as equal to the imine concentration less the increase in bicarbonate concentration. Thus, all required concentrations were known and $K_{\text{Im}}$ could be calculated. The data from two UV experiments are shown in Table 12. The second experiment is considered more reliable since a larger number of measurements was made and a more accurate aldehyde extinction coefficient was obtained (first experiment, $\varepsilon = 14.0 \text{ M}^{-1} \text{ cm}^{-1}$; second experiment, $\varepsilon = 14.9 \text{ M}^{-1} \text{ cm}^{-1}$; lit. $^{46,47,48} \varepsilon = 14.8, 14.6, \text{ and } 15.3 \text{ M}^{-1} \text{ cm}^{-1}$).

The $pK_{\text{app}}$'s of the catalyst at imine equilibrium can be obtained in two ways. If it is assumed that the catalyst basicity is entirely determined by its state of protonation, then the $pK_{\text{app}}$'s at imine equilibrium are only a few hundredths higher than the initial $pK_{\text{app}}$'s. This

47. J. Hine and K. W. Narducy, ibid., 95, 3362 (1973).
Table 12. $K_{\text{Im}}$ from uv Measurements

<table>
<thead>
<tr>
<th>[OAPS], N</th>
<th>[IBA], N</th>
<th>pH</th>
<th>Abs.</th>
<th>% Protonation</th>
<th>$pK_{\text{app}}$</th>
<th>$K_{\text{Im}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.0177</td>
<td>--</td>
<td>0.247</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>0.024</td>
<td>0.00</td>
<td>10.20$^a$</td>
<td>0.045</td>
<td>30</td>
<td>9.75$^b$</td>
<td></td>
</tr>
<tr>
<td>0.024</td>
<td>0.0177</td>
<td>10.1$^a$</td>
<td>0.206</td>
<td>28-29$^a$</td>
<td>9.7-9.8$^{a,c}$</td>
<td>46</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0546</td>
<td>--</td>
<td>0.816</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>0.087</td>
<td>0.00</td>
<td>8.93</td>
<td>0.167</td>
<td>62</td>
<td>9.04$^b$</td>
<td></td>
</tr>
<tr>
<td>0.087</td>
<td>0.0546</td>
<td>8.39</td>
<td>0.754</td>
<td>60.5</td>
<td>8.74$^c$</td>
<td>21</td>
</tr>
</tbody>
</table>

$^a$Approximate values, estimated or calculated from estimates.

$^b$*$pK_{\text{app}}$'s obtained from the Figure 12 graph and the calculated extent of protonation.

$^c*pK_{\text{app}}$'s calculated from the $[H^+]$, [Am], and [AmH$^+$] at imine equilibrium.
calculated increase in $pK_{app}$ comes from the conversion of some carbonate to bicarbonate, leaving the catalyst slightly less protonated and therefore slightly more basic. In the case of the second uv experiment, the initial $pK_{app}$ was calculated to be 9.04, the imine equilibrium $pK_{app}$, 9.06. However, the $pK_{app}$ at imine equilibrium can be calculated a better way, from the pH and the calculated $[Am]$ and $[AmH^+]$ at imine equilibrium. In the case of the same experiment, this method gave a $pK_{app}$ of 8.74, which is believed to be a more accurate value because it does not require the assumption that imine formation has no effect on the basicity of the other amine groups. This example also provides a comparison of the effects of imination and protonation. Starting at 60.5% protonation, the calculated $pK_{app}$ dropped from 9.06 to 8.74 when 17.6% of the amine groups formed imines. Adding the same amount of protonation to the 60.5% protonated catalyst would drop the $pK_{app}$ from 9.06 to 8.67. Hence, in this range, imination is about 80% as effective as protonation in reducing the basicity of the catalyst.

These values of $K_{Im}$ and their $pK_{app}$'s are shown graphically in the Figure 13 plot. The circled points (for primary amines of the type RCH$_2$NH$_2$) represent equilibrium constants determined by uv measurements, pH measurements, or both. $^{48,49}$ It should be noticed that the points lie significantly below the best line through the points for nine other primary amines of the type RCH$_2$NH$_2$. The deviation (an average of 0.11 log units) can be ascribed to several factors. First,
Figure 13. Log-log Plot of the Equilibrium Constant for Imine Formation vs the $pK_a$ of the Protonated Amine.
possibly the $K_{im}'s$ for OAPS actually are slightly lower than predicted for their basicity for reasons of steric crowding. The points for isopropylamine and tertiary butyl amine, which have much less bulk than OAPS but have it much nearer the amino group, also lie significantly below the line in Figure 13.\textsuperscript{48,49} Alternatively, interference from the tail of the imine absorption ($\lambda_{max} 230$ nm, $\varepsilon = 138$ $M^{-1} \text{cm}^{-1}$, for the imine of isobutyraldehyde and 3-methoxypropylamine\textsuperscript{49}) would cause the calculated amount of aldehyde present at imine equilibrium to be high, thus making $K_{im}$ low. For this reason, the values obtained from uv measurements should be regarded as minimum values for the $K_{im}'s$. Also absorbance from an impurity (the catalyst did have considerably more absorbance than could be attributed to its amine groups) that increased as the pH fell would cause erroneously low values of $K_{im}$.

