A STUDY OF SOME REACTION RATES IN THE HOMOGENEOUS SYSTEM GLUCOSE-SODIUM HYDROXIDE-WATER

by

GORDON ARNOLD HARVIE

B.Sc., University of Alberta, 1967

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Chemistry

Accepted for the Faculty of Graduate Studies

[redacted]

27 July, 1971

We accept this thesis as conforming to the required standard

[redacted]

July 1971

ERRATA

1. p.iv -line 17- "eluant" should read "eluent".
2. p.4 -line 23- "saacharinic" should read "saccharinic".
3. p.31 -line 14- "similtaneous" should read "simultaneous".
4. p.35 -line 14- "consistant" should read "consistent".
5. p.36 -line 5- "practiced" should read "practised".
6. p.44 -line 7- "LeMieux" should read "Lemieux".
7. p.55 -line-26- "similtaneously" should read "simultaneously".
8. p.57 -line 16- "aperature" should read "aperture".

To Marilynn

ACKNOWLEDGMENTS

The author would like to express his appreciation to Dr. D. J. MacLaurin for the suggestion of this research topic, for his supervision, and for his financial support. Many thanks are also extended to Dr. A. Wilkinson for his enthusiasm and guidance during the writing of the computer program to solve this complex kinetic problem. And a final vote of thanks goes to my wife, Marilynn, whose patience and "financial support" were essential throughout this period of study.

Supervisor: Dr. D. J. MacLaurin

ABSTRACT

Rate constants for the isomerization, epimerization and degradation reactions occurring in the glucose-sodium hydroxide-water system were calculated in systems using each of the three expected carbohydrate products (glucose, fructose and mannose) in turn separately as starting material. A study of the dependence of the rate constants prevailing in the glucose reaction system on base concentration was accomplished by calculating these rate constants at four different sodium hydroxide concentrations, 0.49, 4.02, 6.07 and 8.08M. Only the magnitude of the fructose to degradation products reaction pathway was found to demonstrate a strong dependence on base concentration.

The materials of interest in the reaction systems were resolved by column chromatography using an anion resin in the borate form. Separation and elution of the carbohydrates required about six hours using a graded eluant. Elution began with 0.12M H_3BO_3 at pH 8.50 by adjustment with KOH and ended with 0.15M $K_2B_4O_7$. The column effluent was monitored continuously and analyzed colorimetrically using automated Technicon equipment. Absorbance was measured at 420 nm using orcinol/H_2SO_4 as the color forming reagent.

Graphical and numerical techniques were used to estimate the rate constants at each sodium hydroxide concentration. These estimates were then refined by a non-linear least squares analysis utilizing an

IBM 360, Model 44 digital computer to give the best-fit to experimentally determined carbohydrate concentration vs reaction time curves.

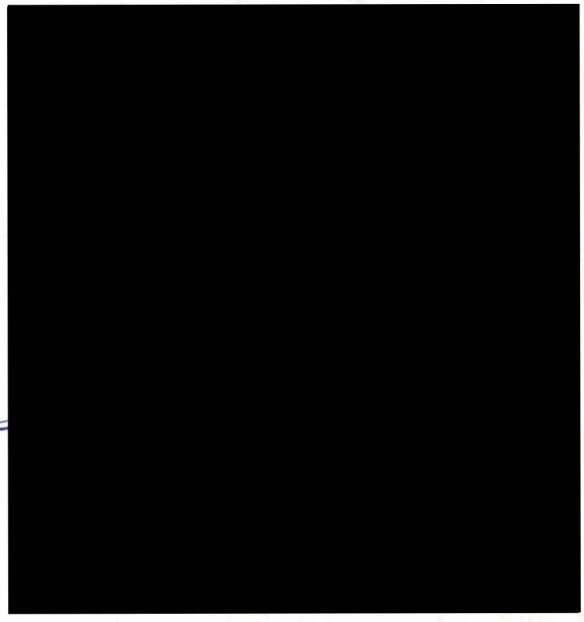


TABLE OF CONTENTS

INTRODUCTION

THE PROBLEM

The kinetics and mechanisms of the chemical reactions of carbohydrates are of important interest, from several points of view. Carbohydrates and their derivatives play a major role in the metabolic processes of man. While the chemical transformations occurring within the body are likely enzyme controlled, an understanding of the enzyme's role may well be improved through the study of less complex systems. The reactions of carbohydrates, particularly in alkaline systems, are important in the several major industries based on cellulose. About forty percent of the weight of all wood consists of cellulose, long chains of anhydro-glucose units, and another twenty percent consists of other carbohydrate material. Much of the chemical wood pulp used in the pulp and paper industry, and other cellulose based industries, is produced by alkaline processes.

In alkali, two general types of carbohydrate reactions are known to occur. Through epimerization or isomerization, other neutral carbohydrates may be formed. In this study, this type of reaction will be termed a transformation reaction. Transformation reactions are accompanied by other base-catalyzed reactions including β-eliminations to form α-dicarbonyl compounds which subsequently undergo benzilic-acid type rearrangements. Fragmentation reactions resulting in aldonic acids having fewer carbon atoms than the starting carbohydrate are also observed in alkali. These reactions will be termed the carbohydrate degradation reactions.

From a more theoretical viewpoint the transformation reactions of carbohydrates in alkali are of interest. An enolization mechanism for the transformation reactions, proposed by Wohl and Neuberg (1), has won acceptance. This mechanism will be detailed in the next section of the report, but it involves the abstraction by base of a proton from the carbon located alpha to the carbonyl group of a carbohydrate. This α-hydrogen abstraction type of mechanism is proposed in other reactions of carbonyl compounds in base, for example, aldol condensations of aldehydes and ketones in dilute alkali. Thus a study of the factors affecting transformation reactions of carbohydrates in alkaline systems may serve to shed light on carbonyl consecutive electron displacement reactions in general.

MacLaurin (2) concluded, after an extensive review of the literature, that because of the lack of reasonable procedures for assay of the reaction systems, little kinetic data were available for carbohydrate isomerization, epimerization, and degradation reactions occurring in aqueous alkaline systems. Because of the important theoretical, physiological, and industrial implications of these reactions, it appeared useful to have kinetic data on them. MacLaurin (2) was able to design an experimental and analytical system for the generation of kinetic data from which the related rate constants prevailing in the reaction system could be derived with an accuracy of ± 2-3%. The development of this general scheme made possible a comprehensive study of these carbohydrate transformation and degradation reactions from a kinetic viewpoint.

MacLaurin suggested that "a program of research involving variation of such parameters as temperature, base concentration, cation type, additional inorganic anions, enzymes, and the structure of the starting molecule should yield kinetic data which could be a penetrating insight to these interesting and important reactions."* The present research was a study of the effects on various reaction rate constants in the homogeneous system, glucose-sodium hydroxide-water, caused by varying sodium hydroxide concentration. Transformation products (glucose, fructose, and mannose) were monitored using the techniques developed by MacLaurin (2), and the rate constants were derived, using the computer, from the kinetic data generated at four different base concentrations.

LOBRY DE BRUYN-ALBERDA VAN EKENSTEIN TRANSFORMATIONS AND DEGRADATION REACTIONS IN ALKALI

Under the influence of alkali, free carbohydrates undergo an aldose-aldose or an aldose-ketose interconversion known as the Lobry de Bruyn-Alberda van Ekenstein transformations. These carbohydrate isomerization and epimerization reactions are named in honor of the Dutch workers (3) who originally reported these reactions. For the sake of brevity these reactions are termed simply the "transformation" reactions.

The proposed mechanism of the transformation and degradation reactions appeared to be the result of speculative inference from the observed products. Evidence in support of the proposed reaction pathways was limited. However, since 1967 some evidence has been presented which lends support to the original proposals, which invoked an enediol intermediate for the transformation and degradation reactions in basic media.

---

*D. J. MacLaurin, personal communication

The proposed mechanism (4) for the transformation reactions is as follows:

The initial and fast stage occurring when an aldohexose(1) is treated with sodium hydroxide (see Figure 1) is the reversible formation of the 1,2-enediolate ion(2). This ion could form the 2-epimer(3) of the original aldose, or by a proton shift to the ion(4), form the 2-ketose(5). Once formed, the 2-ketose undergoes enolization to form the 2,3-enediolate ion(6) and thus gives rise to a 3-ketose(7) by a proton shift, or (6) can give the 3-epimer(8); and so on down the carbon chain. The products expected from a 2,3-enediol intermediate have not been reported to accumulate more than to a very small concentration in any reaction system studied to date. This has been explained (4) by examining the 2,3-enediolate ion(6). Undoubtedly, the steric hinderance between the two large *cis* substituents present in (6) would be much greater than when one of the substituents is hydrogen as in the 1,2-enediolate ion(2). Thus the 2,3-enediolization process would be expected to be a slower process than 1,2-enediolization.

By the mechanism of saccharinic acid formation proposed by Isbell (5), the enediol intermediate(2) can also undergo β-elimination of the hydroxyl (see Figure 2) to an enol(9) which can rearrange to an α-dicarbonyl intermediate(10). The dicarbonyl intermediate could then undergo a benzilic-acid type rearrangement initiated by hydroxyl ion attack to give one of the isomeric saacharinic acids. Hydroxyl ion attack at the aldehydic carbonyl of (10) (see Figure 3) followed by rearrangement would give the metasaccharinic acid(11). Depending which β-hydroxyl group is eliminated, it is possible to form saccharinic(12)

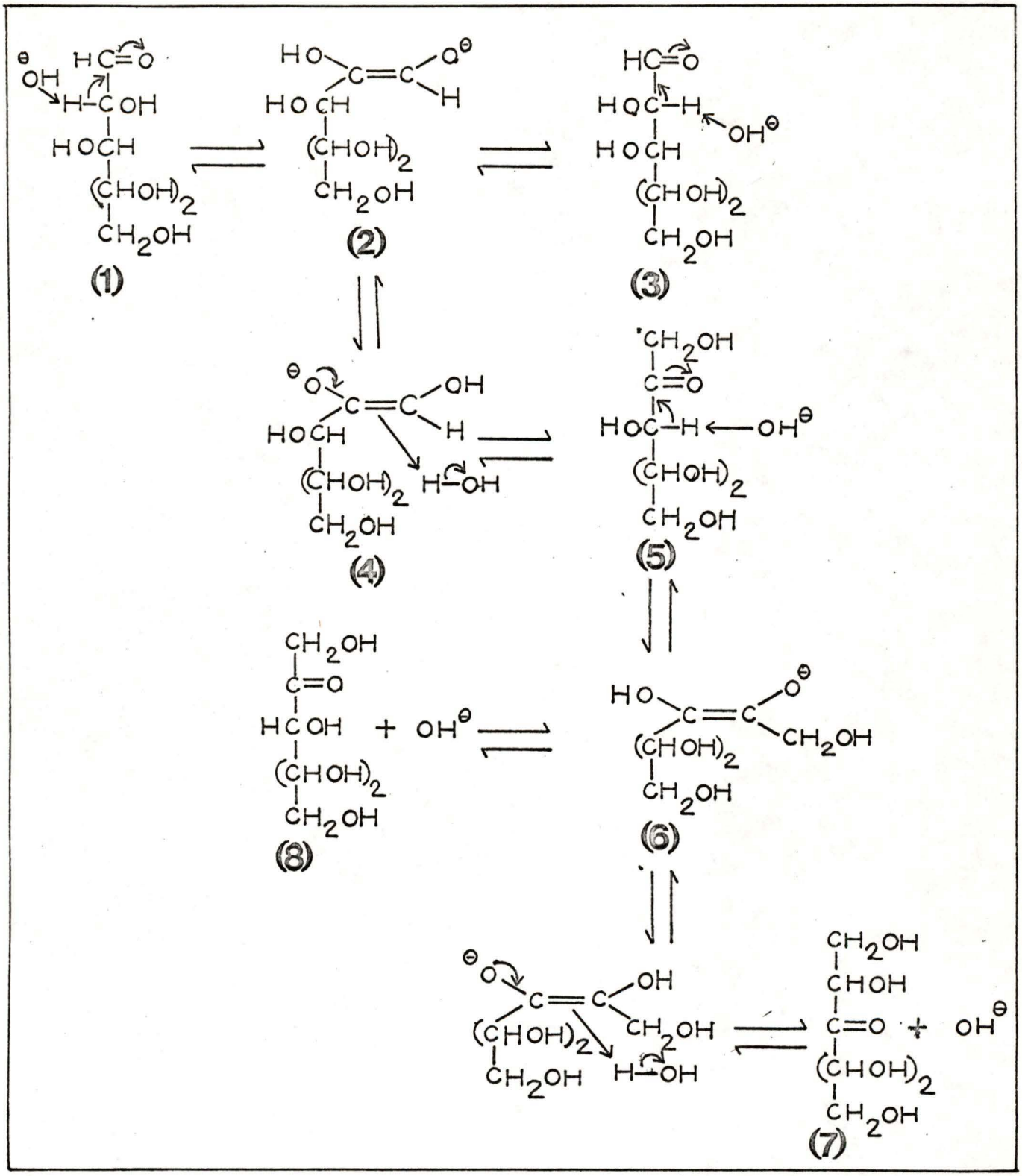


Figure 1. Possible Lobry de Bruyn-Alberda van Ekenstein transformations from an aldohexose in alkali. In the reaction system of the present study: (1)= D-glucose, (3) = D-mannose, (5) = D-fructose, (8) = D-psicose.

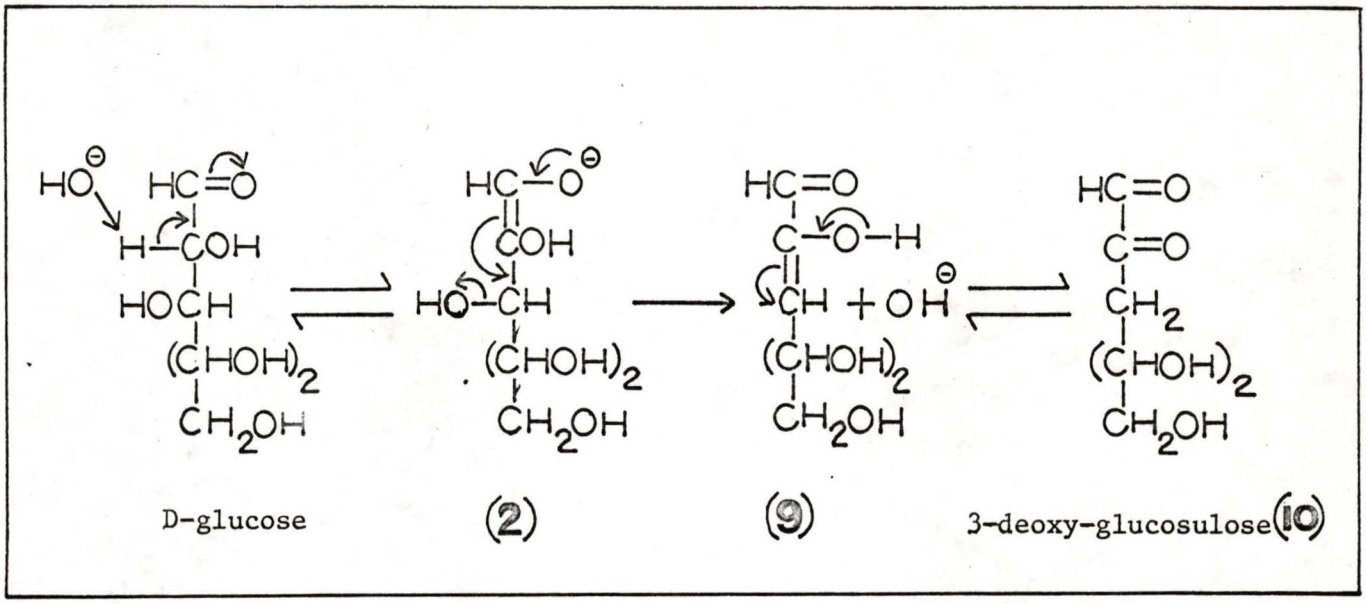


Figure 2. 3-deoxyhexosone formation from D-glucose in alkali

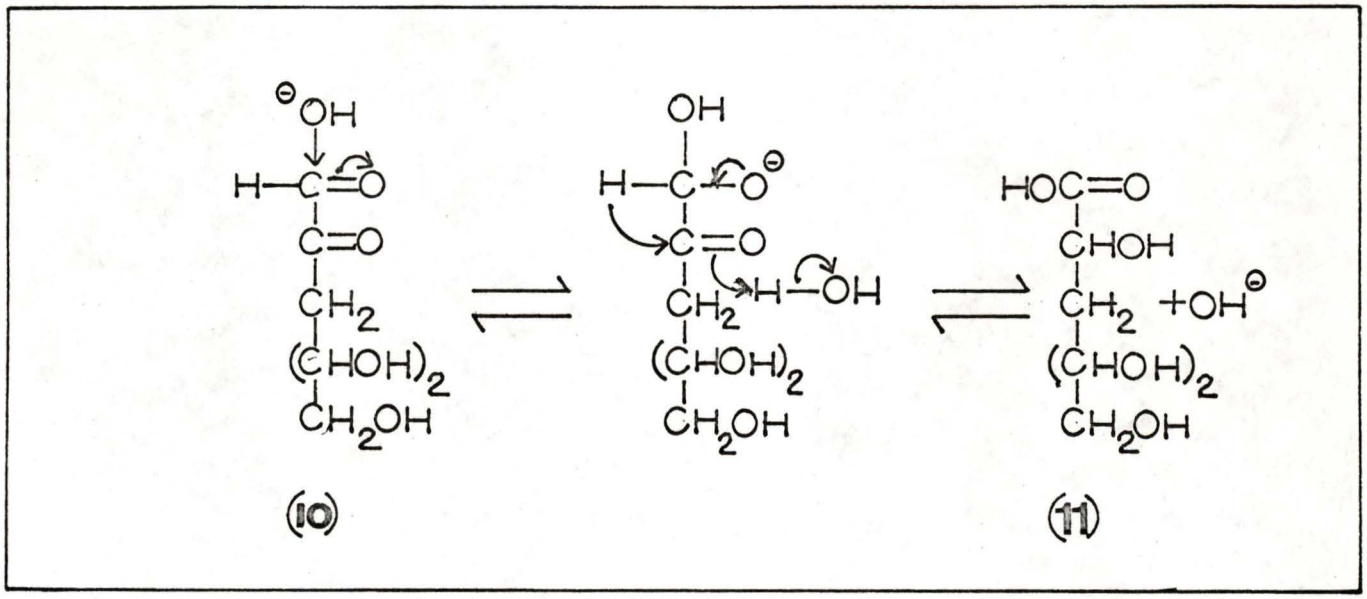


Figure 3. Metasaccharinic acid formation in alkali via a benzilic-acid type rearrangement

and isosaccharinic acids(13) (see Figure 4). The group which migrates in the benzilic-acid type rearrangement is that which is attached to the carbonyl group which undergoes hydroxyl ion attack.

Supporting evidence (6) for the β-elimination mechanism of Isbell was found in the fact that isosaccharinic acid formation was promoted when the C-4 substituent was -OR, (R≠H). This substitution resulted in a better leaving group than a hydroxyl group at the C-4 position. Similarly saccharinic acid formation was promoted by C-1 substitution, and metasaccharinic acid formation was promoted by C-3 substitution.

Alkaline degradation of D-glucose in sodium hydroxide leads mainly to D-glucometasaccharinic acid formation, whereas, in limewater, D-glucose yields D-glucosaccharinic acid (7). The relative amounts of the two acids formed is likely determined by the relative rates for elimination of the C-3 and C-1 hydroxyl groups respectively. Apparently the calcium ion can catalyze either the elimination of the C-1 hydroxyl group or the formation of the 2,3-enediolate ion, which is the precursor of the elimination of the C-1 hydroxyl group (8).

Although the transformation and degradation reactions of carbohydrates in base are discussed here as base-catalyzed reactions, they are found to be acid-base catalyzed (8). However, in acid solutions, the degradation reactions are not analogous to those in base. In acid solutions, the degradation product of hexoses is found to be 5-(hydroxymethyl)-2-furaldehyde(14) rather than saccharinic acids.

Shaw et al. (9) isolated several products from the acid-catalyzed degradation of D-fructose, but those present in major

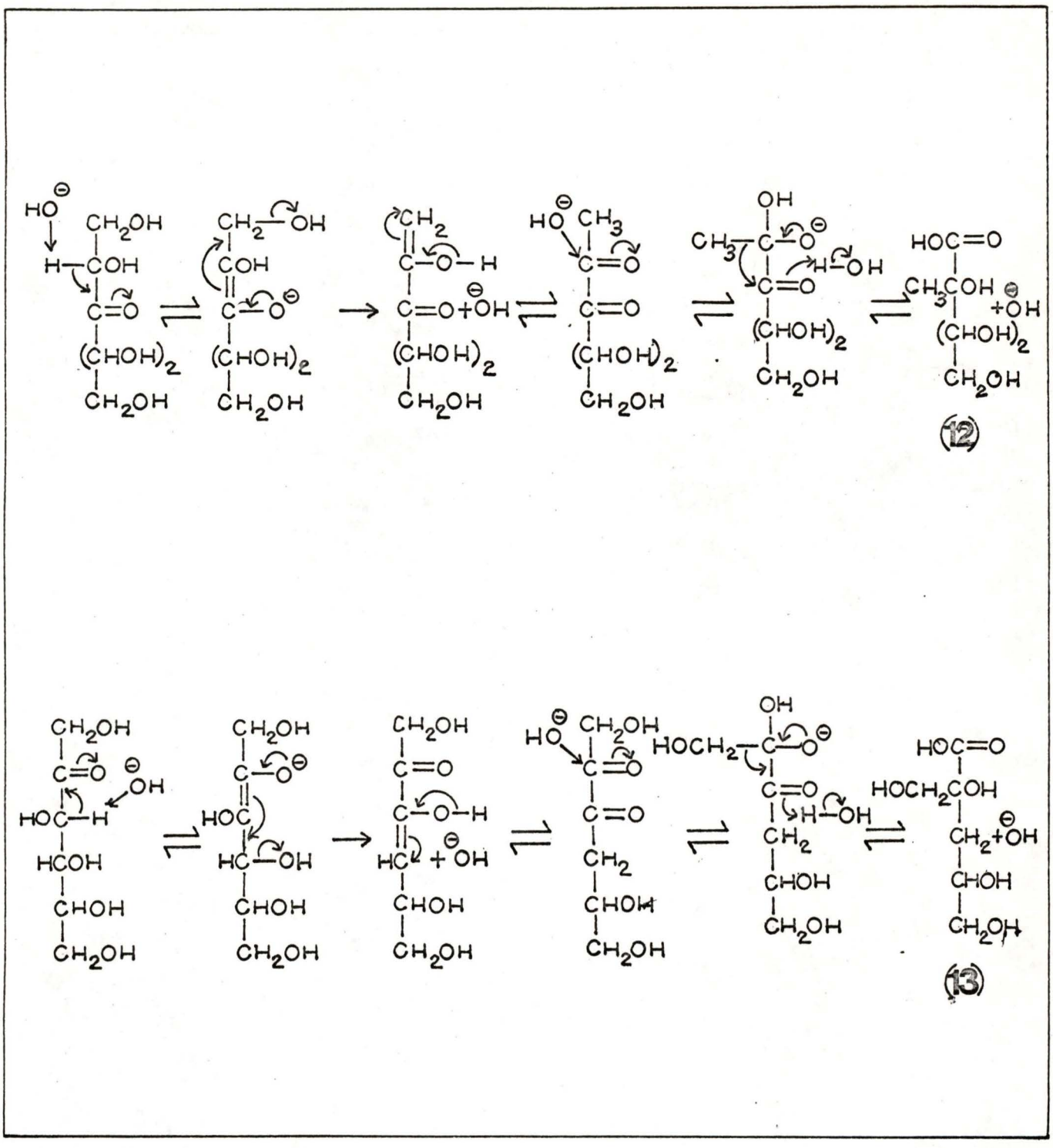


Figure 4. Saccharinic and Isosaccharinic acid formation from hexoses in alkali via a benzilic-acid type rearrangement.

quantities were (14) and 2-(hydroxyacetyl)-furan(15) which were considered to be dehydration products resulting from simple combinations of enolization and dehydration steps (see Figure 5). That the degradation reaction in acidic media went through the 3-deoxy-hexosone intermediate was considered to be supported by Doering and Urban's findings (10) that 3-deoxy-hexosones are converted quantitatively to (14).

Vicinal dicarbonyl compounds which were capable of undergoing benzilic-acid type rearrangement (to saccharinic acids) could also undergo rupture between the carbonyl groups by an oxidative mechanism, giving rise to two shorter-chain carboxylic acids (11,12). The cation employed in the alkali seemed to play an important part. Divalent cations such as calcium and barium gave the highest yields of rearrangement products, whereas monovalent cations, such as sodium and potassium favored fragmentation of the carbon chain. Kenner and Richards (13) have speculated that this was due to an internal complex-formation with divalent cations. Thus the dicarbonyl intermediates gave two distinct degradative reactions with alkali: (a) rearrangements to isomeric saccharinic acids, and (b) oxidative chain-cleavage to aldonic acids.

Fission products have also been observed in reaction mixtures of hexoses in alkali. Formaldehyde(16) and glycolaldehyde(17) were isolated from D-glucose by Komoto (14). His proposed mechanism was that shown in Figure 6. Kenner (15) detected lactic acid(18) from the action of alkali on substituted D-glucose and substituted D-fructose. His proposed mechanism was that shown in Figure 7.

In general, the recent literature has complemented the observations and hypothesis of the past concerning the formation of transformation and degradation products from carbohydrates in alkali. Rowell

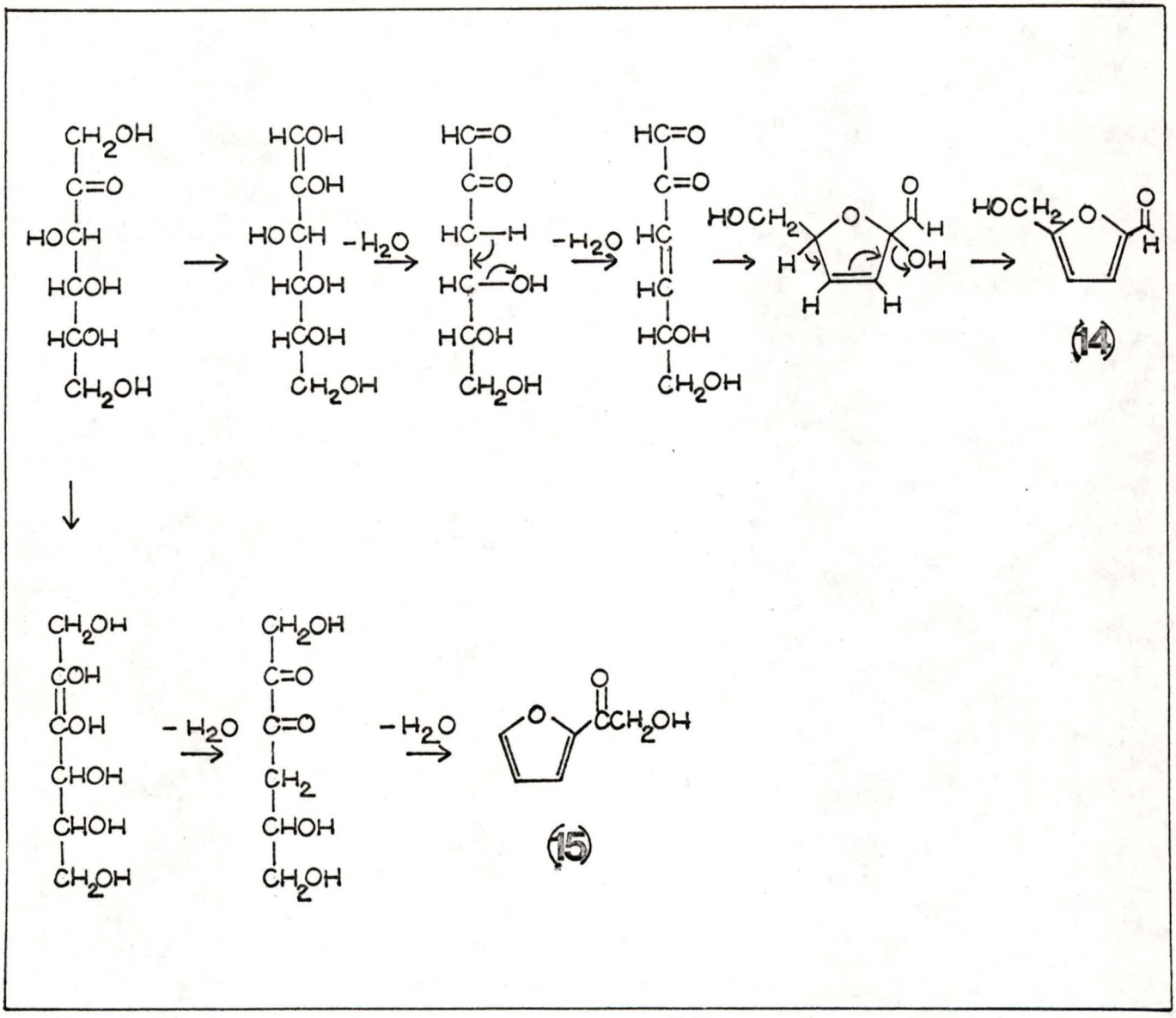


Figure 5. Acidic dehydration products of D-fructose

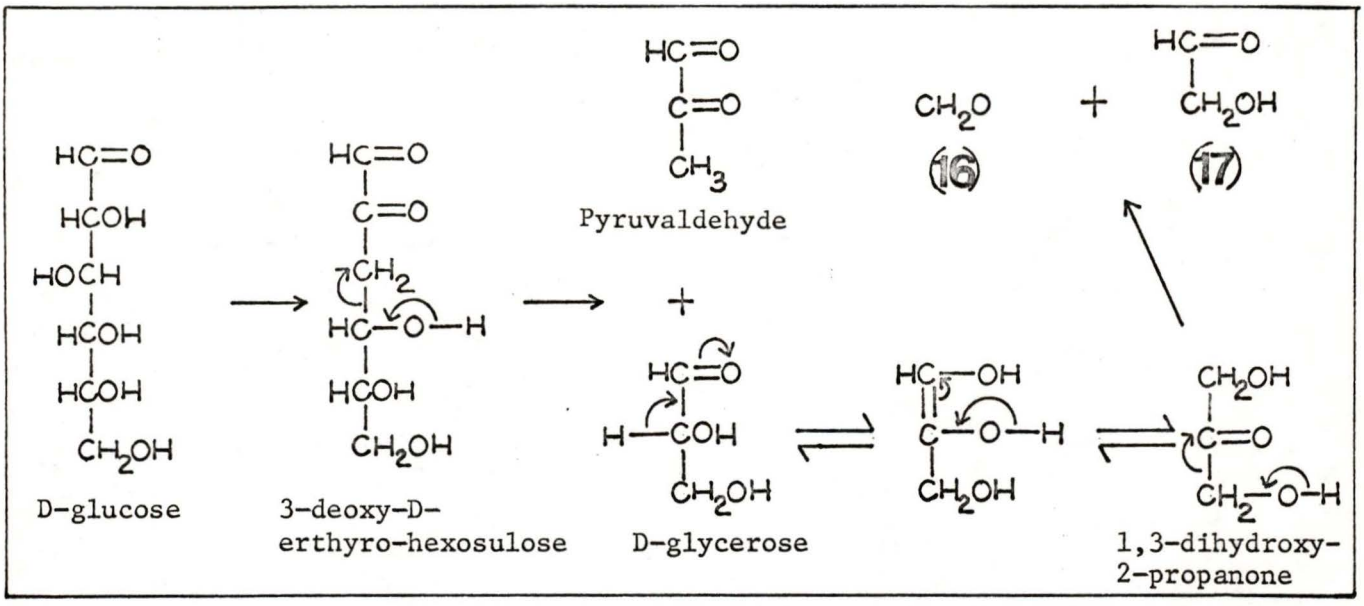


Figure 6. Fragmentation products of D-glucose in alkali

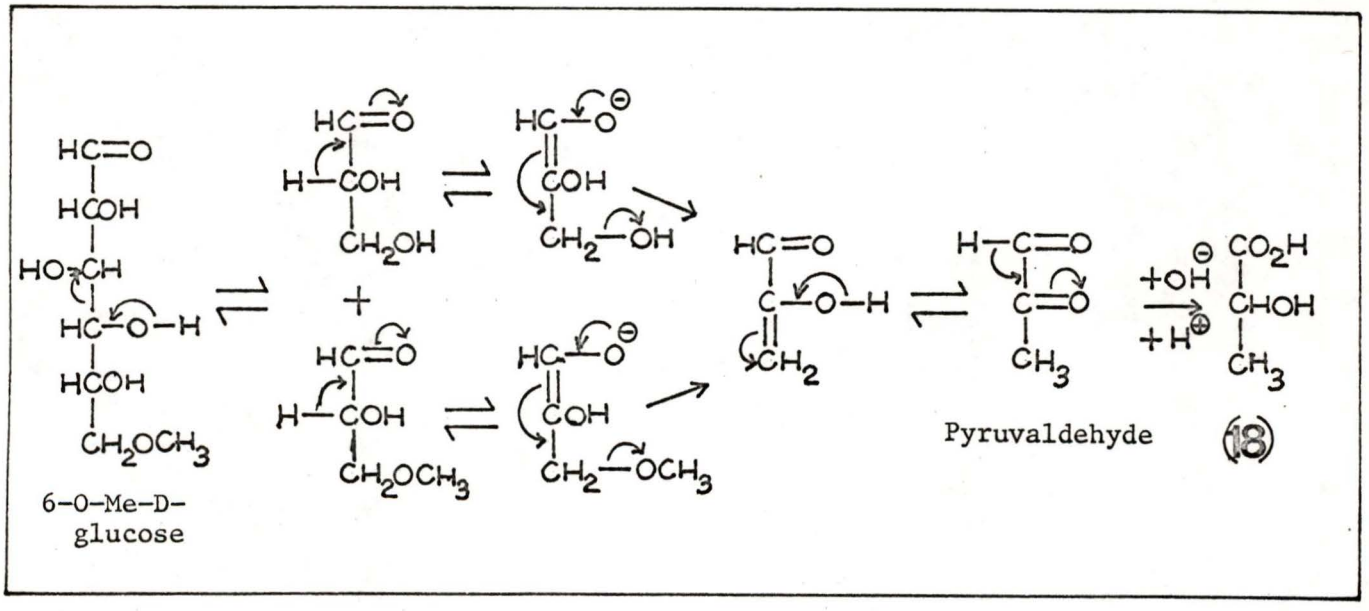


Figure 7. Lactic Acid formation from 6-O-Me-D-glucose in alkali

et al. (16) studied the alkaline degradation of cellobiose under a variety of conditions, and concluded that by introducing oxygen at various concentrations in the reaction system, the oxidation reactions to aldonic acids predominated. These oxidation reactions were effectively eliminated when oxygen was excluded from the system. They also observed that in air barium cations favored the benzilic-acid type rearrangement reaction, whereas sodium cations favored the fragmentation of the carbon chain.

Rowell and Green (17) then studied alkaline degradation of 3-deoxyaldosuloses, the dicarbonyl intermediate proposed in the formation of isomeric saccharinic acids, in the presence of O_2 and of N_2. The results of their experiments in the specific case of the alkaline degradation of 3-deoxy-D-erythro-hexosulose showed that the benzilic-acid rearrangement to D-glucometasaccharinic acid was the preponderant reaction, even in the presence of oxygen. The carbon-carbon bond cleavage to 2-deoxy-D-erythro-pentonic acid was eliminated by excluding oxygen, and only D-glucometasaccharinic acid was formed. Degradation of 3-deoxy-D-threo-hexosulose in the presence of O_2 demonstrated that the C-4, C-5 and C-6 substituent configuration exerts no stereochemical influence on the rearrangement to D-glucometasaccharinic acid. However, the greatest proportion of fragmentation product was found in the presence of O_2 and the divalent barium cation. Rowell and Green have noted that this contradicts previous findings (11,12), and that further work is required in that area.

Feather (18,19,20) has been active in the study of the mechanism of conversion of D-glucose and D-fructose into their degradation products

in base and acid, namely D-glucometasaccharinic acid(11) and 5-(hydroxymethyl)-2-furaldehyde(14) respectively. By conducting deuterium-labelling experiments, Feather and Harris (19) have concluded that the route to formation of (14) in acidic deuterium oxide solution (see Figure 8) does not include the 3-deoxy-D-erythro-hexosulose intermediate(10) known to yield glucometasaccharinic acid in alkaline deuterium oxide solution. Rather, (14) was formed from the enolic intermediate(9) by a direct, irreversible route. Completely analogous results were found by Feather (20) for a pentose and a hexuronic acid in acidic deuterium oxide solutions.

All reaction mechanisms reported (4,8,21) are illustrated as involving the acyclic form of the carbohydrate. Many studies have shown that the acyclic form with its free carbonyl group is present in aqueous solution to only a very small extent. For example, only $\sim$ 2% of D-fructose (22) is present in acyclic keto form in aqueous solution. The pyranose or furanose hemiacetal ring structure predominates in solution. No attempt was made (4,8,21) to explain the initial stages of the reaction, i.e. formation of the enediol from the cyclic form of the carbohydrate by some ring-opening mechanism. This would be necessary if these transformation and degradation reactions are to occur to any extent. Isbell et al. (23) have proposed the formation of enediol intermediates from pseudo-acyclic intermediates arising from transition states in the mutarotational reaction pathway of the cyclic conformers (see Figure 9).

Presumably, then, the alkaline rearrangement of a carbohydrate could begin with the formation of a pseudo-acyclic intermediate by the mutarotation reaction, and will be followed by the formation of an enediol.

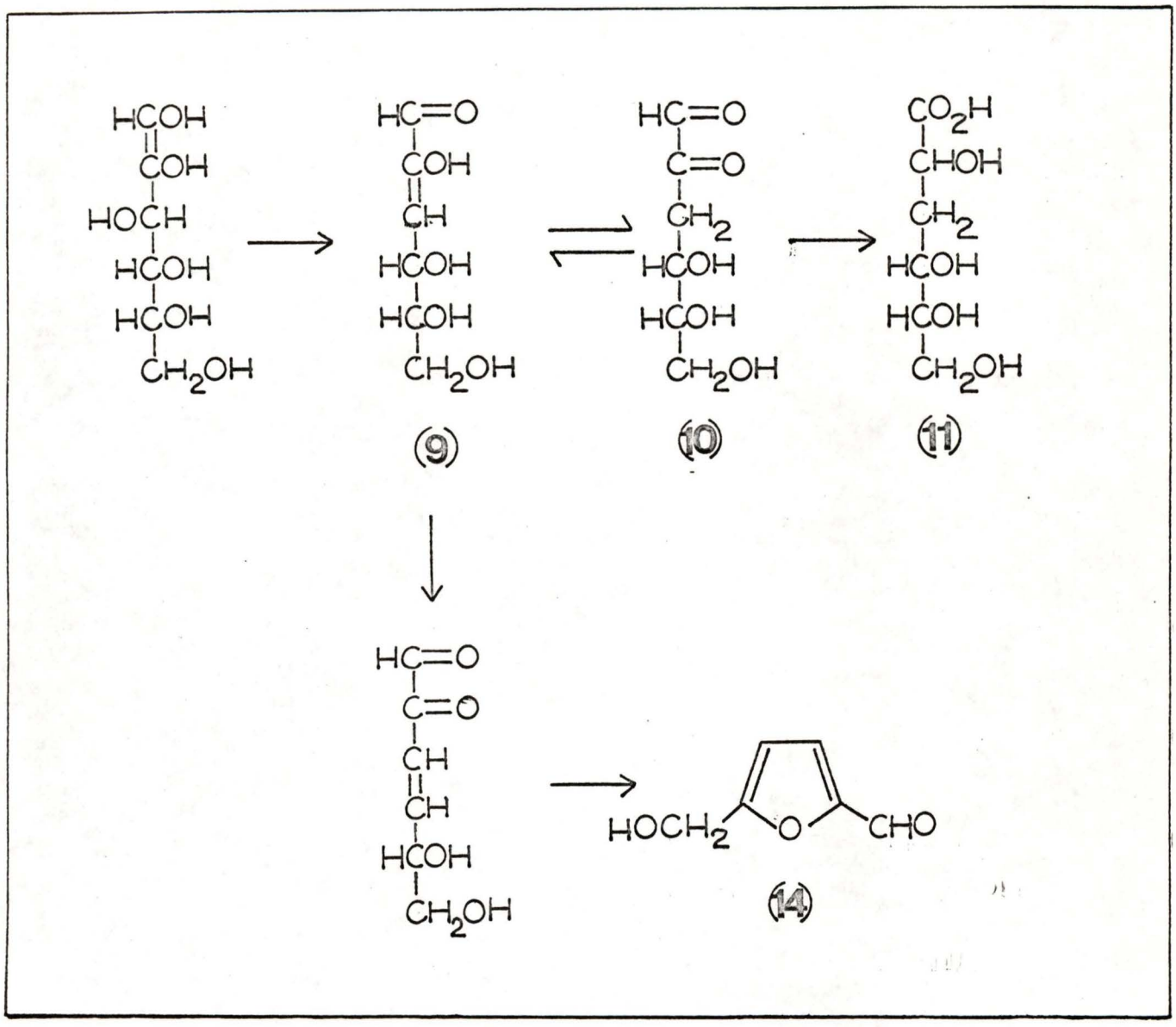


Figure 8. Glucometasaccharinic acid and 5-(hydroxymethyl)-2-furaldehyde formation from a 1,2-enediol.