Calculation of $K_{im}'s$ from pH data was desirable to confirm or modify $K_{im}'s$ obtained from the uv data. When aldehyde is added to an amine buffer, the pH decreases; this decrease occurs because amine bases are transformed into imines, which are of negligible basicity. The equilibrium constants were obtained by first calculating the concentrations of free and protonated amine groups present initially. Then, from the pH at imine equilibrium, the amount of carbonate changed to bicarbonate was calculated, giving a new state of protonation, a new $pK_{app}$, and a new protonated amine concentration. Thus, from the $[\text{AmH}^+]$, pH, and $pK_{app}$ at imine equilibrium, the free amine concentration

\textsuperscript{50} The $pK_{app}$'s at imine equilibrium were estimated empirically, as described later.
was calculated. The difference between this free amine concentration and the initial free amine concentration (allowing for the change of carbonate to bicarbonate) was taken as the imine concentration and the initial aldehyde concentration must have decreased by the same amount. Hence, [Im], [Am], and [Ald] were known and $K_{Im}$ could be computed. The pH data and five calculated $K_{Im}$'s with corresponding $pK_{app}$'s are shown in Table 13. The same five points are shown on the log-log plot in Figure 13.

Table 13. pH Data and Calculated Values of $K_{Im}$ and $pK_{app}$ in Water at 35°.

<table>
<thead>
<tr>
<th>pH of amine buffer</th>
<th>pH after introduction of aldehyde</th>
<th>$pK_{app}$</th>
<th>$K_{Im} (M^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.53</td>
<td>9.04</td>
<td>9.12</td>
<td>36</td>
</tr>
<tr>
<td>9.24</td>
<td>8.71</td>
<td>8.94</td>
<td>35</td>
</tr>
<tr>
<td>9.21</td>
<td>8.68</td>
<td>8.93</td>
<td>36</td>
</tr>
<tr>
<td>8.90</td>
<td>8.39</td>
<td>8.79</td>
<td>25</td>
</tr>
<tr>
<td>8.48</td>
<td>7.98</td>
<td>8.62</td>
<td>24</td>
</tr>
</tbody>
</table>

---

*Total amine concentration. 0.086 M.

*Total isobutyraldehyde concentration, 0.055 M

*Estimated as described in the text.
Calculating the pK_{app}'s according only to the protonation on the catalyst molecule gave unreasonably high K_{Im} values, an average of 0.45 log units above the Figure 13 line. There is no reason to expect OAPS to complex the aldehyde that strongly. The reason for the high calculated values is the overestimated pK_{app}; this gave a free amine concentration too low, an imine concentration too high, and an aldehyde concentration too low. All the errors tended to make the calculated K_{Im} too large.

Instead, the pK_{app} values at imine equilibrium were obtained by assuming that the pK_{app} of the catalyst molecule depends only on the pH of the solution. This is qualitatively reasonable because anything that lowers the pH of the solution, be it protons or aldehyde, should also lower the pK_{app} because the catalyst must be more highly protonated at the lower pH. In the one case where both uv and pH data were available (0.086 N OAPS, 0.0546 M aldehyde, pH = 8.39) a pK_{app} of 8.79 was calculated from the pH alone (by using titration data to calculate the percent protonation and reading the corresponding pK_{app} from the Figure 12 graph); this agreed reasonably well with the value of 8.74 calculated from the [H^+], [Am], and [AmH^+] at imine equilibrium. The pK_{app} values calculated by this empirical method have considerable uncertainty, but nevertheless are probably closer to the true values than are the higher pK_{app}'s, obtained by a method that ignored the effect of imination on the pK_{app} of the remaining amine groups. These K_{Im} values are shown in the Figure 13 plot, with uncertainties corresponding to ± 0.1 uncertainty in pK_{app}. 
It is reasonable that the $pK_{\text{app}}$ of the amine groups remaining at imine equilibrium should be lower than indicated by the amount of protonation on the molecule. If the generalization that an $sp^2$ atom is more electronegative than an $sp^3$ atom is applied to nitrogen, the $\delta^+ \delta^-$ $CH_2N$ bond should have a larger dipole after imination. Since the side-chains are generally directed away from the center of the molecule and since the hydrocarbon segments prefer the extended conformation, it seems that most of the dipoles would be oriented with their positive ends closer to amine groups. Thus, the average amine group should experience a greater net positive charge after imination and, hence, be a weaker base. Also, the amines in a partly iminated OAPS molecule may be weaker bases from the introduction of the hydrocarbon group of the aldehyde. The $\equiv CH-CH(CH_3)_2$ group is much less polar than the water it replaced and the resulting decrease in the polarity of the environment may further reduce the basicity of the amine groups. Also, it could be sufficiently crowded around some amine groups for steric hindrance to solvation to make them weaker bases. Assuming a $K_{\text{Im}}$ on the Figure 13 line, typically (for the conditions under which $K_{\text{Im}}$ measurements were made) each catalyst molecule at imine equilibrium would have complexed about two isobutyaldehyde molecules; this is a considerable change in the structure of the catalyst.

Another complication in these measurements comes from the formation of large-ring aminals to an unknown extent. The formation of heterocycles occurs between polyethylenimine and isobutyaldehyde to a large
extent, but the reaction is expected to be relatively favorable in that case owing to formation of five-membered imidazolidines. With no opportunity for a ring smaller than 13 members, considerably less cyclic aminal should be formed between OAPS and isobutyaldehyde. However, only a small amount is necessary to cause erroneously high values of $K_{\text{Im}}$ calculated from pH measurements. For a qualitative argument, the basicity of the secondary amines formed may be neglected, since their $pK_a$'s should be about 0.5 units lower than the primary amines from which they were formed; also, protonation on one nitrogen of an aminal would make the other nitrogen a weaker base yet.