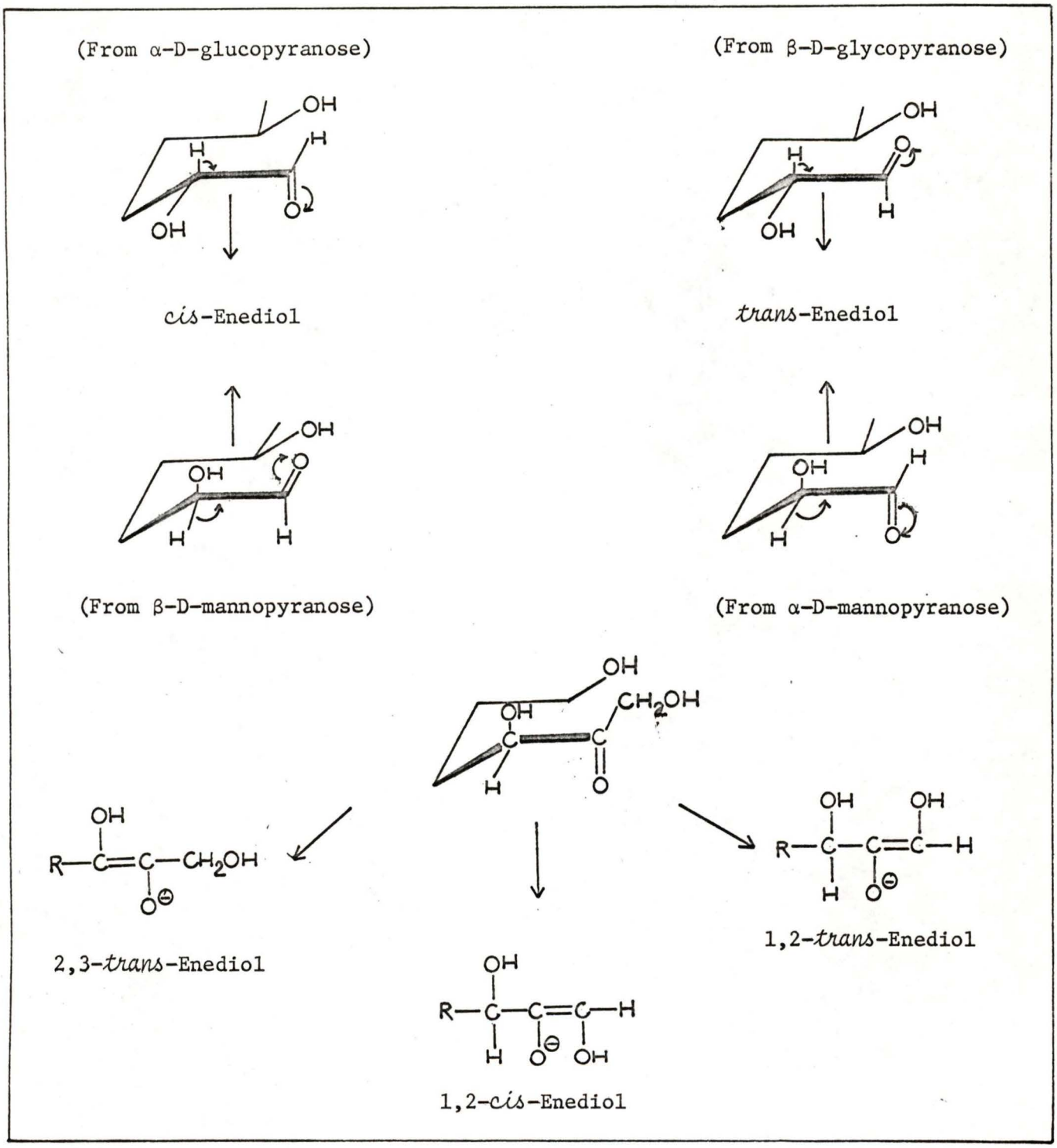


Figure 9. Suggested formation of *cis*- and *trans*-enediols from pseudo-acyclic intermediates of aldoses and 2-ketoses. Note: Some hydroxyl groups attached to carbon atoms have been omitted for clarity.

These pseudo-acyclic intermediates will lead to mixtures of isomeric enediols characteristic of the parent carbohydrate. By removal of the C-2 hydrogen atom through enolization, the pseudo-acyclic intermediates formed from α-D-glucopyranose and β-D-mannopyranose should yield the same *cis*-enediol. Similarly, the same *trans*-enediol will be formed from β-D-glucopyranose and α-D-mannopyranose. The principal enolization for D-fructose would be between C-1 and C-2, but because of the presence of two hydrogen atoms at C-1, the enolization would give both isomeric 1,2-enediols. Formation of a 2,3-*trans*-enediol should also be possible by removal of the C-3 hydrogen atom. Consequently, both an aldose and a ketose may yield mixtures of *cis*- and *trans*-enediols, the proportions of which being determined by the parent carbohydrate.

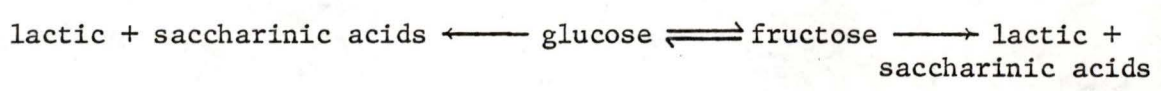
It should be pointed out that Isbell's proposal (23) of *cis*- and *trans*-enediol intermediates formed from different carbohydrates was not new to the literature. Topper and Stetten (24) had explained the C-2 epimerization reaction of D-glucose-1-d to D-mannose in saturated calcium hydroxide solution by a *cis*-enediol--D-fructose--*trans*-enediol mechanistic scheme. Isbell, did, however, provide a mechanism for ring opening which would allow formation of these *cis*- and *trans*-enediol intermediates from the cyclic form of the carbohydrate.

From a comparison of enolization rates studied by carbohydrate tritium (^3H) exchange in basic water-t solutions, Isbell et al. (23) were able to compare the relative enolization rates of several carbohydrates. In general, it was found that the rates of hydrogen-tritium exchange for ketoses is appreciably higher than those for aldoses. With D-glucose as the standard (relative enolization rate = 1), D-mannose

and D-fructose were found to have rates of 0.5 and 10.7 respectively.

Kort (21) reviewed the reactions of free carbohydrates with aqueous ammonia. Low temperatures and short reaction times were found to give transformation and degradation products, whereas, high temperatures and long reaction times were found to give products containing nitrogen.

In addition to the work of MacLaurin (2), two other kinetic studies of carbohydrate transformation reactions appear in the literature. An early paper by Bamford, Bamford and Collins (25) detailed a kinetic study of the degradation and interconversions between glucose and fructose in alkaline solutions. The change in carbohydrate concentration was followed by electrometric titration of the main degradation products, lactic and saccharinic acids, at an alkali concentration which was varied between 1 and 5N. The results were analyzed according to the following kinetic scheme:



and were taken to indicate that all the reactions which occur are first order with respect to carbohydrate concentration. No attempt was made to study interconversions to mannose.

A very recent paper by Garrett and Young (26) was a kinetic study of alkaline transformations among glucose, fructose, and mannose at low sodium hydroxide concentrations, 0.001-0.60M. The kinetic parameters of the aldose-ketose transformation were obtained by measuring the ultraviolet absorption of the acid derived hydroxymethylfurfural which was found to be proportional to the concentration of fructose in

alkali with time. The kinetic model used to evaluate the results differed from that proposed by MacLaurin (2) in that the authors assumed that no mannose was produced from either glucose or fructose, and that the only pathway to degradation products was from fructose. This recent paper will be considered more fully when the results of this present study are discussed in a following section of this thesis.

EXPERIMENTAL RESULTS

The objective of this research, as stated earlier, was to determine the reaction rate constants prevailing in a homogeneous glucose-sodium hydroxide-water system at a variety of base concentrations. The implications of these rate constants and their relationship to base concentration could then be considered.

Neutral carbohydrates arising in any amount greater than 0.5% by weight from D-glucose are expected to be limited to D-mannose and D-fructose only. This conclusion is well supported by theoretical considerations and experimental observations of others. Also, no peaks of significance other than the three carbohydrates monitored are observed on the chromatograms produced in this research.

Each of the carbohydrates known to be present in the glucose reaction system was used as starting material in a series of reaction runs at varying base concentration. The concentration of each of the three carbohydrates present in the reaction system after various reaction times was determined through resolution of a prepared sample of the reaction system by column chromatography and colorimetric analysis of the column effluent. A detailed description of the materials and solutions used is given in Appendix I and of the apparatus and procedures used in Appendix II. In brief summary, the procedures used were as follows.

The reactions were carried out in screw-capped pyrex tubes using 25 ml of the sodium hydroxide solution and one ml of the carbohydrate solution. Reaction solutions were O_2-free and were kept in the dark. The starting molarity of a carbohydrate was about 0.002. Hydroxyl ion

concentrations used were: 0.49 M, 4.02 M, 6.07 M, and 8.08 M. The relative quantities of carbohydrate and base used in a reaction system assured essentially no change in hydroxyl ion concentration during the consumption of at least half of the starting carbohydrate. Thus the expected production of acidic degradation products would have very little affect on the hydroxyl ion concentration. The reaction was quenched at the desired time interval by addition of an aliquot of the reaction mixture to a prepared amount of Amberlite IR-120-H cation-exchange resin. An aliquot from the quenched system was prepared for chromatography by admixture with a buffered boric acid solution. The carbohydrates were resolved in 5-6 hours by anion-exchange resin chromatography. The column effluent was monitored continuously with a Technicon Auto-Analyzer system using an orcinol/sulfuric acid reagent for colorimetric analysis. The analytical results were recorded on a quantitative strip chart chromatogram. Control chromatograms of a prepared mixture of the three carbohydrates of interest arising in a glucose system and chromatograms from reactions of the three different starting materials at the four sodium hydroxide concentrations used in this study are shown in Figure 10. These chromatograms show the acceptable shape of the peaks, the clear-cut resolution of the three carbohydrates, and the precision of reproduction of peak times.

The complete chromatography data for all the reaction run series are given in Appendix III. Also found in that appendix are tables of relative molarities of the three carbohydrates present in reaction systems at various reaction times. From these data the reaction rate constants were calculated using a non-linear least squares curve-fitting computer

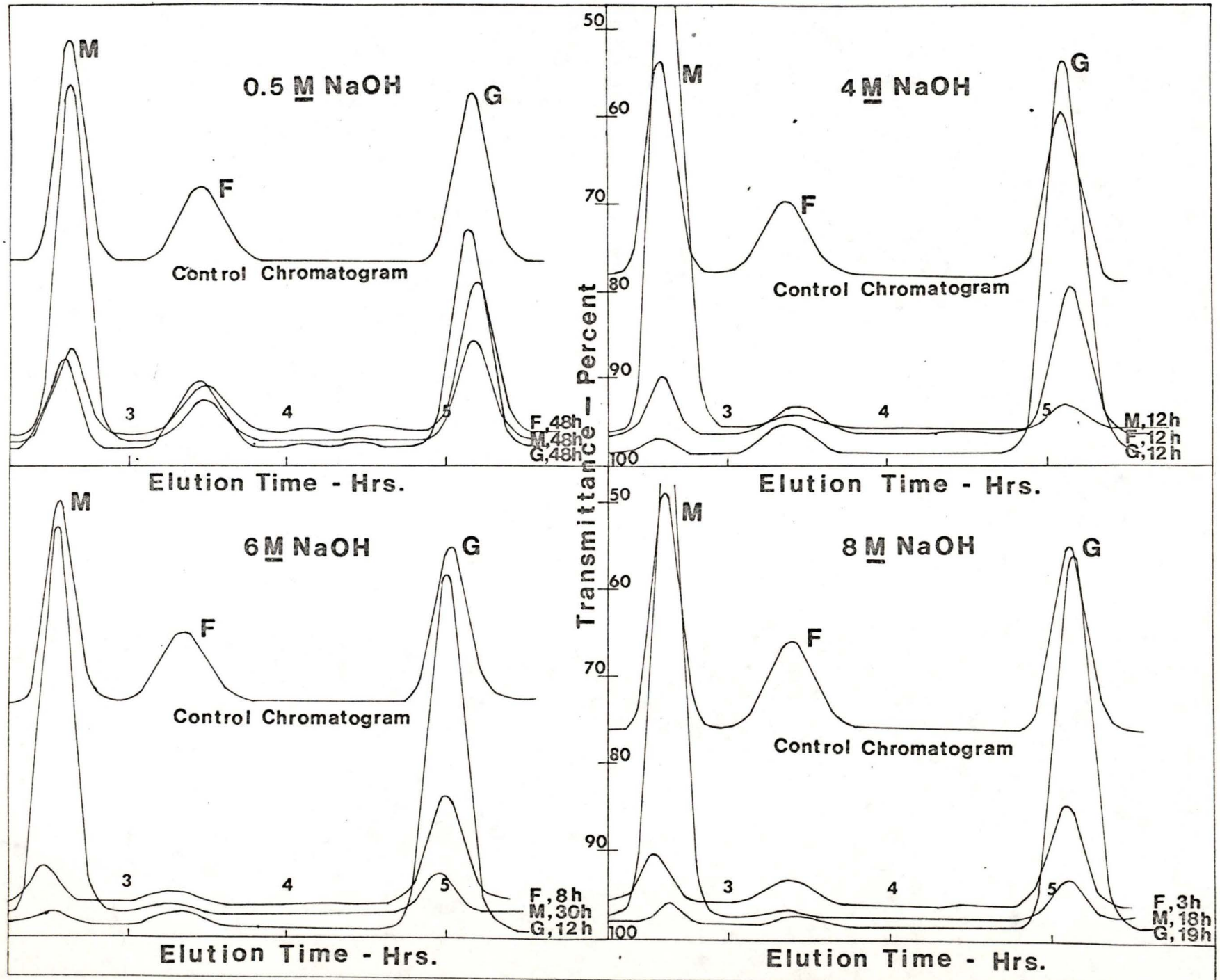


Figure 10. Typical Chromatograms
M=Mannose F=Fructose G=Glucose

program. Details of the derivation of the rate constants from the experimental data are given in Appendix IV.

Analysis of the experimentally obtained data was based on the kinetic model diagrammed in Figure 11. A listing of the computer program used is given in Appendix V. The plots of concentration vs reaction time given in Figures 12 through 14 were drawn from the computer output of the calculated relative molarity values for each experimental reaction time point. The experimental data points are plotted to demonstrate the curve fit to experimentally determined points.

The rate constant "triangles" derived by the computer are given in Figure 15. The computer calculated rate constants and their calculated standard error estimates (discussed in the following section of this thesis) at the four base concentrations are tabulated in Table I. Figure 16 compares the computer calculated rate constant "triangles" for the 0.5M NaOH system when the data is analyzed by three independent approaches. Plots of the rate constants for the various reactions vs hydroxide ion activity are given in Figure 17.

The validity of these experimental results is discussed in the following sections of this thesis along with inferences and conclusions it seems possible to draw from them.

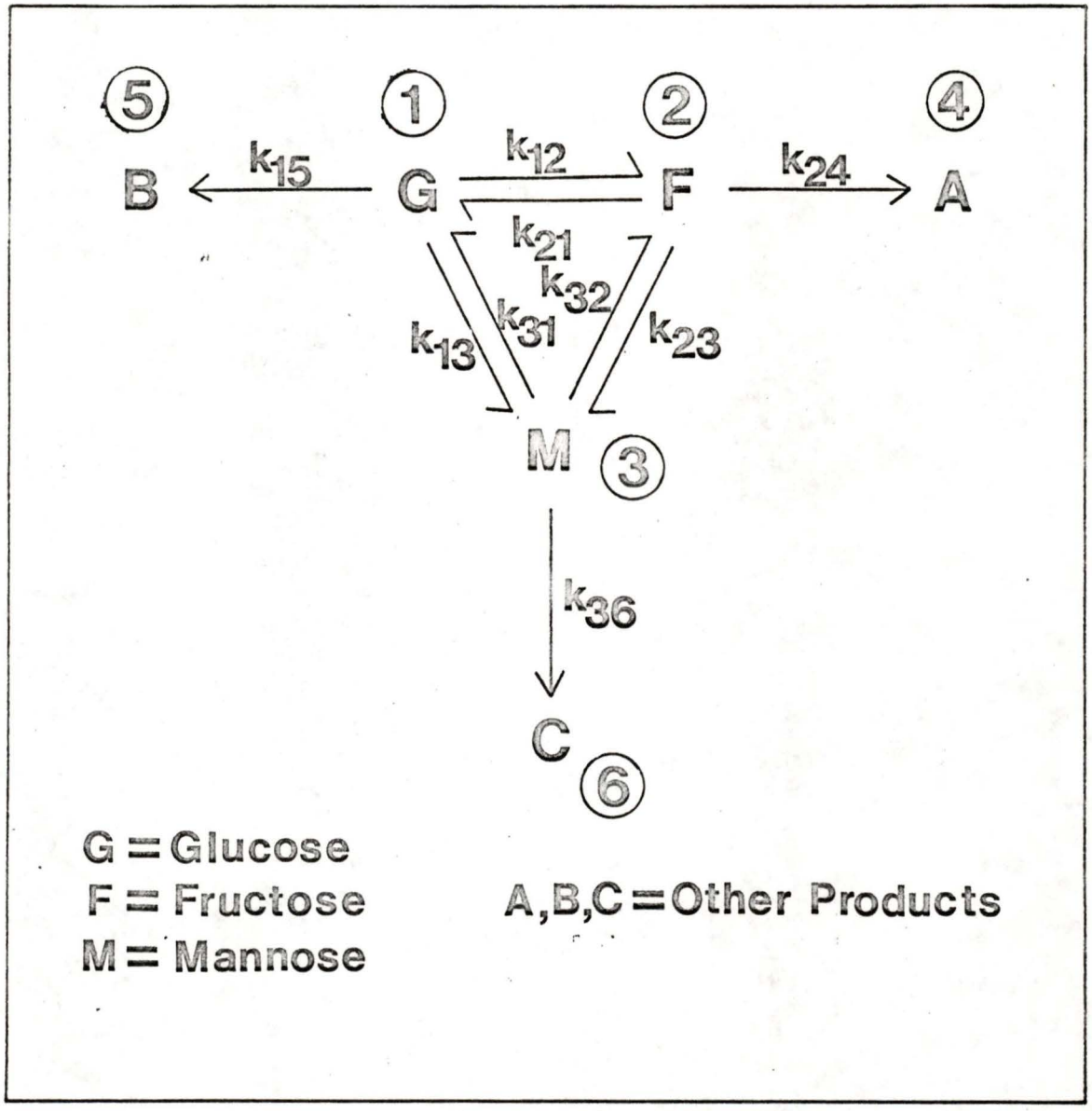


Figure 11. Glucose reaction scheme in sodium hydroxide at 25°C.

Figure 12. Glucose in NaOH at 25°C

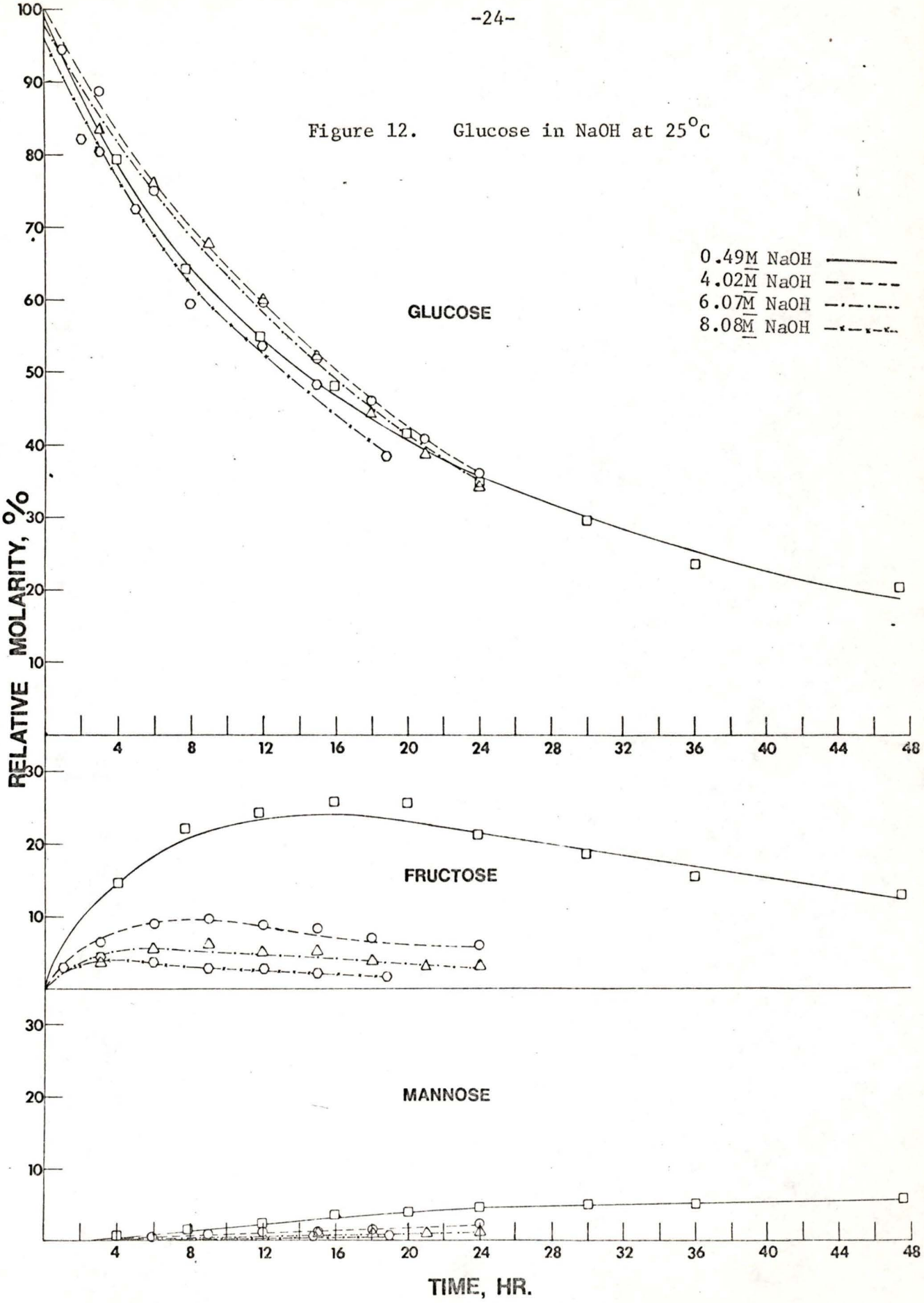


Figure 13. Fructose in NaOH at 25°C

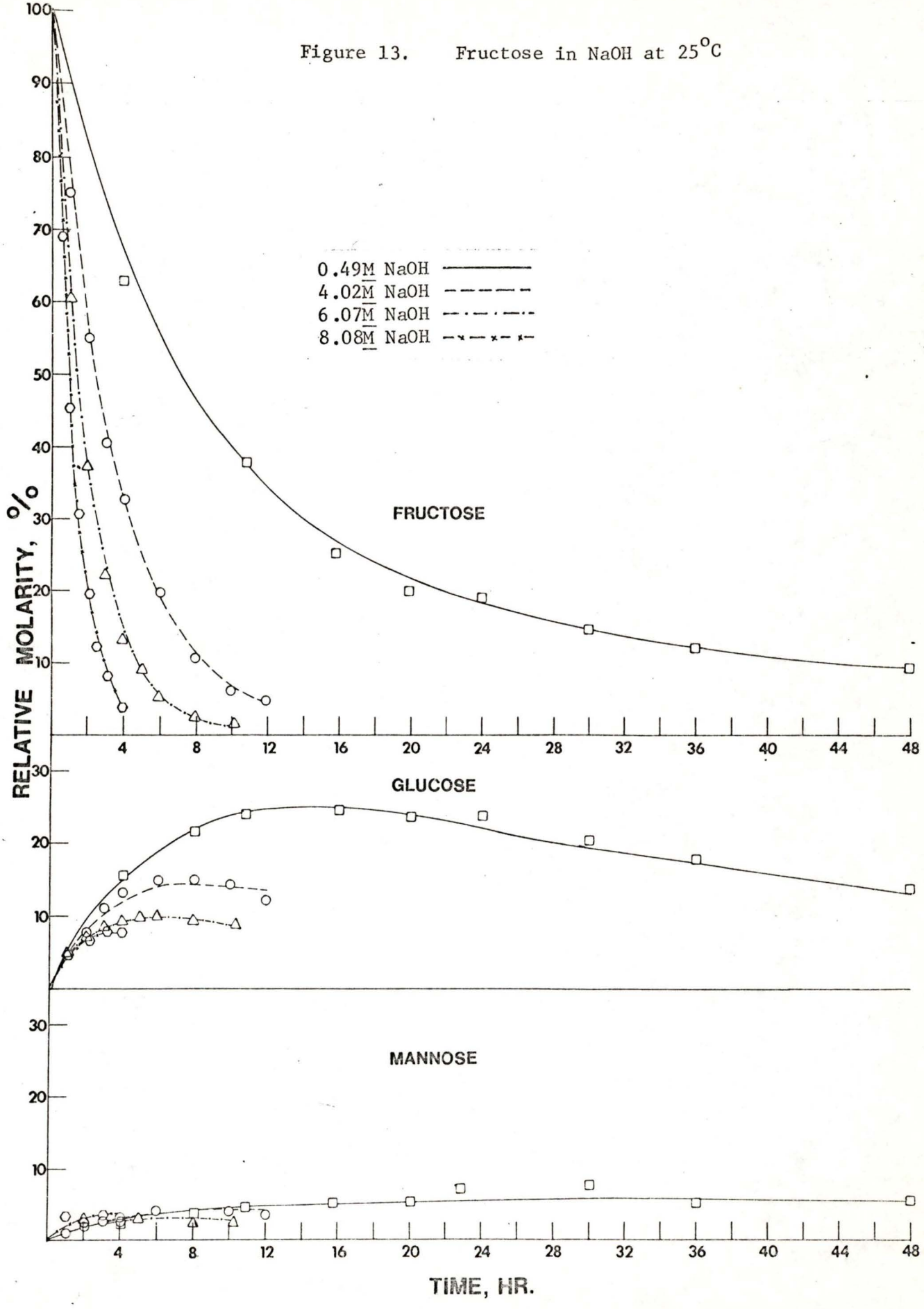
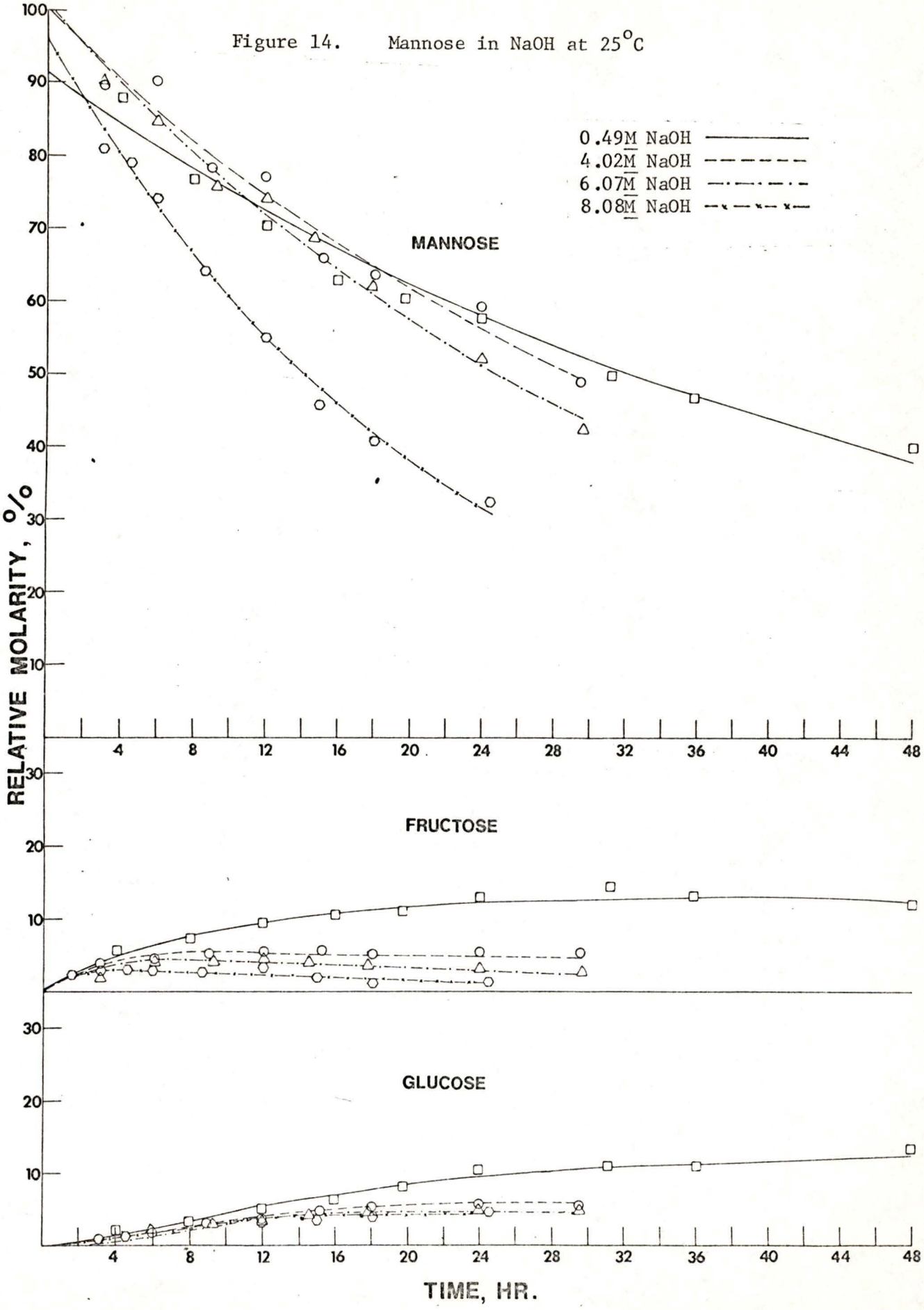
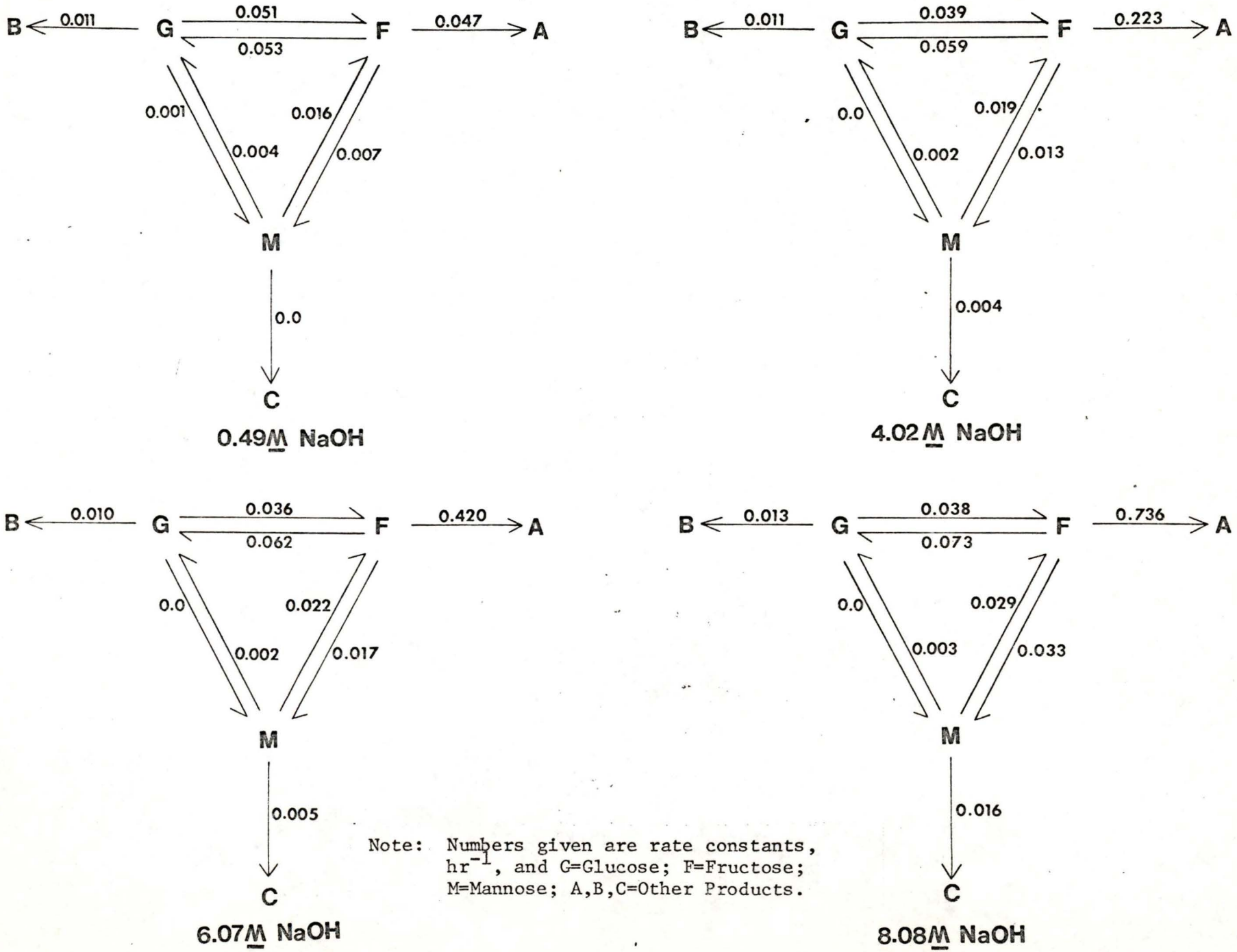


Figure 14. Mannose in NaOH at 25°C





Note: Numbers given are rate constants, hr^{-1}, and G=Glucose; F=Fructose; M=Mannose; A,B,C=Other Products.

Figure 15. Glucose Reaction Scheme in NaOH at 25°C

TABLE I

RATE CONSTANTS IN THE SYSTEM 0.002M GLUCOSE IN SODIUM HYDROXIDE AT 25°C

Reaction Pathway	Rate Constant	Rate Constant Value, hr^{-1}, at indicated NaOH concentration 0.49M	4.02M	6.07M	8.08M
G--F	k_{12}	0.051±0.001	0.039±0.001	0.036±0.001	0.038±0.002
G--M	k_{13}	0.001±0.0	0.0±0.0	0.0±0.0	0.0±0.0
G--B	k_{15}	0.011±0.002	0.011±0.001	0.010±0.002	0.013±0.002
F--G	k_{21}	0.053±0.001	0.059±0.001	0.062±0.001	0.073±0.002
F--M	k_{23}	0.007±0.0	0.013±0.0	0.017±0.001	0.033±0.001
F--A	k_{24}	0.047±0.003	0.223±0.004	0.420±0.008	0.736±0.012
M--G	k_{31}	0.004±0.0	0.002±0.0	0.002±0.0	0.003±0.0
M--F	k_{32}	0.016±0.0	0.019±0.0	0.022±0.001	0.029±0.001
M--C	k_{36}	0.0±0.0	0.004±0.001	0.005±0.001	0.016±0.002

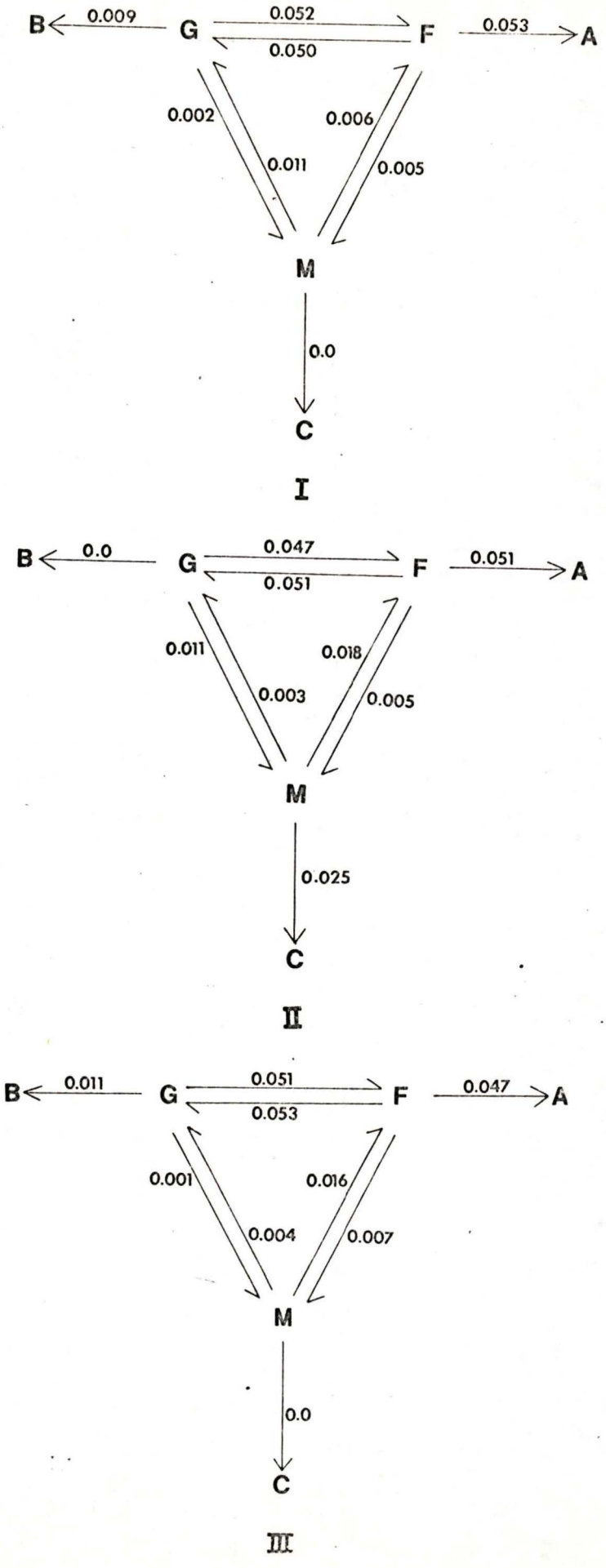


Figure 16. Glucose Reaction Scheme in 0.49M NaOH at 25°C.

I. Rate Constant triangle from analysis of glucose as starting material experimental data.

II. Rate Constant triangle from analysis of fructose as starting material experimental data.

III. Rate Constant triangle from analysis of all experimental data.

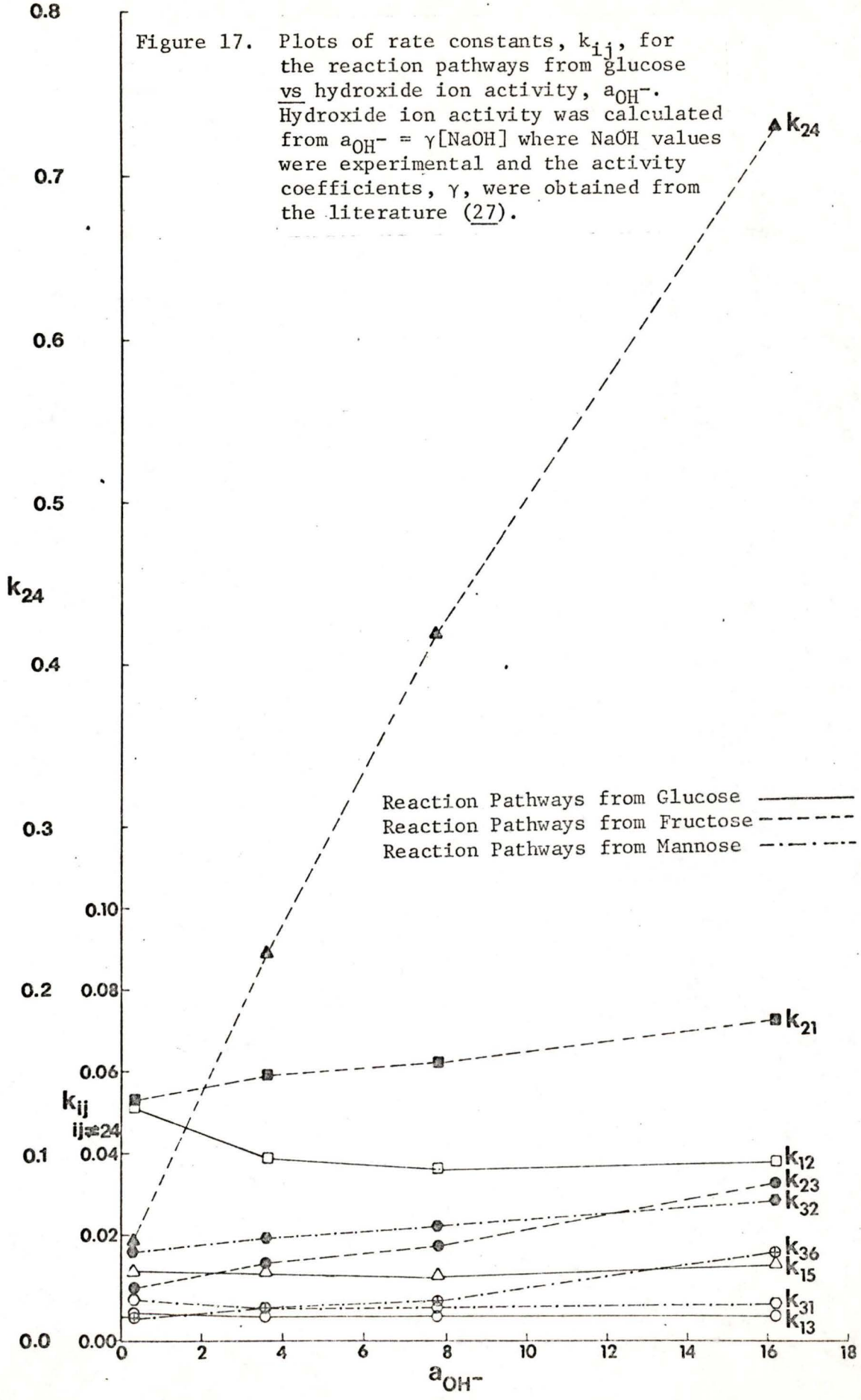


Figure 17. Plots of rate constants, k_{ij}, for the reaction pathways from glucose vs hydroxide ion activity, a_{OH^-}. Hydroxide ion activity was calculated from $a_{OH^-} = \gamma[NaOH]$ where NaOH values were experimental and the activity coefficients, γ, were obtained from the literature (27).