Then the free amine concentration would be correctly calculated from the pH, but the imine concentration would be overestimated and the aldehyde concentration would be underestimated since aminal formation is of different stoichiometry from imine formation. Both errors operate in the same direction, causing $K_{\text{Im}}$ to be overestimated. It was calculated that formation of 10% aminal (90% imine) would reduce the portion of the observed $K$ due to imine by about 0.1 log units.

51. Ethylenediamine is 0.53 $pK$ units more basic than its imidazolidine with isobutyaldehyde.
The same assumption affects the uv calculations much less, causing a drop of only 0.01 log units in the value of the part of the observed $K$ due to imine formation.

There are several complications in measuring the $K_{im}$ for OAPS. From the available data, it seems a reasonable approximation to the true $K_{im}$ values would be those on the line of Figure 13.

**The Mechanism**

**pH-Rate Profile.** The variation of the rate constant as a function of pH is shown in Figure 10, p 79. A bell-shaped curve with its maximum approximately at the pH equal to the $pK_a$ of the conjugate acid of the amine was obtained. This type of curve is required by the mechanism in which the rate controlling step is the deuteration of the iminium ion by the free amine.

\[
\text{:NH}_2\text{R} + \text{D} \quad \text{CH}_3 \quad \text{C} \quad \text{CH}_3 \quad \text{NHR} \quad \text{C} \quad \text{NH}_{2}\text{R} + \text{D} \quad \text{CH}_3 \quad \text{C} \quad \text{NH}_{2}\text{R}
\]

The rate of this step is

\[
v = k [\text{HImD}^+] [\text{Am}]
\]

where $[\text{HImD}^+]$ is the concentration of the protonated deuterium-substituted imine and $[\text{Am}]$ is the concentration of the free amine. By combining the equations for the formation constant of the imine, the acidity
constant of the protonated imine, and the acidity constant of the protonated amine,

\[ K_{\text{Im}} = \frac{[\text{Im}]}{[\text{Ald}][\text{Am}]} \]

\[ K_{\text{ImH}} = \frac{[\text{Im}][H^+]}{[\text{ImH}^+]} \]

(where \([\text{Im}]\) and \([\text{ImH}^+]\) are the concentrations of the imine and protonated imine with unspecified isotopic substitution)

\[ K_{\text{AmH}} = \frac{[\text{Am}][H^+]}{[\text{AmH}^+]} \]

the overall equilibrium constant for the formation of protonated imine from aldehyde and protonated amine was obtained.

\[ \frac{K_{\text{Im}} K_{\text{AmH}}}{K_{\text{ImH}}} = \frac{[\text{ImH}^+]}{[\text{Ald}][\text{AmH}^+]} \]

Substituting this into the rate equation gives

\[ \nu = \frac{K_{\text{Im}} K_{\text{AmH}}}{K_{\text{ImH}}} k [\text{Ald}][\text{AmH}^+][\text{Am}] \]

With the rate equation in this form, it is apparent that the rate of this step should be maximized when the product \([\text{AmH}^+][\text{Am}]\) is maximized, assuming \([\text{Am}^+] + [\text{AmH}^+]\), \([\text{Ald}]\), \(K_{\text{Im}}\), \(K_{\text{AmH}}\), \(K_{\text{ImH}}\), and \(k\) are constants. This assumption will be discussed on p 103. If their sum is fixed, the product of \([\text{AmH}^+]\) and \([\text{Am}]\) will be maximized when they are
equal. This is readily shown by writing an equation for the product of two numbers whose sum is a constant, c,

\[ y = (x)(c - x) = cx - x^2 \]

Equating the first derivative to zero shows that the maximum value of the product occurs when the two numbers are equal

\[ c - 2x = 0 \]

\[ x = \frac{c}{2} \quad \text{or} \quad x = c - x \]

When the amine and protonated amine concentrations are equal, the pH must equal the pK_a of the amine. The rate decreases on the acidic side of the maximum because the concentration of deuterating bases decreases. The rate decreases on the basic side of the maximum because there are not as many imines in the protonated state. Thus, a maximum where the pH equals the pK_a is required by the mechanism of free amine attack on iminium ions. The distinct maximum observed in this case is strong evidence for this mechanism. Furthermore, the fact that the maximum is very prominent is evidence in favor of a bifunctional mechanism. If the competing mechanisms of free amine attack on aldehyde and hydroxide attack dominated sufficiently, the maximum would be largely obscured since the rates of both competing pathways should increase smoothly with increasing pH. The pH maximum is associated only with the amine-attack-on-iminium-ion pathway and the distinctness of the maximum indicates the dominance of that pathway.
The assumption that ([Am] + [AmH⁺]) and [Ald] are constants is not entirely correct. The amount of imine formed is proportional to the amount of free amine present, which decreases with pH due to protonation. Thus, at a lower pH, there would be less imine and more [Ald] and ([Am] + [AmH⁺]). The increase in [Ald] and ([Am] + [AmH⁺]) would tend to shift the maximum to a pH lower than the pKₐ. These statements apply only to that portion of the exchange that proceeds by free amine attack on iminium ions. The other mechanisms that contribute to the total observed rate (amine attack on free aldehyde and hydroxide ion attack on free aldehyde and iminium ion) would tend to shift the maximum to higher pH since the rate by each of these mechanisms increases smoothly with pH in the buffer range of the amine. It is ordinarily expected that all these effects roughly cancel and the maximum is observed approximately at the pH equal to the pKₐ.