DISCUSSION OF THE EXPERIMENTAL RESULTS

THE EXPERIMENTAL DESIGN - AN EVALUATION

Automated anion-exchange chromatography in borate buffers has proven to be a most useful tool in the separation and identification of carbohydrates (mono- and disaccharides). This, when coupled with automatic analysis of the column effluent by a Technicon Auto-Analyzer system, has improved the precision and accuracy of quantitative analysis over that offered by quantitative paper chromatography. Paper chromatography is limited because of the relative insensitivity of the techniques in the detection of materials present in amounts below about 5% of the material present in greatest amount. The analytical technique used by MacLaurin (2) appeared to be limited only in the detection of materials present in amounts lower than 0.5% of the starting material.

It now seems that the rate constants for the several similtaneous reversible reactions occurring in the "monomer triangle" can be separated out by moving in from every corner of the triangle. In retrospect, the experimental design developed to quantitatively analyze this complex reaction system seems adequate.

ACCURACY AND PRECISION OF THE RATE CONSTANT DATA

The overall accuracy of the reaction rate constants and an estimation of their precision will be the cumulative result of combining the accuracy and precision of the basic chromatography data, the conversion of those data to relative molarities, and the fitting of the experimental rate data with computed rate curves. Each step in this sequence will now be considered.

Accuracy and Precision of the Chromatography Data

The accuracy of the chromatography data depends on two factors: (a) the purity of the carbohydrates used, and (b) their quantitative measurement. As discussed in Appendix I, the chromatographic purity of the carbohydrates was established to at least 1 in 500. One of the least accurate operations in all the weighing and volumetric operations used was the measurement of the volume of the sample delivered by microsyringe to the column for chromatography. Another operation which was potentially a source of large error was the quenching procedure using a cation-exchange resin which necessarily had an indeterminate water content.

Extensive calibration of the microsyringe used in this research indicated a delivery accuracy of at least ± 0.002 ml for any volume in the range 0.100 to 2.000 ml, delivered from any portion of the total volume in the syringe. Thus by using a sample volume of from 0.200 to 1.500 ml, an accuracy of at least 1 in 100 was obtained for this operation. During the use of the microsyringe, extreme care had to be observed to eliminate all air bubbles in the liquid sample within the syringe before delivery of a sample to the column for chromatography.

A procedure for preparing the reaction quenching resin was found and checked for reliability. Details for the procedure for the resin preparation are given in Appendix II. A series of five repeat reactions of mannose in 8 M NaOH for a zero hour reaction period were carried out to test the validity of the quenching procedure. The absorbance value found for the peak produced from the same volume of sample applied to the column had a precision of ± 0.005 or 2-3 in 100. It was thus concluded that this reaction quenching procedure was satisfactory for the

purposes of this study.

Because of the straight-line form of the absorbance vs weight relationship, as discussed in Appendix III, it follows that the sensitivity and hence measurable precision of the chromatography data for a given carbohydrate will improve with an increase in the slope of this line. The shape, and hence height of chromatogram peak for a given quantity of material are affected by a number of factors outlined by MacLaurin (2). Essentially the same conditions for chromatography as those used by MacLaurin have been adopted in this research. The only changes were the adoption of a somewhat steeper elution gradient, and a smaller quantity of resin. Both changes were designed to reduce separation times on the column. The acceptable shape of the peaks obtained in this research is clearly demonstrated in Figure 10.

In this study, a change in peak height of the smallest readable amount on the chart (about 0.001 absorbance) was equivalent to $\sim$ 0.1 µg of carbohydrate for glucose and mannose and $\sim$ 0.3 µg of carbohydrate for fructose. By selecting an amount of sample for chromatography containing 20-30 micrograms of the key carbohydrate, the precision of the readability of the chromatogram for that carbohydrate became about 1 in 200.

The precision of the chromatography data was to the major extent dependent upon the overall stability of the chromatography--Auto-Analyzer system. The short life of the acidflex tubes on the peristaltic proportioning pump, their changing performance during their life, and the lot to lot differences in these tubes contributed significantly to run to run variability. It appeared, however, that this system instability

results in a precision scatter about the best line fit of about ± 2-3% for single value determinations of weight of carbohydrate in a given sample. All values given in this study are single values. Replication of the data would, of course, give average values of higher precision.

Precision of the Conversion of the Chromatography Data to Relative Molarity Data

Precision of the converted data could also be affected by a long term drift in calibrations. However, it appears from the recalibration data for the analytical system over the five month period that the main body of kinetic data was collected that any drift in calibration of the system was slight. Hence, it is concluded that precision of the data was less affected by long term factors than by short term factors.

Precision and Accuracy of the Computer Curve-Fitting Step

A non-linear least squares analysis does not permit a simple description of the accuracy and precision of each computer calculated rate constant. However, by examining the scatter to changes in the rate constants, it is possible to reduce the problem to one of a linear approximation in order to arrive at standard error estimates.* This approximation will fail if these estimated standard errors are large in comparison to the rate constants. These estimates can then be used to calculate the usual confidence limits and hypothesis tests in a similar manner to the treatment of a measured standard deviation. The standard

*A useful discussion concerning this problem was contained in Statistics in Physical Science, Hamilton, N.C., Ronald Press Co., New York, 1964.

error estimates calculated by the computer are tabulated with the rate constants listed in Table I.

If the least squares minimum for a parameter, in this instance a rate constant, lies in a "well" rather than at a point, the relative magnitude of the calculated standard error estimate as compared to the calculated rate constant will appear large. This, however, is an indication that data scatter is affecting the program "hunt" for the least squares minimum in a "well" rather than the failure of the program to find a unique solution to the kinetic model.

An examination of the magnitude of the computer calculated standard error estimates given in Table I reveals that the estimated standard error for the large rate constants is less than 5% of the rate constant, as opposed to $\sim$ 20% for the small rate constants. These findings are consistant with the fact that small rate constants are calculated from "flat" curves and are expected to be less sensitive to changes because of data scatter about the best-fit line. From an examination of the standard error estimates it is evident that the dominant rate constants prevailing in the system have been adequately determined.

BASE CONCENTRATIONS USED

Since the major objective of this study was to determine the rate constants prevailing in the system, D-glucose--D-fructose--D-mannose, at a variety of sodium hydroxide concentrations, a discussion of the reasons for choosing the base concentrations used would be appropriate. The carbohydrate transformation reactions reported in

the literature are mostly for systems of low basicity. Therefore it seemed desireable to study these reactions at somewhat higher base concentrations.

Alkaline extraction as a stage in the bleaching and purification of wood pulp has been practiced for many years by the pulp and paper industry. Wood pulps were refined to an alpha-cellulose content of 90-93% by means of a mild caustic (0.5-6% NaOH) extraction at elevated temperatures. However, the viscose rayon industry required pulps of very high alpha-cellulose content (97-98%). Extraction of wood pulps with cold concentrated caustic (up to 17.5% NaOH) was found to yield pulps of this desired alpha-cellulose content. For this reason a sodium hydroxide concentration of 4M was chosen as one of the concentrations for study.

Kenner and Richards (28) found that the degradations of methyl-fructose, glucose and fructose in dilute limewater gave D-glucosaccharinic acids, whereas Nef (29) found mainly D-glucometasaccharinic acids and some D-glucoisosaccharinic acids from glucose in 8N sodium hydroxide. Since there appeared to be a change in mechanism leading to different degradation products at high base concentration, it was decided that a kinetic study in 8M sodium hydroxide could prove useful.

The lower concentration limit of the experimental design was 0.25-0.5M sodium hydroxide. In this region pseudo first-order assumptions remained valid. Consequently, one series of reactions was carried out in 0.5M sodium hydroxide, where the transformation reactions, especially between glucose and fructose predominate over the degradation reactions.

Finally a reaction series was carried out at a sodium hydroxide concentration intermediate between 4 and 8M since it appeared from a preliminary examination of the experimental data obtained for the reaction runs in 0.5, 4 and 8M sodium hydroxide that the greatest change in carbohydrate half-life for glucose and mannose occurred between 4 and 8M. Consequently, a final reaction series was carried out in 6M sodium hydroxide.

RESIN QUENCHING

The absorbance vs quantity of carbohydrate calibration line (discussed in Appendix III) for mannose did not give a straight line when reactions at high base concentration were quenched with hydrochloric acid. This was the reaction quenching procedure used by MacLaurin (2). The high concentration of chloride ion in a quenched reaction sample for reaction runs at high base concentration probably disrupted the separation of the carbohydrates during the anion-exchange resin chromatography employed by the automated analytical scheme. However, only the resolution of mannose was affected because it had the shortest retention time on the column. Once chloride ion was eluted by borate buffer from the column, separation proceeded normally. Fructose and glucose were thus unaffected on the column by the presence of chloride ion.

To overcome this problem, reactions were quenched with a cation-exchange resin in the hydrogen ion form, as discussed in Appendix II. The resin preference for sodium ions allowed quenching of highly alkaline systems without introducing foreign anions, which could disrupt chromatography, to the quenched reaction solutions.

CONCLUSIONS

The rate constants prevailing in the homogeneous system glucose-sodium hydroxide-water have been measured at four different base concentrations, 0.49, 4.02, 6.07 and 8.08M. Derivation of the rate constants based on the kinetic model illustrated in Figure 11 was accomplished through the use of a digital computer.

The validity of the derivation of the rate constants must be examined. Any valid mathematical technique to solve the differential equations (see Appendix IV) which describe the first order reaction system is expected to yield a unique solution to the rate constant "triangle" (Figure 11). This has been adequately demonstrated by MacLaurin (2) and Garrett and Young (26). Thus it should be possible to calculate all rate constants of the "triangle" from the concentration vs time curves produced from any one of the carbohydrates as starting material. In other words, rate constant data generated from any corner of the "triangle" should be similar. This is demonstrated in Figure 16, which shows the rate constant "triangle" for reactions in 0.49M NaOH calculated from an analysis of the experimental data for the glucose decay curve and mannose and fructose appearance curves when glucose is the starting carbohydrate (Figure 16I), and the "triangle" derived from the equivalent treatment when fructose is the starting carbohydrate (Figure 16II). For comparison purposes, the rate constant "triangle" obtained from an analysis of all of the experimental data (nine curves) is also shown (Figure 16III). Further support for both the uniqueness of solution to the "triangle" and the

consistency of the experimental data with the proposed kinetic model (Figure 11) is found in the fact that an analysis of all of the experimental data produces a good fit of computer calculated concentration vs time curves to experimental data.

The rate constant data computed from an analysis of all of the experimental data is expected to minimize errors. Consequently, conclusions which it now seems possible to formulate will be based on the computer-fitted concentration vs time curves (Figures 12 through 14) and the computer calculated rate constants (Table I and Figure 15), all of which are calculated from an analysis of the total of the experimental data.

Since MacLaurin (2) had not detected any other transformation products in more than trace amounts, this study was confined to an analysis for glucose, fructose and mannose only. As is evident in Figure 10, the chromatograms of fructose and glucose as starting materials in 0.49M NaOH for 48 hours display one or two shallow peaks in that region of the chromatogram between the fructose and glucose peaks. Further work in systems of low alkalinity would be necessary to attempt an identification of these peaks which likely represent only trace amounts of other neutral carbohydrates. Chromatograms produced in this research at base concentrations above 4M NaOH gave little indication of other neutral carbohydrates as reaction products.

A preliminary examination of the carbohydrate decay curves (Figures 12 through 14) reveals that mannose is the most stable of the carbohydrates in sodium hydroxide at all base concentrations studied. Its stability is relatively constant in the sodium hydroxide concentration range 0.5-6M (half-life of $\sim$ 25-30 hours) but alkali sensitivity

increases sharply in the concentration range 6-8M, the half-life in 8M NaOH being reduced to 14 hours. Glucose stability in sodium hydroxide appears to be independent of alkali concentration. A 16-fold increase in the base concentration produces almost no change in the half-life for glucose (13-16 hours at all base concentrations). Fructose is the least stable of the carbohydrates to alkali attack, and in fact, the preferred reaction pathways are those leading from fructose in all systems, and especially at higher base concentrations. A 16-fold increase in the sodium hydroxide concentration produces a decrease in the half-life for fructose of a similar magnitude.

MacLaurin (2) observed that the forward and reverse reactions between carbohydrates had rate constants of similar magnitude. It appears from this research (Figure 15) that for the gluco-manno and fructo-manno reaction pathways that this observation is somewhat valid at all base concentrations studied. MacLaurin also observed that the gluco-fructo pathway had approximately equal rate constants. This is confirmed in this research only at the lowest base concentration studied, but Garrett and Young (26) also found approximately equal rate constants for the gluco-fructo path (G$\rightarrow$F, 0.067 hr^{-1}; F$\rightarrow$G, 0.070 hr^{-1}) for reactions in 0.20M NaOH at 25°C. Thus the equality of the rate constants in the gluco-fructo pathway may not be fortuitous at sodium hydroxide concentrations of 1M or less and at low temperatures (20-25°C).

Another observation made by MacLaurin (2) was that the magnitudes of the rate constants for the reversible pathways fall into three levels, a very low level for the gluco-manno system, a higher level for

the manno-fructo system and the highest value for the gluco-fructo pathway. This observation is applicable to all base concentrations studied in this research as well. The isomerization pathways must have a much lower energy barrier compared to the epimerization pathway.

From the very small magnitude of the gluco-manno epimerization rate constants, it is apparent that mannose formed from glucose arises via the "detour" route through fructose at all base concentrations studied. The "direct" route to formation of mannose from glucose is perhaps important only during the initial stages of reaction when fructose quantities are small.

The degradation pathway leading from fructose (F→A) is the most significant of the three degradation pathways (see Table I and Figure 15) and, particularly at the higher base concentrations, this reaction pathway dominates the entire system. This finding was expected since Isbell et al. (23) found that the enolization rate for fructose was considerably higher than that for glucose or mannose. At high sodium hydroxide concentrations the formation of 2,3-enediols and their related degradation products such as isosaccharinic acids from fructose must be more significant. As well, degradative reactions via a 1,2-enediol are expected to be prevalent since fructose can form both the *cis* and *trans*-1,2-enediols (see Figure 9).

Garrett and Young (26) based their analysis of the glucose-fructose-mannose system on a kinetic model which had been simplified from that proposed by MacLaurin (2). Simplifying assumptions made were: (a) that negligible ammounts of mannose are formed from either glucose

or fructose, (b) that the gluco-fruc equilibrium is fast compared to the degradation to other products, and (c) that the only allowed pathway to degradation products was fr fructose. It appears from this research that these assumptions ar alid for sodium hydroxide concentrations up to 0.5-0.6M only. At her concentrations the validity of all of the assumptions is que onable. Consequently, it is evident that MacLaurin's proposed kine model (see Figure 11) most accurately describes the kinetics of the system at all sodium hydroxide concentrations to 8M.

In apparent contrast to Garrett and Young's findings (26) that all of the first-order rate constants were negative functions of hydroxide ion activity, a_{OH^-}, except that for the fructose degradation route, in a low sodium hydroxide concentration range, it appears from the results of this research (see Figure 17) that some of the reaction pathways show either no hydroxide dependence, only a little, or a high degree of dependency. The reaction pathways from glucose have relatively consistent rate constant values throughout the entire base concentration range studied here. These values are k_{12}(G→F):∿0.04-0.05 hr^{-1}, k_{13}(G→M):negligible, and k_{15}(G→B):∿0.01 hr^{-1}. Two of the reaction pathways from mannose have rate constant values which demonstrate some hydroxide ion activity dependence. The rate constant values for the mannose to fructose pathway (k_{32}) vary from 0.016 to 0.029 hr^{-1} over the sodium hydroxide concentration range, and those for the mannose to degradation products pathway (k_{36}) vary from a negligible value to 0.016 hr^{-1} in 8M NaOH. The rate constant for the third pathway from mannose, i.e. mannose to glucose (k_{31}), has a constant value of ∿0.003 hr^{-1}.

The reaction rate constant values for the pathways from fructose all demonstrate a definite dependence on hydroxide ion activity. These values are k_{21}(F→G):∿0.05 to 0.075 hr^{-1}, k_{23}(F→M):∿0.005 to 0.030 hr^{-1} and k_{24}(F→A):∿0.05 to 0.75 hr^{-1}.

Corbett and Kenner (6) have postulated that metasaccharinic and isosaccharinic acids are formed from fructose in strong base by a pathway involving divalent and trivalent enediolate anions. These anions are formed from fructose by the removal of protons from hydroxyl groups as well as hydrogens attached to carbons which are located alpha to the carbonyl group. The large hydroxide ion activity dependence demonstrated by the fructose to degradation products pathway (F→A) in this research perhaps reflects the increasing ease of removal of protons from hydroxyl groups by hydroxide ion to form di- and trivalent anions as the hydroxide ion concentration increases.

LITERATURE CITED

1. Wohl, A. and Neuberg, C., Ber., 33:3099(1900).

2. MacLaurin, D.J. and Green, J., Can. J. Chem., 47:3947(1969) and 47:3957(1969).

3. Lobry de Bruyn, C.A. and Alberda van Ekenstein, W., Rec. trav. chim., 19:1(1900) and earlier publications.

4. LeMieux, R.U. In Molecular Rearrangements, edited by de Mayo, P., Interscience Publishers, New York, 1964, p. 743.

5. Isbell, H.S., J. Research Natl. Bur. Standards, 32:45(1944).

6. Kenner, J. and Richards, G.N., J. Chem. Soc. (London),278, 1784, 3277(1954); 2916,2921(1956); Corbett, W.M. and Kenner, J., J. Chem. Soc. (London), 3274,3281(1954); and Corbett, W.M., Kenner, J. and Richards, G.N., J. Chem. Soc. (London), 1709(1955).

7. Sowden, J.C., Advan. Carbohyd. Chem., 12:35(1957).

8. Speck, J.C., Advan. Carbohyd. Chem., 13:63(1958).

9. Shaw, P.E., Tatum, J.H. and Berry, R.E., Carbohyd. Res., 5:266(1967).

10. Doering, W. von E. and Urban, R.S., J. Am. Chem. Soc., 78:5938(1956).

11. Ishizu, A., Lindberg, B. and Theander, O., Carbohyd. Res., 5:329(1967).

12. Lindberg, B. and Theander, O., Acta Chem. Scand., 22:1782(1968).

13. Kenner, J. and Richards, G.N., J. Chem. Soc., 1932(1960).

14. Komoto, M., Nippon Nogei Kagaku Kaishi, 36:461,464,546(1962).

15. Kenner, J. and Richards, G.N., J. Chem. Soc., 2916(1956).

16. Rowell, R.M., Somers, P.J., Barker, S.A. and Stacey, M., Carbohyd. Res., 11:17(1969).

17. Rowell, R.M. and Green, J., Carbohyd. Res., 15:197(1970).

18. Feather, M.S., Carbohyd. Res., 7:86(1968).

19. Feather, M.S. and Harris, J.F., Carbohyd. Res., 15:304(1970).

20. Feather, M.S., Tetrahedron Letters, 48:4143(1970).

21. Kort, M.J., Advan. Carbohyd. Chem., 25:311(1970).

22. Litowsky, I., Avigad, G. and Englard, S., Carbohyd. Res., 14:365 (1970).

23. Isbell, H.S., Frush, H.L., Wade, C.R.W. and Hunter, C.E., Carbohyd. Res., 9:163(1969).

24. Topper, Y.J. and Stetten, D., J. Biol. Chem., 191(1951).

25. Bamford, C.H., Bamford, D. and Collins, J.R., Proc. Roy. Soc., 204:85(1950).

26. Garrett, E.R. and Young, J.F., J. Org. Chem., 35:3502(1970).

27. Stokes, R.H., J. Am. Chem. Soc., 67:1689(1945).

28. Kenner, J. and Richards, G.N., J. Chem. Soc., 1784(1954).

29. Nef, J.V., Ann., 89:376(1910).

30. Kesler, R.B., Anal. Chem. 39, no.12:1416(1967).

31. Matsen, F.A. and Franklin, J.L., J. Am. Chem. Soc., 72:3337(1950).

APPENDIX I

MATERIALS AND SOLUTIONS

CARBOHYDRATES

The glucose, fructose, and mannose used in this research were from commercial sources. The glucose and mannose samples were supplied by Dr. D. J. MacLaurin and had already been tested for chromatographic purity (2). The commercially available fructose was purified before use. At no time did a chromatogram of the fructose used as a calibration standard show even a minor impurity peak (greater than 1%) although chromatographed at least one dozen times, and at differing elution gradients.