The maximum in the kp vs pH plot (Figure 10) occurred at pH 8.4-8.5, which was noticeably different from the pKₐ,app of the catalyst under those conditions, namely 8.74. The difference of about 0.4 pH units could be caused by the factors described above or could be caused by an additional complication with OAPS—the fact that K_{Im}, K_{AmH}, K_{ImH}, and k are not constants. From the slope of the Brønsted plot to be shown in Figure 14 (slope = -0.40), the quantity log
\[
\frac{K_{Im} K_{AmH}}{K_{ImH}} k'_{py}
\]
was predicted to decrease with increasing pKₐ of the amine. The plot predicts the reactivity of different imines with a constant

52. This difference is composed of 0.3 (for the difference between the measured pH and pKₐ,app) and 0.10 for log γ.
base, pyridine. The rate constant for attack of free amine on the iminium ion, $k'_{\text{am}}$, should increase with increasing $pK_a$ of the attacking base, according to the Brønsted plot of $\log \left( \frac{K_{\text{imH}} K_{\text{amH}}}{K_{\text{imH}} k'_B} \right)$ vs $pK_a$ ($\text{slope} = 0.42$). To the extent that the absolute values of the slopes are equal, these two factors offset each other. Thus, the difference between the optimum pH and $pK_{\text{app}}$ is probably due to the increased aldehyde and total amine concentrations at lower pH.

The Effect of Catalyst Concentration on Rate. The dependence of the rate constant on the catalyst concentration is shown in Figure 11, p 80. The curve starts upward from the origin, but has leveled off considerably at a catalyst concentration of 0.2 N. There was not sufficient catalyst available to conduct runs at higher concentrations, but from the direction of curvature and from analogy to the same kind of curve for polyethylenimine, the curve should level off at higher concentration.

A rate constant vs catalyst concentration curve that levels off is good evidence for the bifunctional mechanism. If the catalysis were monofunctional (i.e., a base in another molecule deuterated the protonated imine), then the rate constant should increase monotonically with catalyst concentration. This was demonstrated with N,N-dimethylethylene-diamine, in which there is catalysis by the monofunctional mechanism only, within the limits of detection. On the other hand, with the bi-

53. This plot will be discussed in greater detail on p 117.

functional mechanism the curve is expected to level off because nearly all the exchange occurs through the complex. If there is enough catalyst present to convert all the aldehyde to complex, then additional catalyst should not increase the rate. The maximum rate constant obtainable can be calculated from the data on kinetic run number 5 from Table 11. UV data indicated that 28% of the aldehyde was complexed. Hence, if all the aldehyde were complexed, under the same conditions, the rate constant would be \( k = (20.0 \times 10^{-5})/0.28 = 71 \times 10^{-5} \text{ sec}^{-1} \).

Comparison with Other Catalysts. Further support for the bifunctional mechanism comes from comparison with other catalysts. Several factors must be considered in addition to just the rate constants: the catalyst concentration, the solvent, the temperature, the pH of the solution, the difference between the solution pH and the optimum pH for that catalyst, the basicities of the amines removing the deuteron, the reactivities of the iminium ions, the contribution of catalysis by hydroxide and other bases if present, the equilibrium constant for imine formation, etc. How many of these factors must be taken into account depends on the purpose of the comparison and the accuracy required. In some comparisons, conceivably, none of the factors should be considered, e.g., a purely pragmatic consideration of catalysis in an industrial process, where the reaction rate is the only fact of importance (assuming the product purity and operating costs were the same). However, OAPS was prepared for a mechanistic study, and the details of the mechanism, especially the rate enhancement due to the proximity effect, were of prime importance.
Hence, all of these factors should be considered in a detailed comparison of OAPS catalysis with monofunctional amine catalysis. Such a comparison will be made in the next section, beginning on p 108.

First, some evidence in favor of a bifunctional mechanism can be gleaned by comparison with polyethylenimine. The polymer of molecular weight 1800 at a concentration of 0.104 M, with 0.053 M isobutyraldehyde at 35°, gave an observed first-order rate constant of $7.0 \times 10^{-5}$ sec$^{-1}$ at pH 8.5. For comparison, 0.096 N OAPS gave an observed first-order rate constant of $21.5 \times 10^{-5}$ sec$^{-1}$ with the same pH, temperature, and aldehyde concentration. Thus, OAPS gave an observed rate constant over three times as large. The pH of 8.5 was optimal for OAPS, but about 0.5 units too basic for polyethylenimine; the non-optimal pH would make the polymer's reaction about 10% slower, but this is largely offset by the difference in concentration of 8%. Thus, a factor of three is a reasonable estimate of the difference in catalytic efficiency under these conditions. Since the polymer has been established to be a bifunctional catalyst, it seems likely that OAPS also acts bifunctionally.

A considerable amount of study has been done on methylamine catalysis. It seems the best comparison would be made with data for a run at pH near its $pK_a$ and a concentration as close as possible to 0.1 N. The best fit to this description is a kinetic run with 0.237 N methylamine, 0.050 M isobutyraldehyde, at pH 10.58 and 35°. The observed pseudo first-order rate constant was $5.49 \times 10^{-5}$ sec$^{-1}$. The correction

for the pH being 0.13 units higher than optimum is a very small one; the product of \([\text{Am}]\) and \([\text{AmH}^+]\) in this run was only 2.3% less than the product if the total amine were divided equally between free and protonated forms. Since \(k_p\) was roughly proportional to the total amine concentration for \(N,N\)-dimethylethylenediamine (a monofunctional catalyst) at a given pH, it was assumed the methylamine-catalyzed rate could be predicted at a lower concentration by the same relationship. Thus, the methylamine rate constant was estimated to be \(2.0 \times 10^{-5} \text{ sec}^{-1}\) for 0.086 N methylamine at pH 10.58. This may be compared to the OAPS rate constant of \(20.0 \times 10^{-5} \text{ sec}^{-1}\) for 0.086 N catalyst at pH 8.39 (essentially the pH\(_{\text{max}}\) for OAPS). It is seen that the rate constant for OAPS is 10 times as large in this comparison. This ratio is in qualitative agreement with bifunctional catalysis for OAPS. However, it is not an accurate measure of the rate enhancement due to bifunctional catalysis because too many differences were ignored. For example, the 150-fold greater hydroxide ion concentration in the methylamine run would cause a 150-fold larger contribution from hydroxide catalysis to the methylamine rate constant.

Another useful model compound is 2-methoxyethylamine; it resembles OAPS closely, both in steric bulk around the amine group and in basicity with a \(pK_a\) of 9.09 at 35°. A kinetic run with 0.086 N 2-methoxyethylamine at pH 9.09 gave a \(k_p\) of \(1.60 \times 10^{-5} \text{ sec}^{-1}\). This compares with a \(k_p\) of \(20.0 \times 10^{-5} \text{ sec}^{-1}\) under comparable conditions (pH = 8.39, 0.086 N) for OAPS. The ratio of the \(k_p\)'s is 12.5, which is regarded as convincing qualitative evidence for a bifunctional mechanism for OAPS catalysis.
The Components of the Rate Constant for Catalysis by 2-Methoxyethylamine. There are several competing mechanisms in monofunctional primary-amine-catalyzed exchange. From previously published data each contribution to the observed rate can be calculated or estimated. Isobutyraldehyde may exchange through the uncomplexed aldehyde (forming an intermediate enol or enolate) or through the protonated imine (forming an intermediate enamine). The aldehyde or the iminium ion may be attacked by any of the bases in solution. Thus, the observed rate of disappearance of isobutyraldehyde-2-d is

\[- \frac{d}{dt} [AD] = [AD] \sum k_{Bi}[Bi] + [HImD^+] \sum k'_{Bi}[Bi] \quad (1)\]

where \([AD]_T\) is the total stoichiometric concentration of isobutyraldehyde-2-d (including free and hydrated aldehyde, imine, and all other forms), \([AD]\) is the concentration of free and hydrated isobutyraldehyde-2-d, \([HImD^+]\) is the concentration of the iminium ion of isobutyraldehyde-2-d, \(k_{Bi}\) is the second order rate constant for the attack of the \(i^{th}\) base on the aldehyde, \([Bi]\) is the concentration of the \(i^{th}\) base, and \(k'_{Bi}\) is the second order rate constant for the attack of the \(i^{th}\) base on the iminium ion. The observed pseudo first-order rate constant, \(k_p\), is defined by

\[- \frac{d}{dt} [AD]_T = k_p [AD]_T \quad (2)\]

Combining equations (1) and (2) and solving for \(k_p\) gives
or

\[ k_p = \frac{[AD]}{[AD]^n} \sum k_{B_1}[B_1] + \frac{[HImD^+]}{[AD]^n} \sum k'_B[B_1] \]

or

\[ k_p = \sum k_{B_1}[B_1] + \frac{[HImD^+]}{[AD]} \sum k'_B[B_1] \quad (3) \]

where \( f = [AD]/[AD]^n \), the fraction of the aldehyde that is uncomplexed.

The following are the equilibrium constant equations for imine formation, protonated amine acidity, and protonated imine acidity,

\[ K_{Im} = \frac{[ImD]}{[Am][AD]} \]

\[ K_{AmH} = \frac{[Am][H^+]}{[AmH^+]} \]

\[ K_{ImH} = \frac{[ImD][H^+]}{[HImD^+]} \]

where \([ImD]\) is the concentration of deuterium-substituted imine and \([HImD^+]\) is the concentration of protonated deuterium-substituted imine.

Then, ignoring secondary isotope effects on the equilibrium constants, the overall formation constant for iminium ions from aldehyde and protonated amine may be written as

\[ \frac{[HImD^+]}{[AD][AmH^+]} = \frac{K_{Im} K_{AmH}}{K_{ImH}} \]
This relationship may be substituted into equation (3) giving

\[ k_p = f \sum k_{B_i}[B_i] + f \frac{K_{Im} K_{AmH}}{K_{ImH}} [AmH^+] \sum k'_{B_i}[B_i] \]  

(4)

which is a useful substitution because the elusive iminium ion concentration is eliminated and because the \( k'_{B_i} \)'s available are combined with the three equilibrium constants.

The bases which must be considered are water, hydroxide ion, and free amine. Attack of the basic group of the imine has been shown to contribute negligibly in the case of methylamine catalysis and the contribution by the small amount of the weakly basic carbinolamine should also be negligible. With these assumptions equation (4) can be expanded.

\[ k_p = f \left\{ k_w[H_2O] + k_h[OH^-] + k_{am}[Am] \right\} + \]

\[ f[AmH^+] \frac{K_{Im} K_{AmH}}{K_{ImH}} \left\{ k'_{w}[H_2O] + k'_{h}[OH^-] + k'_{am}[Am] \right\} \]  

(5)

The first collection of terms represents exchange by attack of bases on the aldehyde and the second collection of terms represents exchange by attack of bases on the iminium ion. The rate constants \( k_w, k_h, \) and \( k_{am} \)
are for attack on the aldehyde by water, hydroxide ion, and free amine, respectively, and $k'_w$, $k'_h$, and $k'_am$ are rate constants for the attack on the iminium ion by the same bases.