D-glucose:

Baker AR Dextrose, anhydrous powder. Found to be ∿ 99.9% pure and dry as received. Used as received.

D-fructose:

Fisher Scientific, crystals. Recrystallized twice from 95% ethanol. Used after drying over Drierite.

D-mannose:

Matheson, Coleman and Bell, anhydrous powder. Recrystallized from methanol-isopropanol and ethanol-acetone. Used after drying over Drierite.

SOLUTIONS

0.12 M H_3BO_3, pH 8.50

30.00 ± 0.01 g H_3BO_3 (Matheson, Coleman and Bell) + 4000 ± 1 ml distilled water + nominal 12 M KOH (∿ 10 ml) to pH 8.50 ± 0.05. Solution filtered through 2 Whatman GF/A, 5.5-cm glass filter papers supported on a Millipore sintered disk-and-funnel unit. Stored at room temperature (R.T.) in bulk quantities (∿ 20 ℓ) in a clear glass bottle. The pH of the solution was checked occasionally, but no change occurred over a 1-2 month period.

0.15 M $K_2B_4O_7$, pH 9.4

184.0 ± 0.05 g $K_2B_4O_7.4H_2O$ (Allied Chemicals) + 4000 ± 1 ml distilled water and filtered as above. Stored at R.T. in bulk quantity (∿ 20 ℓ) in a clear glass bottle.

0.50 M $K_2B_4O_7$, pH 9.7

611.0 ± 0.5 g $K_2B_4O_7.4H_2O$ (Allied Chemicals) + 4000 ± 1 ml distilled water and filtered as above. Stored at R.T. in a clear glass bottle.

Orcinol/70% H_2SO_4

95.5-96.5%, sp. gr. 1.84, H_2SO_4 (Allied Chemicals) slowly added with external cooling to 300 ± 0.5 ml distilled water in a 1-liter volumetric flask and to 60 ± 0.1 ml distilled water in a 200-ml volumetric flask. The addition of the final 1-2 ml of H_2SO_4 to the calibration line was done after the well-mixed aqueous H_2SO_4 solution had stood at least 12 hours at R.T. The 1200 ml of 70% H_2SO_4 was slowly poured onto 2.10 ± 0.001 g of orcinol monohydrate

(K and K Laboratories, used as received) in a 2-liter wide mouth brown glass bottle. The orcinol/H_2SO_4 was stirred for ½ hour with a magnetic stirring bar to complete solution of the orcinol. Solutions were not used until all traces of orcinol had dissolved. No solutions more than 12 hours old were used for quantitative work, since the solution color deepens with time. This volume of color reagent was sufficient for two consecutive 6 hour chromatography runs or ∿ 14 hours of operation.

NaOH Solutions

In a teflon beaker, 500 g NaOH (Fisher, pellets) was dissolved in 500 ml of freshly prepared, boiled, N_2-saturated distilled water. This "lye oil" (∿ 20 M in NaOH) was let stand under N_2 in a thick-walled, 1-liter, tightly capped polypropylene bottle at R.T. for at least 2 weeks prior to use to allow Na_2CO_3 to settle out. Aliquots of the clear supernate were transferred by pipet to the required amount of freshly prepared, boiled, N_2-saturated, distilled water in a polyethylene bottle in which the stock NaOH solution was to be stored. The NaOH solution was standardized against $HKC_8H_4O_4$ (Mallinckrodt Primary Standard) to a phenolpthalein endpoint. Using this procedure, MacLaurin (2) found the carbonate content of NaOH solutions prepared in this way to be entirely satisfactory.

APPENDIX II

APPARATUS AND EXPERIMENTAL PROCEDURES

CONSTANT-TEMPERATURE BATH

A Colora Ultra-Thermostat Constant-Temperature Bath and Circulator was used. The water level was kept at a constant level by a feed line which would add water to the bath when the water level fell below the desired level. Cold tap water was run at a low flow rate through the cooling coil of the bath at all times during operation. This assembly maintained the temperature with a precision of ± 0.1°C.

RESIN COLUMN CHROMATOGRAPHY AND AUTOMATED ANALYTICAL EQUIPMENT

A schematic diagram showing the equipment and its arrangement as used for the chromatographic separation and quantitative analysis of the compounds of interest in reaction mixtures is shown in Figure 18. All components and accessories except the Haake type FJ constant-temperature circulator were supplied by the Technicon Corporation, Tarrytown, N.Y.

REACTION RUN PROCEDURES

A separate small reaction batch was run for any given reaction time. The procedures used were:

A 1.000 ± 0.001-ml volume of an aqueous solution (boiled, N_2-saturated, distilled water) of the chosen starting carbohydrate (1.000-1.010 ± 0.0001 g/100.0 ml) was delivered under nitrogen by micro-syringe to a 200 mm x 25 mm pyrex tissue-culture tube which had a Teflon-lined black plastic screwtop. Onto the carbohydrate solution

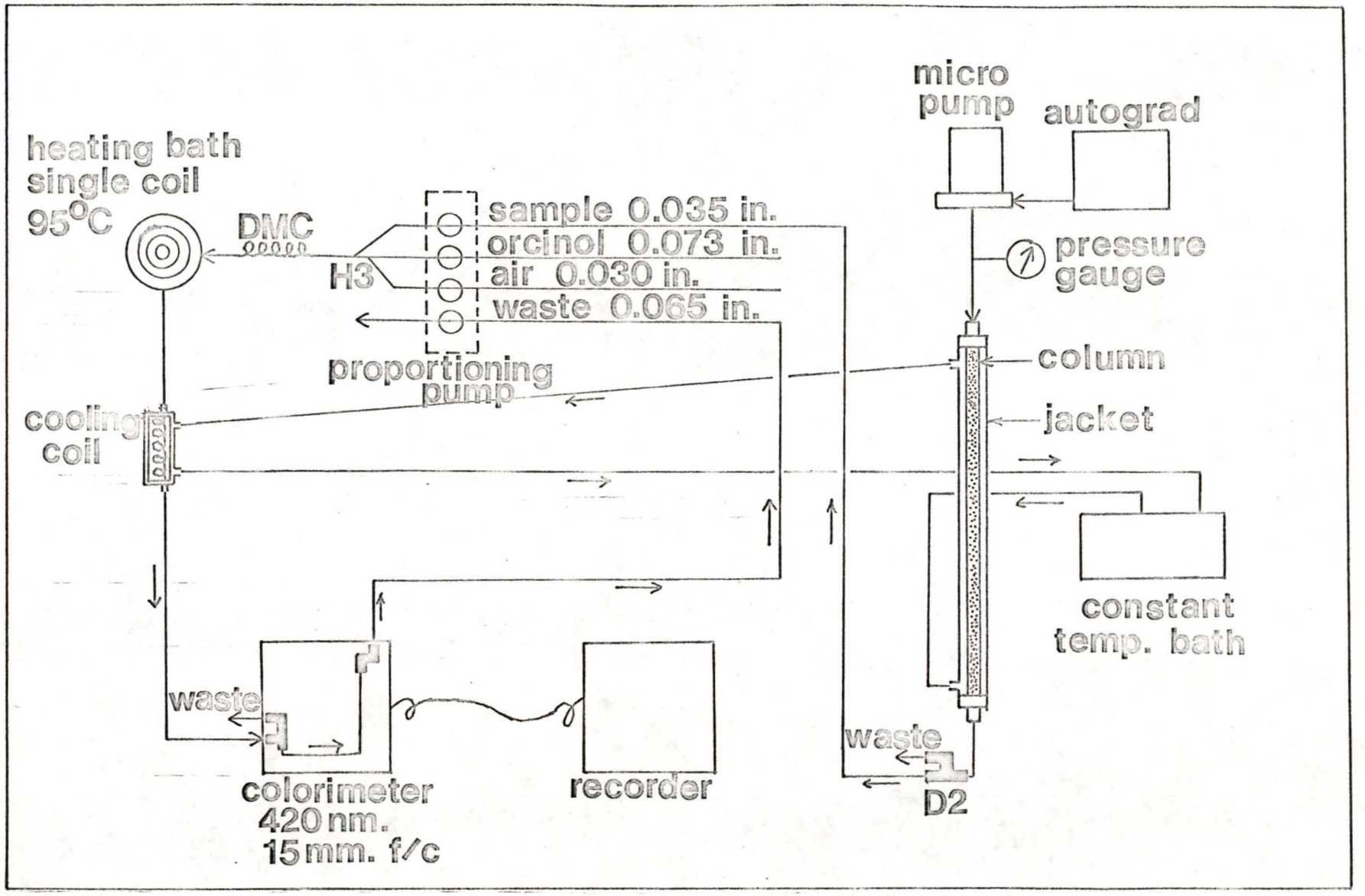


Figure 18. Apparatus -- Schematic Diagram.
Note: All tubing carrying orcinol/H_2SO_4 reagent is Acidflex.

was then delivered by pipet under nitrogen, 25.00 ± 0.01 ml of the sodium hydroxide solution. The tube cap was screwed on snugly and the system mixed by shaking. The tube was then wrapped in several layers of aluminum foil and marked for identification. The wrapped tube was suspended by wires into the constant-temperature bath where it was free to be gently rocked by the swirling waters in the bath. The time at which the first NaOH hit the carbohydrate solution was taken as the zero of reaction time. The reaction was quenched at the end of the desired reaction period, and a sample was prepared for column chromatography as described in the next section.

REACTION RUN CHROMATOGRAPHY SAMPLE PREPARATION

A 5.00 ± 0.01-ml (25°C) volume of a reaction mixture was delivered by pipet onto the required amount of Amberlite IR-120-H cation-exchange resin for quenching (See Table II). The stopping time of the reaction was defined as when the last of the 5-ml reaction sample entered the vigorously stirring slurry of resin. Within 5 seconds complete quenching had occurred and the resin slurry was then cooled in the constant-temperature bath.

The resin for the quenching of each reaction mixture was prepared as follows. Approximately 50 grams of bulk resin stored in distilled water was vacuum filtered through a Millipore sintered disk-and-funnel unit. A 35 gram portion of the filtered resin was then placed in a large centrifuge tube and spun at high speed for 5 minutes. Excess water was displaced to the bottom of the centrifuge tube. The required amount of resin for quenching a particular reaction mixture was then weighed into a beaker from the

top half of the centrifuge tube. This ensured a quantity of resin of constant water content. To the weighed sample of resin in the beaker was added 10.00 ± 0.01-ml (R.T.) of distilled water. This enabled the resin slurry to be stirred with a magnetic stirring bar to reduce quenching time.

A 5.00 ± 0.01-ml (25°C) aliquot of the slightly acidic quenched reaction mixture was combined in a capped glass vial with 5.00 ± 0.01-ml (R.T.) volume of 0.24 M H_3BO_3, pH 8.0. This gave a solution, pH ∿ 8, 0.12 M in H_3BO_3. The H_3BO_3 concentration was thus the same as at the beginning of the subsequent elution.

Table II

NaOH Concentration	Weight of IR-120-H resin used for quenching
0.5 M	10.00 ± 0.05 g
4 M	15.00 ± 0.05 g
6 M	20.00 ± 0.05 g
8 M	25.00 ± 0.05 g

CHROMATOGRAPHY AND AUTO-ANALYZER CONDITIONS

The following conditions were established for column chromatography and Auto-Analyzer operation.

Column:

3 mm i.d. x 750 mm water-jacketed glass tube column. Resin bed length of 690 mm.

Resin:

Aminex A-15, Bio-Rad Laboratories, Richmond, California. Spherical beads. Particle size: 17.5 ± 2 μ. The resin was cleaned before use by the following sequence of treatments with various reagents. The resin was vacuum filtered and rinsed thoroughly with distilled water between reagent treatment steps.

Sequence:

1. distilled water
2. acetone
3. methanol
4. 0.2 M NaCl
5. ∿ 1 M HNO_3, let stand in a gently boiling water bath for several hours.
6. 0.5 M potassium tetraborate
7. distilled water

Column Temperature:

30.0 ± 0.1°C, measured in the Haake circulator bath.

Eluent Flow Rate:

A Positive Displacement Pump setting of 20.0, which gave a flow rate of ∿ 0.5 ml/min. The precision of the flow rate was found to be ± 0.01 ml/min and was independent of the column back pressure. This pump setting gave a maximum pressure drop of about 800 p.s.i.g. across the column, which occurred during column washing with 0.5 M $K_2B_4O_7$.

Elution System:

In the nine-chambered, rectangular-form Autograd:

Chambers 1-2, each 100 ml of 0.12 M H_3BO_3, pH 8.5

Chambers 3-5, each 100 ml of 0.15 M $K_2B_4O_7$, pH 9.4

Chambers 6-9, empty

Proportioning Pump Tubing:

Sample from column	color code - orange, Tygon, 0.035 in. i.d.
Air Supply	color code - black, Tygon, 0.030 in. i.d.
Orcinol/H_2SO_4 reagent	color code - green, Acidflex, 0.073 in. i.d.
From colorimeter cell	color code - blue, Acidflex, 0.065 in. i.d.

Micropipet:

Gilmont Ultraprecision Microsyringe: 2.5 ml capacity, CANLAB, Toronto, Ontario.

CHROMATOGRAPHY RUN PROCEDURES

The top column fitting was unclamped with the positive displacement pump off. The pump was then started on 0.12 M H_3BO_3, pH 8.50, and the top fitting was slowly ejected until it could be lifted free. Pumping was continued with the column fitting inverted until all air was removed from the fitting. The fitting was then clamped in the inverted position while a sample was being applied to the column.

The liquid was then removed with a disposable pipet down to the resin bed surface. This could be easily done without disturbing the resin. The flange seat and wide area above the resin bed was wiped with a facial tissue before application of a column sample. The reaction sample, generally 0.200-1.500 ml, was then delivered slowly to the column with

the microsyringe. The syringe needle was placed against the column wall during this operation. The liquid above the resin was then forced into the resin with nitrogen at 50 p.s.i.g. The nitrogen supply was shut off when the liquid was about 1/4 inch above the resin bed, and the pressure was released slowly by unclamping the nitrogen fitting, and manually easing it off just as the liquid met the resin bed surface. About 0.3 ml of 0.12 $\underline{M}$ H_3BO_3, pH 8.50, was then delivered to the column above the resin in such a manner that the column inner walls were thoroughly rinsed. This liquid was also forced into the resin with nitrogen as before. The column was then filled to near overflowing with 0.12 $\underline{M}$ H_3BO_3, pH 8.50, and the top column fitting was inverted, being careful to leave a drop hanging from the fitting. This drop was meshed with the convex liquid surface at the top of the column in such a manner that air would be excluded from the column system. The column fitting was then forced into the column against a significant back pressure. Extruded liquid as a result of this operation was caught with a facial tissue. The back pressure was so strong, if air had not been entrained in the system, that it was necessary to use a vinyl-coated column clamp to force the fitting completely into the column. Once the fitting was in place and the pressure had dropped below 100 p.s.i.g., the vinyl-coated clamp was repositioned, and a second clamp was placed in position over the coated clamp. The positive displacement pump suction line was then connected to the Autograd. The Autograd stirrer was switched on, and the inter-chamber valves opened in sequence, beginning with the one farthest from the outlet chamber. The positive displacement pump, and the recorder "chart drive" were then switched on similtaneously, and approximately

one minute later, the Auto-Analyzer sample line was connected to the bottom of the column when the system pressure was greater than 600 p.s.i.g.

It was found that although no obvious air bubbles appeared in the positive displacement pump inlet line, occasionally there was a pressure drop during the first hour of pumping. This was indicative that air bubbles were trapped in the pump head. The situation could be quickly remedied by the following procedure. The pump and recorder "chart drive" were switched off, and the outlet valve of the Autograd was closed. The pump outlet line was removed and the end immersed in 0.12 M H_3BO_3, pH 8.50. The Autograd outlet valve was then lifted and closed immediately. This operation forced any trapped air through the pump head, but occasionally the Autograd valve needed to be opened and closed two or three times more to remove all air. With the outlet of the pump full to near overflowing, the outlet line was reconnected, then the Autograd outlet valve was re-opened, and the pump and recorder "chart drive" were switched on again.

Upon completion of a run, the column was immediately washed for 1/2 hour with 0.5 M $K_2B_4O_7$ and then for 1/2 hour with 0.12 M H_2BO_3, pH 8.50, by pumping through to the drain with the positive displacement pump.

AUTO-ANALYZER PROCEDURES

The Auto-Analyzer was started about 45 minutes prior to being connected to the column. The recorder "instrument" switch and the colorimeter "power" switch were turned on. Then the proportioning pump sample line was placed into a 0.12 M H_3BO_3, pH 8.50, solution and the reagent line into the freshly prepared orcinol/H_2SO_4 solution. The proportioning

pump manifold tubing array end block was placed in position on the pump platen alignment pegs and the tubing checked for correct positioning. The roller assembly was swung down into place and checked for firm latching. Then the proportioning pump switch was turned on. In about 35 minutes, liquid progressed through the Auto-Analyzer system to the colorimeter. When a constant liquid flow through the colorimeter was established, the colorimeter flow cell was then cleared of entrained air by manually pinching off the line from the flow cell to the proportioning pump at a point close to the flow cell. The pinch was suddenly released after four or five slugs of air had passed the cell. Occasionally the pinching procedure had to be repeated several times. The recorder "chart drive" was then turned on (the chromatography set-up was in operation at this point) and the photoelectric circuitry was adjusted to 95% transmittance with the "%T" control. A blank (0) aperture was inserted between the light source and the colorimeter flow cell, and the recorder pen was adjusted to infinite absorbance (2%) with the "zero" control. The blank aperature was then removed.

After 1 hour of operation a final baseline adjustment to 98% transmittance was made, and the infinite absorbance setting was rechecked.

At the completion of a run, the recorder "chart drive", "instrument", and colorimeter "power" switches were turned off. The sample line was disconnected from the column and placed into distilled water. The reagent line was removed from the orcinol/H_2SO_4 solution, rinsed with distilled water, wiped with a tissue, and immersed in a separate container of distilled water. Distilled water was allowed to pump through the entire system for 15 minutes. The line ends were then taken out of the distilled

water, and pumping was continued until the system was pumped free of water (a further 10 minutes). The proportioning pump was switched off, and then the roller assembly was unlatched and left in a vertical position. The end block at the suction end of the tubing manifold was lifted from its positioning pegs and laid on the pump platen to permit the tubes to relax under no tension. A set of platen manifold tubing was replaced due to slight deterioration (cracking and splitting) after every 20 column runs.

APPENDIX III

THE CHROMATOGRAPHY DATA FROM THE REACTION RUNS AND CONVERSION OF IT TO CONCENTRATIONS OF CARBOHYDRATES

CHROMATOGRAPHY DATA FROM REACTION RUNS

Twelve series of reaction runs were made for the generation of final kinetic data using the materials and solutions described in Appendix I, and the procedures detailed in Appendix II. Reaction series A, B and C with glucose, fructose, and mannose respectively as starting materials were completed at one base concentration before beginning a reaction series at a different base concentration. Four base concentrations for each of the series and the chromatography data obtained are given in Tables III through XIV.

CALIBRATION OF ABSORBANCE AGAINST WEIGHT OF CARBOHYDRATE

Calibration of chromatography data against weights of carbohydrate materials of interest in the sample applied to the column (and hence concentrations in the corresponding reaction system) was done as follows. Kesler (30) and MacLaurin (2) had shown that the Lambert-Beer's law applied for the color reaction between various carbohydrates and orcinol in H_2SO_4. Using the equipment and procedures of this research, rectangular plots of absorbance against quantity of the color producing material gave straight lines through the origin. This straight line relationship was found to be true below a 40 microgram (μg) sample of any of the carbohydrates analyzed for. Accordingly, chromatography data was calibrated and converted to concentrations of carbohydrates in the reaction systems in

this study. Typical data obtained for an absorbance vs µg of carbohydrate plot is given in Table XV, and is plotted in Figure 19.

Chromatograms for calibration of kinetic data were run from solutions (boiled, N_2-saturated, distilled water, and 0.12 M H_3BO_3 at pH 8.50) containing a single carbohydrate or all three carbohydrates each at 0.0050-0.0100 ± 0.00005 g/100.0 ml. Large scale plots (500 mm x 450 mm) of absorbance vs µg carbohydrate were made from the calibration data generated by chromatographing the standard carbohydrate solutions. The best-fit (visual) straight lines were then drawn through the origin and the experimental points. Each time the platen manifold tubing on the proportioning pump was replaced, the system was recalibrated by chromatographing the standard carbohydrate solutions at intervals throughout the twenty column run life-time of the tubing.

From the absorbance vs µg carbohydrate plots, the weight of each carbohydrate present in a reaction mixture could be read off. These weight values were converted to the relative molar data given in Tables XVI through XXVII by comparing the ratio of the weight of carbohydrate in a reaction mixture divided by the volume of sample applied to the column to the ratio of the weight of carbohydrate used as starting material for that particular series in a 0.0 hr reaction system divided by the volume of sample applied to the column; i.e.