For the kinetic run with 0.0863 N 2-methoxyethylamine at pH 9.09 at 35°, the required concentrations were calculated from initial conditions of 0.0544 N amine and 0.0318 N protonated amine, using a $K_{Im}$ of 38 M$^{-1}$ and a total aldehyde concentration of 0.0546 M.

$$[H_2O] = 55 \text{ M}$$
$$[OH^-] = 3.04 \times 10^{-5} \text{ M}$$
$$[Am] = 0.0518 \text{ M}$$
$$[AmH^+] = 0.0268 \text{ M}$$
$$f = 0.506$$

The following values of the rate constants were obtained as described on the next few pages.

$$k_w = 3.3 \times 10^{-10} \text{ M}^{-1} \text{ sec}^{-1}$$
$$k_h = 3.1 \times 10^{-2} \text{ M}^{-1} \text{ sec}^{-1}$$
$$k_{am} = 4.9 \times 10^{-4} \text{ M}^{-1} \text{ sec}^{-1}$$

$$k'_w = \frac{K_{Im} K_{AmH}}{K_{ImH}}$$
The rate constants \( k_w \) and \( k_h \) were taken directly from earlier work, \(^{12}\) where they were determined at 35°.

The rate constant \( k_{am} \) could not be measured directly because the attack of amine on aldehyde is kinetically indistinguishable from the attack of hydroxide ion on the iminium ion. However, the value of the term containing both rate constants,

\[
\left( k_m + \frac{k'_h K_w}{K_{TH}} \right) K_{MH}
\]

was determined from data on methylvamine catalysis, where \( k_m \) is the rate constant for attack of methylvamine on the aldehyde, \( K \) is the equilibrium constant for imine formation from methylvamine and isobutyraldehyde, \( K_w \) is the ion-product constant for water, \( K_{IH} \) is the acidity constant for protonated methylviminium ions, and \( K_{MH} \) is the acidity constant for methylvammonium ions. Values of \( 6.25 \times 10^{-14} \text{ sec}^{-1} \) in the presence of \(^{56}\) 2,6-lutadiene buffers and \( 9.7 \times 10^{-14} \text{ sec}^{-1} \) in the presence of methylvamine buffers \(^{55,56}\) were obtained for the term. It was later found that

In a plot of $\log (k_B^{KM} K_{A M} H) vs \log K_B$, the fit to the line described by five other buffer bases was optimized for the methylamine and hydroxide ion points when 91\% of the term was attributed to the methylamine component and 9\% to the hydroxide ion component. Assuming $k_m$ contributed 91\% and using the average of these two values and the appropriate value of $K_{MH}$ ($3.57 \times 10^{-11} M$ at 35° and 0.5 M ionic strength), $k_m$ was calculated to be $2.0 \times 10^{-3} M^{-1} \text{sec}^{-1}$. This is the rate constant for attack of methylamine on the aldehyde. This rate constant can be converted into the rate constant for attack of 2-methoxyethylamine on the aldehyde with information from the Brønsted plot for bases attacking isobutyraldehyde. A Brønsted slope of 0.5 was found for attack of phenoxide ions or of 3- and 4-substituted pyridines on isobutyraldehyde. From that fact and the $pK_a$'s of methylamine and 2-methoxyethylamine (10.31 and 9.09 at 35° and zero ionic strength) the rate constant for 2-methoxyethylamine can be estimated.

$$\log k_{am} = \log k_m - (0.5) (\Delta pK_a)$$

$$= -2.70 - (0.5)(1.22)$$

$$= -3.31$$

Thus, $k_{am} = 4.9 \times 10^{-4} M^{-1} \text{sec}^{-1}$


58. In reference 57, the value of $k_m$ was miscalculated. The correct value is $2.0 \times 10^{-3} M^{-1} \text{sec}^{-1}$. 
The term \( \frac{K_{\text{Im}}}{K_{\text{ImH}}} k'_{\text{w}} \) (the product of the rate constant for attack of water on 2-methoxyethyliminium ions and three equilibrium constants) was estimated from the similar quantity describing the attack of water on methyliminium ions. The Brønsted plot of Figure 14 was used. Its data were determined by studying the kinetics of isobutyaldehyde exchange in pyridine buffers in the presence of different primary amine salts. Thus, a fixed base, pyridine, attacked a variety of iminium ions, and the relative reactivities of the iminium ions were determined. Using the slope of the log-log plot (-0.40), the pKₐ's of methylamine and 2-methoxyethylamine, and the value of the term for methyliminium ions, the similar term may be estimated for 2-methoxyethylamine. For methyliminium ions, \( \log \left( \frac{K_{\text{Im}}K_{\text{AmH}}}{K_{\text{ImH}}} k'_{\text{w}} \right) \) was determined to be -8.55 and -8.14 in 2,6-lutadiene and acetate buffers, respectively. Using the average, the similar term was estimated for 2-methoxyethyliminium ions

\[
\log \left( \frac{K_{\text{Im}}K_{\text{AmH}}}{K_{\text{ImH}}} k'_{\text{w}} \right) = -8.35 + (0.40)(1.22) = -7.86
\]

Thus, \( \frac{K_{\text{Im}}K_{\text{AmH}}}{K_{\text{ImH}}} k'_{\text{w}} = 1.38 \times 10^{-8} \text{ M}^{-2} \text{ sec}^{-1} \)

Using a similar procedure, the product of \( k'_{\text{h}} \) and the three equilibrium constants was obtained for 2-methoxyethyliminium ions. Using the

Figure 14. Bronsted Plot of Over-all Rate Constants for Attack of Pyridine on the Iminium Ion vs the pKₐ of the Protonated Amine from which the Iminium Ion Was Formed.
average of two values of $\frac{K_{Im} K_{AmH}}{K_{ImH}} k'_h$ for methyliminium ions (0.17 M$^{-2}$ sec$^{-1}$), the slope of 0.40, and the pK$_a$ difference of 1.22, the value for hydroxide ion attacking 2-methoxyethyliminium ions was obtained. For methyliminium ions,

$$\log \left( \frac{K_{Im} K_{AmH}}{K_{ImH}} k'_h \right) = -0.77$$

Thus, for 2-methoxyethyliminium ions,

$$\log \left( \frac{K_{Im} K_{AmH}}{K_{ImH}} k'_h \right) = -0.77 + (0.40)(1.22) = -0.28$$

$$\frac{K_{Im} K_{AmH}}{K_{ImH}} k'_h = 0.52 \text{ M}^{-2} \text{ sec}^{-1}$$

To estimate the rate constant for attack of 2-methoxyethyamine on 2-methoxyethyliminium ions, two corrections were applied to the methylamine data. An average value of -1.72 was determined for log $\left( \frac{K_{Im} K_{AmH}}{K_{ImH}} k'_B \right)$ for the attack of methylamine on methyliminium ions. First it was changed, as before, to allow for the greater reactivity of the 2-methoxyethyliminium ions. For attack of methylamine on 2-methoxyethyliminium ions,

$$\log \left( \frac{K_{Im} K_{AmH}}{K_{ImH}} k'_B \right) = -1.72 + (0.40)(1.22) = -1.23$$

The second correction allows for the fact that 2-methoxyethylamine is a weaker base than methylamine. The slope of the Brønsted plot of log
\[
\left( \frac{K_{\text{Im}} \cdot K_{\text{AmH}}}{K_{\text{ImH}}} \right) \text{ vs } pK_a \quad \text{must be about 0.42, since the slope of the } \log k_B \text{ vs } pK_a \text{ plot is 0.5}^{12} \quad \text{and the slope of the } \log \left( \frac{K_{\text{Im}} \cdot K_{\text{AmH}}}{K_{\text{ImH}}} \right) \text{ vs } \log k_B \text{ plot is 0.84. Thus, for attack of 2-methoxyethylamine on 2-methoxy-ethylinium ions,}
\]

\[
\log \left( \frac{K_{\text{Im}} \cdot K_{\text{AmH}}}{K_{\text{ImH}}} \right) k_{\text{am}} = -1.23 - (0.42)(1.22) = -1.74
\]

\[
\frac{K_{\text{Im}} \cdot K_{\text{AmH}}}{K_{\text{ImH}}} k_{\text{am}} = 1.82 \times 10^{-2} \text{ M}^{-2} \text{ sec}^{-1}
\]

Thus, all the required rate constants and concentrations have been obtained. It should be added that the estimates for \( k_{\text{am}} \) and \( k'_{\text{am}} \) may be a little high, due to small steric differences between methylamine and 2-methoxyethylamine. That the size of the substituent on the \( \alpha \)-carbon matters is shown by the fact that trimethylamine is considerably above, but triethylamine considerably below, the Brønsted line for phenoxide ions attacking free aldehyde. The steric factors for the approach of bases to deuterium in the iminium ion are virtually identical to the steric factors for approach of bases to deuterium in the aldehyde, so this argument applies to both \( k_{\text{am}} \) and \( k'_{\text{am}} \).

Substituting the values obtained into equation (5),

\[
k_p = (0.506) \left[ (3.3 \times 10^{-10})(55) + (3.1 \times 10^{-2})(3.04 \times 10^{-5}) + (5.0 \times 10^{-4})(0.0318) \right] + (0.506)(0.0268) \left[ (1.4 \times 10^{-8})(55) + (0.52)(3.04 \times 10^{-5}) + (1.82 \times 10^{-2})(0.0318) \right]
\]
= \left\{ 9.2 \times 10^{-9} + 4.77 \times 10^{-7} + 8.05 \times 10^{-8} \right\} \\
+ \left\{ 1.04 \times 10^{-8} + 2.14 \times 10^{-7} + 7.85 \times 10^{-8} \right\} \\
= 8.54 \times 10^{-8} + 8.07 \times 10^{-8} \\
= 1.66 \times 10^{-5} \text{ sec}^{-1}

which is in very good agreement with the experimental value of $1.60 \times 10^{-5}$ sec\(^{-1}\). Considering the number of corrections that were made from Bronsted slopes, the uncertainty of the data to which the corrections were applied, and the uncertainty in the experimental value, the agreement must be regarded as largely fortuitous. Nevertheless, it does illustrate that the observed rate constant for monofunctional catalysis can be calculated adequately from fundamental data.