Relative Molarity %

$$= \frac{\text{molarity of carbohydrate (product, x hr reaction time)}}{\text{molarity of carbohydrate (starting material, zero hr reaction time)}} \times 100$$

$$= \frac{\mu g \text{ (product)} \times 10^{-6}/180.16 \times \text{vol of column sample in ml} \times 10^{-3}}{\mu g \text{ (starting material)} \times 10^{-6}/180.16 \times \text{vol of column sample in ml} \times 10^{-3}} \times 100$$

$$= \frac{\mu g \text{ (product)/ml (column sample)}}{\mu g \text{ (starting material)/ml (column sample)}} \times 100$$

The relative molar data of Tables XVI through XXVII were used to generate reaction rate constants as described in Appendix IV, and to obtain the plots of relative molarities vs reaction time shown as Figures 12 through 14 in the main body of this thesis.

TABLE III.

REACTION SERIES A (GLUCOSE) - CHROMATOGRAPHY DATA

$[G]_o$ = 0.00215; $[OH^-]_o$ = 0.49 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time, hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	158	0.400	--	0.	--	--	0.	--	5:15	0.218	24.1
4.03	161	0.600	2:36	0.003	0.2	3:28	0.021	5.1	5:11	0.260	28.8
7.75	162	0.800	2:36	0.009	0.7	3:27	0.043	10.6	5:09	0.280	31.0
11.85	163	0.900	2:35	0.015	1.2	3:27	0.054	13.2	5:09	0.271	30.0
16.00	165	1.000	2:36	0.023	1.9	3:27	0.064	15.6	5:08	0.263	29.1
19.95	167	1.100	2:38	0.030	2.4	3:29	0.069	17.0	5:10	0.252	27.8
24.08	170	1.200	2:36	0.037	3.0	3:27	0.064	15.6	5:08	0.228	25.2
30.00	169	1.200	2:33	0.038	3.1	3:26	0.054	13.2	5:07	0.193	21.3
36.05	168	1.200	2:35	0.041	3.3	3:27	0.045	11.0	5:07	0.153	17.0
47.50	159	1.200	2:36	0.050	4.0	3:28	0.036	8.8	5:09	0.132	14.7

TABLE IV.

REACTION SERIES B (FRUCTOSE) - CHROMATOGRAPHY DATA

$[F]_o$ = 0.00214; $[OH^-]_o$ = 0.49 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	129	0.400	--	0	--	3:30	0.096	23.2	--	0	--
3.98	132	0.500	2:38	0.008	0.6	3:29	0.076	18.3	5:11	0.041	4.5
7.97	134	0.600	2:38	0.015	1.2	3:29	0.083	20.0	5:12	0.070	7.6
10.88	135	0.750	2:36	0.022	1.8	3:27	0.068	16.5	5:09	0.095	10.3
15.87	136	1.000	2:36	0.037	2.9	3:28	0.061	14.8	5:10	0.132	14.3
19.92	137	1.200	2:37	0.046	3.7	3:28	0.058	14.0	5:11	0.149	16.2
23.90	142	1.200	2:38	0.061	4.8	3:29	0.053	13.1	5:12	0.153	16.5
30.02	143	1.200	2:38	0.069	5.4	3:28	0.041	10.2	5:10	0.133	14.3
36.00	145	1.200	2:38	0.050	3.9	3:29	0.034	8.5	5:10	0.115	12.4
47.98	130	1.200	2:38	0.047	3.7	3:29	0.026	6.3	5:13	0.085	9.3

TABLE V.

REACTION SERIES C (MANNOSE) - CHROMATOGRAPHY DATA

$[M]_o$ = 0.00214; $[OH^-]_o$ = 0.49 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	141	0.400	2:37	0.306	23.9	--	0	--	--	0	--
3.93	148	0.500	2:37	0.338	26.3	3:27	0.006	1.6	5:11	0.005	0.6
7.93	149	0.600	2:40	0.355	27.6	3:30	0.010	2.5	5:12	0.010	1.1
11.92	150	0.700	2:38	0.384	29.8	3:29	0.015	3.8	5:11	0.019	2.1
16.05	151	0.800	2:37	0.389	30.2	3:27	0.020	5.0	5:09	0.028	3.1
19.73	153	0.800	2:38	0.375	29.1	3:29	0.021	5.3	5:10	0.035	3.8
23.97	154	1.000	2:36	0.444	34.6	3:27	0.031	7.7	5:09	0.058	6.2
31.15	155	1.000	2:38	0.385	29.9	3:27	0.034	8.5	5:11	0.060	6.5
35.86	157	1.000	2:36	0.362	28.1	3:27	0.031	7.7	5:11	0.066	7.1
48.02	146	0.800	2:38	0.249	19.4	3:29	0.023	5.7	5:10	0.057	6.2

TABLE VI.

REACTION SERIES A (GLUCOSE) - CHROMATOGRAPHY DATA

$[G]_o$ = 0.00215; $[OH^-]_o$ = 4.02 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	185	0.500	--	0	--	--	0	--	5:04	0.245	27.5
3.00	188	0.600	2:34	0.002	0.1	3:27	0.009	2.2	5:09	0.262	29.3
5.93	190	0.750	2:34	0.004	0.3	3:23	0.014	3.5	5:06	0.276	31.0
9.13	191	0.900	2:33	0.007	0.5	3:22	0.019	4.8	5:05	0.340	38.0
11.97	193	1.000	2:35	0.009	0.7	3:23	0.019	4.8	5:07	0.294	32.9
14.95	194	1.100	2:34	0.011	0.9	3:23	0.020	5.0	5:04	0.282	31.5
18.08	196	1.200	2:36	0.014	1.1	3:25	0.018	4.6	5:07	0.274	30.6
20.97	197	1.500	2:43	0.045	3.6	3:32	0.045	11.3	5.13	0.304	34.0
23.97	187	1.500	2:36	0.024	1.9	3:27	0.020	5.0	5:08	0.269	30.1

TABLE VII.

REACTION SERIES B (FRUCTOSE) - CHROMATOGRAPHY DATA

$[F]_o$ = 0.00214; $[OH^-]_o$ = 4.02 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	172	0.500	--	0	--	3:29	0.100	24.5	--	0	--
1.00	174	0.750	2:35	0.006	0.5	3:27	0.113	27.8	5:08	0.016	1.8
2.02	175	1.000	2:35	0.012	1.0	3:27	0.110	27.0	5:09	0.034	3.7
3.03	177	1.200	2:35	0.020	1.6	3:25	0.098	24.1	5:06	0.060	6.6
3.98	178	1.200	2:35	0.024	1.9	3:25	0.077	19.3	5:07	0.069	7.7
5.95	179	1.500	2:35	0.035	2.8	3:26	0.058	14.6	5:08	0.098	11.0
7.98	181	1.500	2:39	0.035	2.8	3:30	0.032	8.0	5:10	0.098	11.0
10.07	182	1.500	2:36	0.034	2.7	3:24	0.019	4.7	5:10	0.095	10.6
12.00	173	1.500	2:35	0.030	2.4	3:26	0.015	3.7	5:08	0.081	9.0

TABLE VIII.

REACTION SERIES C (MANNOSE) - CHROMATOGRAPHY DATA

$[M]_o$ = 0.00214; $[OH^-]_o$ = 4.02 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	198	0.500	2:34	0.316	26.0	--	0	--	--	0	--
3.00	200	0.600	2:37	0.336	28.0	3:21	0.004	1.2	5:04	0.004	0.5
6.05	252	0.700	2:32	0.378	33.0	3:15	0.006	1.6	4:55	0.006	0.7
8.95	204	0.700	2:35	0.344	28.5	3:23	0.0065	1.9	5:02	0.010	1.2
11.98	253	0.800	2:32	0.370	32.2	3:15	0.008	2.3	4:57	0.013	1.5
15.17	205	0.900	2:34	0.373	31.1	3:20	0.009	2.6	5:00	0.019	2.3
18.00	206	1.000	2:32	0.396	33.1	3:17	0.010	2.8	4:58	0.024	2.8
24.00	199	1.500	2:37	0.530	46.6	3:21	0.013	3.6	5:06	0.038	4.5
29.47	207	1.000	2:35	0.311	25.6	3:21	0.008	2.3	5:03	0.024	2.8

TABLE IX.

REACTION SERIES A (GLUCOSE) - CHROMATOGRAPHY DATA

$[G]_o$ = 0.00215; $[OH^-]_o$ = 6.07 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	226	0.500	--	0	--	--	0	--	5:01	0.225	27.0
3.00	228	0.600	2:32	0.001	0.1	3:22	0.004	1.1	5:03	0.226	27.1
5.93	229	0.600	2:30	0.004	0.3	3:21	0.006	1.7	5:01	0.207	24.8
9.02	231	0.750	2:30	0.005	0.4	3:19	0.009	2.5	4:59	0.230	27.6
12.00	233	0.900	2:31	0.007	0.6	3:20	0.009	2.5	5:00	0.243	29.2
14.95	234	1.000	2:30	0.008	0.7	3:19	0.010	2.8	5:00	0.236	28.3
18.08	235	1.200	2:32	0.010	0.9	3:21	0.008	2.4	5:02	0.241	28.9
21.02	236	1.200	2:40	0.010	0.9	3:24	0.007	2.0	5:05	0.209	25.0
23.98	227	1.200	2:32	0.012	1.1	3:21	0.007	2.0	5:00	0.185	22.2

TABLE X.

REACTION SERIES B (FRUCTOSE) - CHROMATOGRAPHY DATA

$[F]_o$ = 0.00214; $[OH^-]_o$ = 6.07 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	215	0.500	--	0	--	3:21	0.091	25.1	--	0	--
1.00	217	0.750	2:29	0.008	0.8	3:18	0.083	23.0	5:00	0.016	1.9
2.03	218	1.000	2:30	0.016	1.4	3:21	0.068	18.7	5:03	0.031	3.6
2.92	219	1.200	2:30	0.020	1.7	3:21	0.049	13.5	5:02	0.043	5.2
3.98	220	1.200	2:29	0.019	1.6	3:19	0.029	8.0	5:00	0.048	5.8
4.97	221	1.500	2:29	0.024	2.1	3:19	0.025	6.9	5:00	0.061	7.4
6.02	222	1.500	2:31	0.023	2.0	3:21	0.015	4.2	5:01	0.064	7.6
8.00	223	1.500	2:29	0.020	1.7	3:16	0.007	2.0	4:59	0.058	7.0
10.25	216	1.500	2:34	0.019	1.7	3:21	0.005	1.4	5:01	0.056	6.8

TABLE XI.

REACTION SERIES C (MANNOSE) - CHROMATOGRAPHY DATA

$[M]_o$ = 0.00214; $[OH^-]_o$ = 6.07 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	238	0.500	2:33	0.276	24.0	--	0	--	--	0	--
2.98	241	0.600	2:31	0.300	26.1	3:15	0.002	0.6	4:57	0.004	0.5
5.97	242	0.600	2:34	0.281	24.4	3:17	0.004	1.1	4:58	0.005	0.6
9.23	244	0.750	2:34	0.315	27.4	3:17	0.005	1.4	4:58	0.009	1.0
11.95	245	0.800	2:34	0.326	28.4	3:17	0.006	1.6	4:59	0.011	1.3
14.62	247	0.900	2:33	0.341	29.7	3:17	0.006	1.6	4:57	0.014	1.6
17.88	248	1.000	2:34	0.344	30.0	3:18	0.006	1.6	4:58	0.016	1.9
23.92	250	1.100	2:34	0.317	27.6	3:17	0.005	1.4	4:58	0.021	2.5
29.57	240	1.200	2:33	0.283	24.6	3:17	0.005	1.4	4:58	0.023	2.7

TABLE XII.

REACTION SERIES A (GLUCOSE) - CHROMATOGRAPHY DATA

$[G]_o$ = 0.00215; $[OH^-]_o$ = 8.08 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	102	0.500	--	0	--	--	0	--	5:08	0.223	23.8
0.53	103	1.000	2:35	0.001	0.1	3:25	0.002	0.6	5:17	0.413	44.2
0.98	113	0.500	2:39	0.0015	0.15	3:25	0.003	0.7	5:09	0.211	43.0
2.02	105	1.000	2:34	0.002	0.2	3:27	0.006	1.5	5:08	0.367	39.2
3.00	106	1.000	2:34	0.003	0.25	3:23	0.007	1.75	5:05	0.358	38.4
5.97	109	1.000	2:36	0.004	0.3	3:27	0.006	1.5	5:09	0.322	34.4
8.98	110	1.500	2:37	0.008	0.6	3:26	0.008	2.0	5:08	0.400	42.6
12.02	111	1.500	2:38	0.008	0.6	3:25	0.008	2.0	5:09	0.361	38.4
14.73	107	1.000	2:36	0.006	0.5	3:25	0.004	1.1	5:08	0.215	23.0
18.87	112	1.500	2:39	0.011	0.8	3:25	0.006	1.5	5:09	0.254	27.3

TABLE XIII.

REACTION SERIES B (FRUCTOSE) - CHROMATOGRAPHY DATA

$[F]_o$ = 0.00214; $[OH^-]_o$ = 8.08 M; T °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	87	0.600	--	0	--	3:26	0.109	27.1	--	0	--
0.50	88	0.600	--		--	3:24	0.075	18.7	--		--
0.50	92	1.800	2:31	0.023	2.2	--		--	5:06	0.024	2.5
1.00	89	0.600	--		--	3:23	0.050	12.4	--		--
1.00	93	1.800	2:29	0.034	3.3	--		--	5:02	0.039	4.1
1.50	90	1.800	2:31	0.037	3.6	3:23	0.101	25.2	5:08	0.047	4.9
2.10	91	1.800	2:32	0.036	3.4	3:25	0.064	15.9	5:06	0.055	5.8
2.48	94	1.800	2:30	0.034	3.3	3:21	0.040	10.0	5:04	0.056	5.9
3.10	95	1.800	2:29	0.032	3.1	3:19	0.027	6.7	5:03	0.063	6.6
3.97	96	1.800	2:32	0.024	2.3	3:23	0.012	3.0	5:06	0.059	6.2

TABLE XIV.

REACTION SERIES C (MANNOSE) - CHROMATOGRAPHY DATA

$[M]_o$ = 0.00214; $[OH^-]_o$ = 8.08 M; T, °C = 25 ± 0.1 Absorbance (Abs.) measured at 420 nm

			MANNOSE			FRUCTOSE			GLUCOSE		
Reaction Time hr	Column Run No.	Volume of Sample	Peak Time hr:min	Abs.	µg M	Peak Time hr:min	Abs.	µg F	Peak Time hr:min	Abs.	µg G
0.00	120	0.500	2:40	0.290	22.6	—	0	—	—	0	—
1.48	118	0.500	2:39	0.287	22.4	3:27	0.002	0.5	5:11	0.002	0.3
2.95	117	1.000	2:38	0.472	36.8	3:25	0.004	1.0	5:11	0.007	0.7
4.48	121	1.000	2:37	0.458	35.8	3:26	0.005	1.2	5:07	0.007	0.8
6.03	122	1.000	2:38	0.429	33.4	3:26	0.005	1.2	5:09	0.009	1.0
8.63	123	1.000	2:38	0.372	29.0	3:25	0.005	1.2	5:09	0.011	1.2
12.03	125	1.000	2:38	0.320	25.0	3:26	0.006	1.5	5:09	0.013	1.5
14.97	126	1.500	2:36	0.402	31.3	3:24	0.005	1.2	5:08	0.021	2.3
18.00	127	1.500	2:38	0.358	27.9	3:23	0.004	1.0	5:08	0.022	2.5
24.47	114	1.500	2:35	0.284	22.1	3:27	0.004	1.0	5:08	0.027	2.9

TABLE XV.

TYPICAL ABSORBANCE VS WEIGHT DATA
FROM CHROMATOGRAPHY CALIBRATION STUDIES

GLUCOSE

Column Run Number	Wt. Applied µg	Abs. 420 nm	Peak Time hr:min
79	10.4	0.108	5:09
85	15.6	0.150	5:07
80	20.8	0.199	5:04
84	26.0	0.239	5:08

FRUCTOSE

79	10.8	0.042	3:27
82	15.6	0.061	3:23
80	21.6	0.088	3:21
81	31.2	0.127	3:24

MANNOSE

79	10.6	0.135	2:39
78	12.5	0.166	2:33
80	21.2	0.258	2:36
83	25.0	0.332	2:38

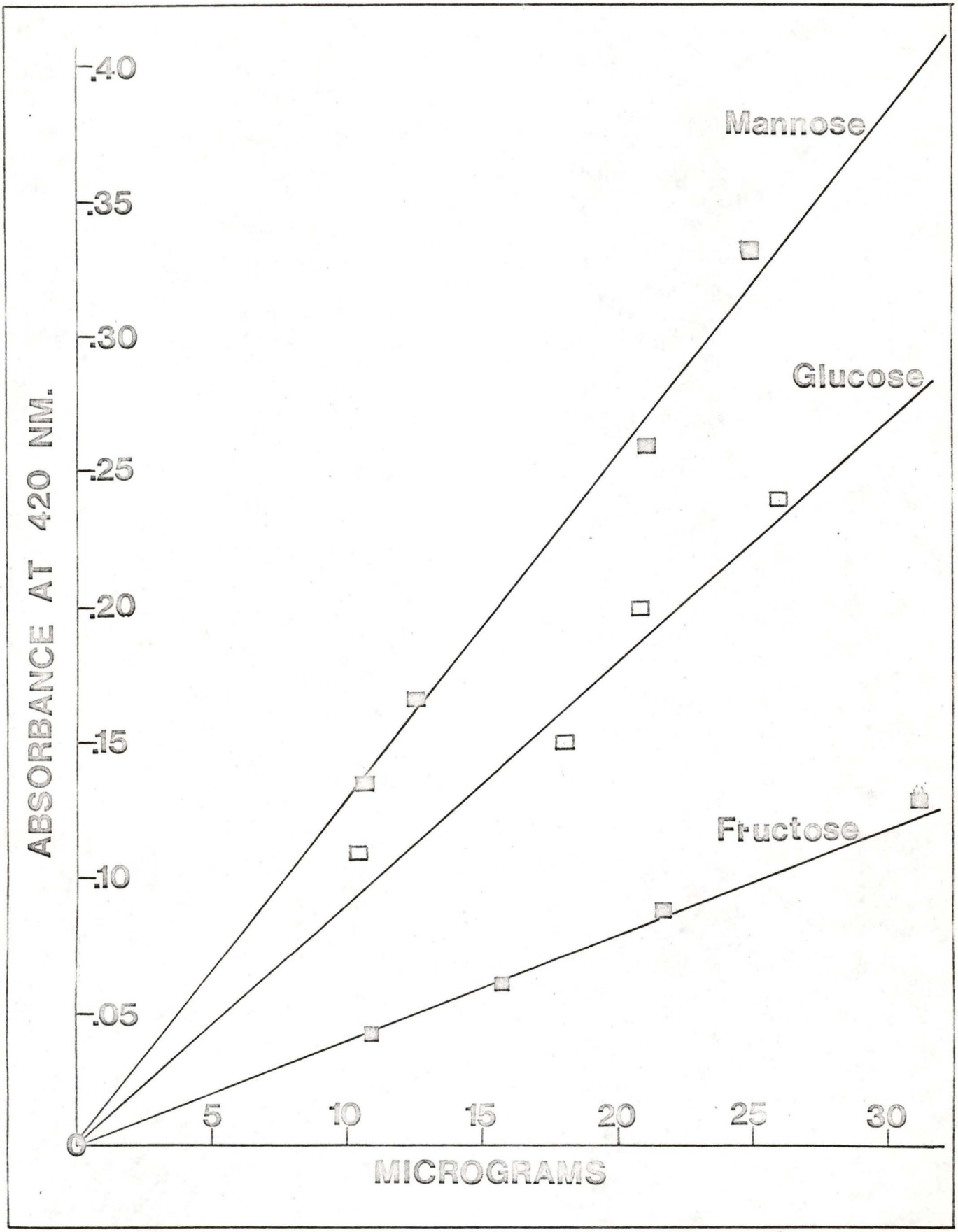


Figure 19. Typical Calibration Plots

TABLE XVI.

REACTION SERIES A (GLUCOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[G]_o = 0.00215$; $[OH^-]_o = 0.49$ M; T, $^oC = 25 \pm 0.1$

Reaction Time, hr	Glucose, % M G_o	Mannose, % M G_o	Fructose % M G_o
0.00	100.0	0.0	0.0
4.03	79.7	0.6	14.1
7.75	64.3	1.5	22.0
11.85	55.3	2.2	24.3
16.00	48.3	3.2	25.9
19.95	41.9	3.6	25.7
24.08	34.9	4.1	21.6
30.00	29.5	4.3	18.3
36.05	23.5	4.6	15.2
47.50	20.3	5.5	12.2

TABLE XVII.

REACTION SERIES B (FRUCTOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[F]_o = 0.00214$; $[OH^-]_o = 0.49$ M; T, $^oC = 25 \pm 0.1$

Reaction Time, hr	Fructose, %M F_o	Mannose, %M F_o	Glucose, %M F_o
0.00	100.0	0.0	0.0
3.98	63.1	2.1	15.5
7.97	57.5	3.4	21.8
10.88	37.9	4.1	23.7
15.87	25.5	5.0	24.6
19.92	20.1	5.3	23.3
23.90	18.8	6.9	23.7
30.02	14.7	7.8	20.5
36.00	12.2	5.6	17.8
47.98	9.0	5.3	13.4

TABLE XVIII.