The Components of the Rate Constant for Catalysis by OAPS. A treatment similar to that in the last section can be carried out for OAPS. This will be done for run number 5, Table 11, p 78, which gave the largest rate constant on the pH-rate curve. From the measured pH of the kinetic solution, the total amine concentration, a calculated $pK_{\text{app}}$ of 8.74, and the assumption that $K_{\text{im}}$ is on the Bronsted line ($K_{\text{im}} = 29 \text{ M}^{-1}$), the required concentrations were calculated.
\[ [\text{H}_2\text{O}] = 55 \text{ M} \]
\[ [\text{OH}^-] = 6.4 \times 10^{-8} \text{ M} \]
\[ [\text{Am}] = 0.016 \text{ M} \]
\[ [\text{AmH}^+] = 0.053 \text{ M} \]
\[ f = 0.72 \]

The required rate constants were calculated exactly as before, using a \( \text{pK}_{\text{app}} \) of 8.74.

\[
K_w = 3.3 \times 10^{-10} \text{ M}^{-1} \text{ sec}^{-1}
\]
\[
K_h = 3.1 \times 10^{-2} \text{ M}^{-1} \text{ sec}^{-1}
\]
\[
K_{am} = 2.9 \times 10^{-4} \text{ M}^{-1} \text{ sec}^{-1}
\]

\[
\frac{K_{\text{Im}} K_{\text{AmH}}}{K_{\text{ImH}}} \cdot k'_w = 1.32 \times 10^{-8} \text{ M}^{-2} \text{ sec}^{-1}
\]

\[
\frac{K_{\text{Im}} K_{\text{AmH}}}{K_{\text{ImH}}} \cdot k'_h = 0.50 \text{ M}^{-2} \text{ sec}^{-1}
\]

\[
\frac{K_{\text{Im}} K_{\text{AmH}}}{K_{\text{ImH}}} \cdot k'_{am} = 1.78 \times 10^{-2} \text{ M}^{-2} \text{ sec}^{-1}
\]

Now \( k_p \) can be predicted for OAPS at pH 8.39. Substituting these values into equation (5)
\[ kp = (0.72) \left\{ (3.3 \times 10^{-10})(55) + (3.1 \times 10^{-2})(6.4 \times 10^{-6}) + (2.9 \times 10^{-4})(0.016) \right\} + (0.72)(0.053) \left\{ (1.32 \times 10^{-8}) (55) + (0.50)(6.4 \times 10^{-8}) + (1.78 \times 10^{-2})(0.016) \right\} \]

\[ = \left\{ 1.3 \times 10^{-8} + 1.4 \times 10^{-7} + 3.3 \times 10^{-6} \right\} + \left\{ 2.8 \times 10^{-8} + 1.2 \times 10^{-7} + 10.9 \times 10^{-8} \right\} \]

\[ = 0.35 \times 10^{-5} + 1.10 \times 10^{-5} \]

\[ = 1.45 \times 10^{-5} \text{ sec}^{-1} \]

However, this calculation has considered only monofunctional catalysis. We attribute the difference between the observed rate constant (observed \( kp = 20.0 \times 10^{-5} \text{ sec}^{-1} \)) and this calculated rate constant to bifunctional catalysis. Thus, about 93\% of the exchange proceeds through the bifunctional amine-catalyzed mechanism and the six other contributing mechanisms amount to only 7\% of the observed rate. Catalysis by water (through both the aldehyde and iminium ion) contributed only about 0.02\% of the observed rate. Catalysis by hydroxide ion (through both the aldehyde and iminium ion) also made a small contribution, amounting to about 0.1\% of the total observed rate. About 1.7\% of the observed rate is attributed to attack of free amine on the aldehyde. Hence, the remaining 98\% of the observed rate proceeded by attack of free amine groups on iminium ions. This pathway has been made very favorable by
The bimetallic catalyst and strongly dominates the reaction. Catalysis by OAPS was 20.0/1.45 = 13.8 times as fast as was predicted under the same conditions for a mononuclear whose amine groups had the same properties as OAPS amine groups.

If the assumption is made that rate enhancement over monofunctional catalyst occurred entirely because of the greater effective concentration of amine groups around iminium ions, that ratio of the rates is the ratio of the square of the average amine group concentration in solution, or 0.29.

The possibility of other reasons for the rate enhancement over monofunctional catalysis seems unlikely. For example, more effective substrate complexing through hydrophobic bonding is a tenable idea because the equilibrium constant for isoniazid formation is ordinary for a catalytic basicity. There is no evidence to support any kind of mechanism other than intramolecular deuteration of protonated iminium ions.

Hence the effective concentration of amine groups around iminium ions was 19.7/0.09 = 18 times the average amine group concentration in solution, or 0.29. Hence the effective concentration of amine groups around iminium ions was 19.7/0.09 = 18 times the average amine group concentration in solution, or 0.29.

The observed rate constant. Since whether intra- or intermolecular, the same kind of amine group is attacking the same kind of iminium ion, the observed rate constant can be calculated. The amine group concentration around iminium ions can be calculated.
Summary. Octakis-\(\text{-}(3\text{-aminopropyl})\)sucrose was prepared by reduction of the corresponding nitrile. Purification of the product was difficult; the best material obtained was found to have 7.0 of a possible 8 amino groups per molecule. The catalyst was also hydrolyzed to its monosaccharide components to a small extent, less than 15%. Several foreign materials were present, including a large amount of carbon dioxide, which existed as ammonium carbonates and bicarbonates.

CAPS catalyzed \(\alpha\)-hydrogen exchange in isobutyraldehyde-2-d at a rate about 1.4 times as fast as was predicted for the same amine groups in separate molecules. This was taken as strong evidence that bifunctional catalysis was occurring. Further support for bifunctional catalysis was inferred from the plots of rate constant vs catalyst concentration and rate constant vs pH. All the evidence obtained on whether the reaction might be intra- or intermolecular pointed to strongly dominating intramolecular deuteration, i.e., bifunctional catalysis.
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