REACTION SERIES C (MANNOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[M]_o = 0.00214$; $[OH^-]_o = 0.49$ M; T, $^oC = 25 \pm 0.1$

Reaction Time, hr	Mannose, %M M_o	Fructose, %M M_o	Glucose, %M M_o
0.00	100.0	0.0	0.0
3.93	87.9	5.3	2.1
7.93	76.9	7.0	3.1
11.92	71.2	9.1	5.0
16.05	63.1	10.4	6.5
19.73	60.8	11.1	7.9
23.97	57.8	12.9	10.4
31.15	50.0	14.2	10.9
35.86	47.0	12.9	11.9
48.02	40.5	11.9	13.0

TABLE XIX.

REACTION SERIES A (GLUCOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[G]_o = 0.00215$; $[OH^-]_o = 4.02$ M; T, $^oC = 25 \pm 0.1$

Reaction Time, hr	Glucose, %M G_o	Mannose, %M G_o	Fructose, %M G_o
0.00	100.0	0.0	0.0
3.00	88.8	0.3	6.7
5.93	75.2	0.7	8.5
9.13	76.7	1.0	9.7
11.97	59.8	1.3	8.7
14.95	52.1	1.5	8.3
18.08	46.4	1.7	7.0
20.97	41.2	4.4*	13.7*
23.97	36.5	2.3	6.1

*These values were not included as data for computer least squares curve fitting.

TABLE XX.

REACTION SERIES B (FRUCTOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[F]_o = 0.00214$; $[OH^-]_o = 4.02$ M; T, °C = 25 ± 0.1

Reaction Time, hr	Fructose, %M F_o	Mannose, %M F_o	Glucose, %M F_o
0.00	100.00	0.0	0.0
1.00	75.6	1.4	4.9
2.02	55.1	2.0	7.6
3.03	41.0	2.7	11.2
3.98	32.8	3.2	13.1
5.95	19.9	3.8	15.0
7.98	10.9	3.8	15.0
10.07	6.4	3.7	14.4
12.00	5.0	3.3	12.2

TABLE XXI.

REACTION SERIES C (MANNOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[M]_o = 0.00214$; $[OH^-]_o = 4.02$ M; T, °C = 25 ± 0.1

Reaction Time, hr	Mannose, %M M_o	Fructose, %M M_o	Glucose, %M M_o
0.00	100.0	0.0	0.0
3.00	89.7	3.8	1.6
6.05	90.7	4.4	1.9
8.95	78.3	5.2	3.1
11.98	77.4	5.5	3.6
15.17	66.5	5.6	4.9
18.00	63.7	5.4	5.4
24.00	59.7	4.6	5.8
29.47	49.2	4.4	5.4

TABLE XXII.

REACTION SERIES A (GLUCOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[G]_o = 0.00215$; $[OH^-]_o = 6.07$ M; T, °C = 25 ± 0.1

Reaction Time, hr	Glucose, %M G_o	Mannose, %M G_o	Fructose, %M G_o
0.00	100.0	0.0	0.0
3.00	83.6	0.3	3.4
5.93	76.5	0.9	5.2
9.02	68.1	1.0	6.2
12.00	60.1	1.2	5.2
14.95	52.4	1.3	5.2
18.08	44.6	1.4	3.7
21.02	38.6	1.4	3.1
23.98	34.3	1.7	3.1

TABLE XXIII.

REACTION SERIES B (FRUCTOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[F]_o$ = 0.00214; $[OH^-]_o$ = 6.07 M; T °C = 25 ± 0.1

Reaction Time, hr	Fructose, %M F_o	Mannose, %M F_o	Glucose, %M F_o
0.00	100.0	0.0	0.0
1.00	61.1	2.1	5.0
2.03	37.3	2.8	7.2
2.92	22.4	2.8	8.6
3.98	13.3	2.7	9.6
4.97	9.2	2.8	9.8
6.02	5.6	2.7	10.1
8.00	2.7	2.3	9.3
10.25	1.9	2.3	9.0

TABLE XXIV.

REACTION SERIES C (MANNOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[M]_o = 0.00214$; $[OH^-]_o = 6.07$ M; T, $^oC = 25 \pm 0.1$

Reaction Time, hr	Mannose, %M M_o	Fructose, %M M_o	Glucose, %M M_o
0.00	100.0	0.0	0.0
2.98	90.6	2.1	1.7
5.97	84.7	3.8	2.1
9.23	76.1	3.9	2.8
11.95	74.0	4.2	3.4
14.62	68.8	3.7	3.7
17.88	62.5	3.3	4.0
23.92	52.3	2.7	4.7
29.57	42.7	2.4	4.7

TABLE XXV.

REACTION SERIES A (GLUCOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[G]_o = 0.00215$; $[OH^-]_o = 8.08$ M; T, $^oC = 25 \pm 0.1$

Reaction Time, hr	Glucose, %M G_o	Mannose, %M G_o	Fructose, %M G_o
0.00	100.0	0.0	0.0
0.53	92.9	0.2	1.3
0.98	94.5	0.3	2.9
2.02	82.4	0.4	3.2
3.00	80.7	0.5	3.7
5.97	72.3	0.6	3.2
8.98	59.7	0.8	2.8
12.02	53.8	0.8	2.8
14.73	48.3	1.1	2.3
18.87	38.2	1.1	2.1

TABLE XXVI.

REACTION SERIES B (FRUCTOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[F]_o = 0.00214$; $[OH^-]_o = 8.08$ M; T, °C = 25 ± 0.1

Reaction Time, hr	Fructose, %M F_o	Mannose, %M F_o	Glucose, %M F_o
0.00	100.0	0.0	0.0
0.50	69.0	2.2	3.1
1.00	45.8	3.3	5.0
1.50	31.0	3.6	6.0
2.10	19.6	3.4	7.1
2.48	12.3	3.3	7.3
3.10	8.2	3.1	8.1
3.97	3.7	2.3	7.6

TABLE XXVII.

REACTION SERIES C (MANNOSE) -
RELATIVE CARBOHYDRATE PERCENT MOLARITIES

$[M]_o = 0.00214$; $[OH^-]_o = 8.08$ M; T, °C = 25 ± 0.1

Reaction Time, hr	Mannose, %M M_o	Fructose, %M M_o	Glucose, %M M_o
0.00	100.0	0.0	0.0
1.48	99.1*	2.2	1.3
2.95	81.4	2.2	1.5
4.48	79.2	2.7	1.8
6.03	73.9	2.7	2.2
8.63	64.2	2.7	2.7
12.03	55.3	3.3	3.3
14.97	46.2	1.8	3.4
18.00	41.2	1.5	3.7
24.47	32.6	1.5	4.3

*This value was not included as data for computer least squares curve fitting.

APPENDIX IV

DERIVATION OF THE REACTION RATE CONSTANTS FROM THE EXPERIMENTAL DATA

MacLaurin (2) demonstrated that the several reactions involving the starting carbohydrate in 1M sodium hydroxide were pseudo first order. Plots of the logarithm of the relative concentration of the starting material against time gave quite good straight lines during the early stages of the reaction, and the experimental design was such that there was essentially no change in concentration of the base during the disappearance of at least half of the starting material.

For the reaction system (refer to Figure 11) with first order reactions, the following equations apply.

$$D(G) = -aG + k_{21}F + k_{31}M$$

$$D(F) = k_{12}G - bF + k_{32}M$$

$$D(M) = k_{13}G + k_{23}G - cM$$

where $a = k_{15} + k_{12} + k_{13}$

$$b = k_{21} + k_{24} + k_{23}$$

$$c = k_{31} + k_{32} + k_{36}$$

It can be shown that the time dependent concentrations G, F and M can be expressed as:

$$\begin{bmatrix} G \\ F \\ M \end{bmatrix} = \begin{bmatrix} \quad \\ X \\ \quad \end{bmatrix} \begin{bmatrix} \ell^{-\omega_1 t} \\ \ell^{-\omega_2 t} \\ \ell^{-\omega_3 t} \end{bmatrix} \qquad (1)$$

where ω_1, ω_2, and ω_3 are the roots of the characteristic equation:

$$\begin{bmatrix} \lambda-a & k_{21} & k_{31} \\ k_{12} & \lambda-b & k_{32} \\ k_{13} & k_{23} & \lambda-c \end{bmatrix} = 0$$

The X matrix is given by the six independent equations:

$$\begin{bmatrix} \omega_i-a & k_{21} & k_{31} \\ k_{12} & \omega_i-b & k_{32} \\ k_{13} & k_{23} & \omega_i-c \end{bmatrix} \begin{bmatrix} X_{1i} \\ X_{2i} \\ X_{3i} \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix} \qquad i = 1,2,3$$

and by the three normalizing equations:

$$\begin{bmatrix} G_o \\ F_o \\ M_o \end{bmatrix} = \begin{bmatrix} & X & \end{bmatrix} \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix}$$

where G_o, F_o, and M_o are the initial concentrations. For glucose as the starting carbohydrate, $F_o = M_o = 0$, and G_o was determined by the computer program, i.e., G_o was not set equal to 100, but was instead calculated. Fructose and mannose as the starting carbohydrate were treated similarly.

A useful paper concerning the mathematics necessary to solve the rate constant "triangle" was that by Matsen and Franklin (31).

A general linear search subroutine, VA04A, proved efficient at adjusting initial approximations of rate constants so that (1) gave a good fit to experimental data. When provided with a set of parameters and a subroutine which calculates a function, F(x), from the parameters, VA04A refined the parameter set to find the minimum value of F(x).

F(x) was made to be the sum of squares of weighted residuals, thus giving a non-linear least squares solution.

Initial approximations of the nine rate constants prevailing in the chosen reaction system of glucose in aqueous sodium hydroxide were made as follows. The slopes of plots of the logarithm of the relative concentrations of the starting material and products in 8M NaOH vs time for the initial stages of the reaction gave approximations of the nine rate constants applicable to that system. Since it was expected that the study would be one of mass effect by hydroxide ion, it was thought that multiplication of the computer refined rate constants for the 8M NaOH system by the base concentration ratio would give approximations of the rate constants prevailing in other NaOH systems. Thus, multiplication factors of 1/2 and 3/4 gave adequate initial guesses for the 4M and 6M NaOH systems respectively. The rate constants determined by MacLaurin (2) for glucose in 1M NaOH at 22°C were used as initial guesses for the 0.5M NaOH system of this research since the experimental data appeared similar.

Details of the computer program used in this research are found in Appendix V.

APPENDIX V

THE COMPUTER PROGRAM

Initial values for the rate constants in the rate equations were obtained by a combination of graphical and numerical means as discussed in Appendix IV.

These initial values were refined by an iterative process on an IBM 360, Model 44 digital computer. The Fortran IV program was written by Dr. A. Wilkinson of this department, and utilized available subroutines to refine the initial approximations of the rate constants at each base concentration. The program also gave a calculated value for the relative molarities of each carbohydrate at each reaction time point. As well, an estimate of the exactness of fit to the experimental data was given.

A test of the validity of the computer program used in this study was conducted by employing MacLaurin's data for glucose in 1M NaOH at 22°C (2). First the rate constants determined by MacLaurin were not refined by the computer program and the curves generated by the two computer programs were compared. All curves appeared identical. Next the rate constants determined by MacLaurin were allowed to be refined by the minimization subroutine. The rate constants proved to be only a little changed, and MacLaurin's conclusions based on the rate constants remained completely valid. It was thus concluded that the mathematical and iterative approach used in obtaining a solution to the kinetic model (see Figure 11) was correct. The fact that the same solution was found by two different approaches (that used by MacLaurin

(2) and the one used in this study) was supporting evidence that the kinetic model does have a unique solution.

In retrospect, it appears that the direct numerical solution via matrix algebra and employment of a least squares analysis offers little improvement to the approach used by MacLaurin (2), but it is less tedious in terms of man-hours spent in converting raw experimental data to rate constant data.

A description of VA04A, the minimization subroutine, is given immediately following. This is then followed by a complete computer program listing.

VA04A

PURPOSE: To find a minimum of a function of several variables. The procedure is iterative and the user must supply initial approximations to the values of the variables at the minimum, and a subroutine to evaluate the function for any values of the variables. Derivatives are not required.

ARGUMENT LIST: CALL VA04A(X,E,N,F,ESCALE,IPRINT,ICØN,MAXIT,CALCFX)

All arguments except F must be set before VA04A is called. On return arguments X and F will have been changed.

N the number of variables

X a one-dimensional array, of length N at least, such that X(I) contains the initial approximation to the I^{th} variable on entry to the subroutine. On return X(I) will contain the value of I^{th} variable at the minimum position to the required accuracy.

E a one-dimentsional array, of length at least N, such that E(I) is set to the absolute accuracy required on the I^{th} variable X(I). It is assumed that the E(I) are roughly proportional to the X(I).

ESCALE set by the user to limit the length of step taken by the subroutine at each iteration. X(I) will be changed by not more than ESCALE * E(I). This parameter is used to prevent the subroutine moving away from the required minimum and possibly converging to a neighbouring one.

IPRINT controls printing from the subroutine as follows:

IPRINT = 0 no printing

IPRINT = 1 the variables and value of the function are printed at every search along a line (approximately every other function value)

IPRINT = 2 variables and value of the function at every iteration i.e., every (N+1) searches along a line

ICØN set to 1 or 2 to control the ultimate convergence criterion (see below). Normally ICØN will be set to 1, but if a more thorough check on the ultimate convergence is required (at the expense of computation time!) set ICØN = 2.

MAXIT the subroutine will return to the calling program regardless after MAXIT iterations have been made.

F will be set by VA04A to the minimum value of the function.

METHOD See M. J. D. Powell, Computer Journal, 1965, Vol. 7, No. 4. The minimum will almost never be found in less than N iterations, each iteration using at least 2N values of the function. The iteration is such that each iteration causes the function to decrease, except when the ultimate convergence criterion is being applied with ICØN = 2.

USER SUBROUTINE CALCFX:

SUBROUTINE CALCFX(N,X,F)

This subroutine must be supplied by the user and set F to the value of the function when values of the variables are supplied in X. CALCFX must be specified in an EXTERNAL statement in the program which calls VA04A.

ULTIMATE CONVERGENCE CRITERION:

The convergence criterion will normally be satisfactory, i.e., ICØN is set to 1. However, if low accuracy is required, or if it is suspected that the required accuracy is not being achieved, ICØN should be set to 2. In this case a more thorough check will be made, possibly increasing the computation time by as much as 30%.

With ICØN = 1 convergence will be assumed when an iteration changes each variable by less than 10% of the required accuracy.

With ICØN = 2 such a point is found and it is then displaced by 10 times the required accuracy in each variable. Minimization is then continued from the new point until a change of less than 10% is again made by an iteration. The two estimates of the minimum are then compared.

PRINTING:

Apart from the printing requested via the parameter IPRINT, the only output will be relevant diagnostics if it is believed that the minimum has not been found to the required accuracy.

COMMON: The first element in CØMMØN must be an array of length N(N+3) to be used by VA04A as working space.

RECOMMENDATIONS:

(1) Set ESCALE as large as is reasonable, remembering that it should prevent the maximum step jumping from one "valley" to another.

(2) Set the required accuracy so that ESCALE>100.

(3) If the results appear unreasonable, try with different initial values of the variables X(I).

```
                                      MAIN
                C    CALLING PROGRAM TO USE VA04A FOR LEAST SQUARES
                C      UC   - (OBSERVED - CALCULATED) VALUE
                C      E    - ACCURACY REQUIRED FOR EACH PARAMETER
                C    ESCALE - MAXIMUM STEPSIZE CONTROL
                C    IPRINT - CONTROLS PRINTING BY VA04A
                C        0 - NO PRINTING
                C        1 - PRINTS APPROX EVERY SECOND CALCULATION OF F
                C        2 - PRINTS EVERY (N+1)TH EVALUATION
                C     ICON - TESTS FOR CONVERGENC IF 2 BUT NOT IF 1
                C    MAXIT - NUMBER OF ITERATIONS
                C      DUM - WORKING VECTOR FOR VA04A
                C      F   - FUNCTION VALUE PROVIDED BY CALCFX
                C      NM  - CONTROLS PRINTING IN CALCFX.  VALUE .TRUE. ALLOWS PRINTING
                C      NV NUMBER OF DEGREES OF FREEDOM USED IN NORMALIZING COEFFICIENTS
                C
0001                    IMPLICIT REAL*8 (A-H,O-Z)
0002                    LOGICAL*4 NM
0003                    EXTERNAL CALCFX
0004                    EQUIVALENCE (RK,P)
0005                    EQUIVALENCE (DUM(1),COR(1)),(DUM(46),SIGP(1))
0006                    DIMENSION X(200),Y(200),W(200),YC(200),C(3,3),N(3,3)
0007                    DIMENSION SIGP(9),COR(45)
0008                    DIMENSION TITLE(10),OC(200),E(9),P(9),RK(3,3),CCW(200)
0009                    DIMENSION NPTS(9),NWT(9)
0010                    COMMON /FUNC/ X,Y,W,YC,C,N,NM,NPTS
0011                    COMMON DUM(108)
                C    READ TITLE AND SEARCH INSTRUCTIONS
0012                 99 READ(5,1,END=100) TITLE
0013                  1 FORMAT(10A8)
0014                    WRITE(6,2) TITLE
0015                  2 FORMAT('1',10A8)
0016                    READ(5,3) IPRINT,ICON,MAXIT,NV,ESCALE,E
0017                  3 FORMAT(2I1,2I3,2X,7D10.4/3D10.4)
0018                    WRITE(6,4) IPRINT,ICON,MAXIT,ESCALE
0019                  4 FORMAT('0IPRINT',I5,8X,'ICON',I5,8X,'MAXIT',I5,8X,'ESCALE',F10.2//
                       *'0',10X,'INITIAL VALUES OF RATE CONSTANTS AND ACCURACIES'//31X,'PR
                       *ODUCT'/' REACTANT',12X,'1',34X,'2',34X,'3')
0020                    DO 7 IREAC=1,3
0021                    READ(5,5)(RK(IREAC,IPROD),IPROD=1,3)
0022                  5 FORMAT(3D20.5)
0023                  7 WRITE(6,6) IREAC,(RK(IREAC,IPROD),E((IREAC-1)*3+IPROD),IPROD=1,3)
0024                  6 FORMAT(' ',I4,4X,3(F10.6,5X,F10.6,10X))
                C    READ INITIAL CONCENTRATIONS AND OBSERVATIONS
                C    FOR THE THREE DATA SETS AND CALCULATE WEIGHTS
0025                    N2=0
0026                    NX=0
0027                    DO 9 IREAC=1,3
0028                    READ(5,8)(N(IREAC,IPROD),IPROD=1,3),(C(IREAC,IPROD),IPROD=1,3),W1,
                       *W2
0029                  8 FORMAT(3I3,1X,5F10.4)
0030                    DO 95 IPROD=1,3
0031                 95 NPTS(IPROD+(IREAC-1)*3)=N(IREAC,IPROD)+NX
0032                    NX=NX+N(IREAC,3)
0033                    N1=N2+1
0034                    N2=N2+N(IREAC,3)
0035                    DO 9 I=N1,N2
0036                    READ(5,19) X(I),Y(I)
0037                 19 FORMAT(2D10.4,10X,D10.4)

0038                  9 W(I)=1.0/(W1+W2*Y(I)**2)
0039                199 READ(5,98,END=100) NWT
0040                 98 FORMAT(9I1)
0041                    N1=1
0042                    DO 97 I=1,9
0043                    IF(NWT(I).EQ.0) GO TO 97
0044                    IF(I.NE.1) N1=NPTS(I-1)+1
0045                    N2=NPTS(I)
0046                    DO 96 J=N1,N2
0047                 96 W(J)=W(J)/1000.0
0048                 97 CONTINUE
                C    CALL VA04A TO PERFORM MINIMIZATION
0049                    IF(MAXIT.EQ.0) GO TO 15
0050                    NM=.FALSE.
0051                    CALL VA04A(P,F,9,F,ESCALE,IPRINT,ICON,MAXIT,CALCFX)
0052                 15 NM=.TRUE.
0053                    WRITE(6,2) TITLE
                C    CALL CALCFX TO OBTAIN EIGENVALUES AND NORMALIZED EIGENVECTORS
0054                    CALL CALCFX(P,F)
0055                    NM=.FALSE.
0056                    CALL CORREL(P,E,F)
                C    WRITE AGREEMENT FACTORS
0057                    WRITE(6,10) F
0058                 10 FORMAT('0 VALUE OF F RETURNED IS',D20.5)
0059                    F=DSQRT(F/(N2-9-NV))
0060                    WRITE(6,11) F
0061                 11 FORMAT('0 SQRT(W*(O-C)**2/(NO-NV)) =',F10.4)
                C    MAKE ALL ELEMENTS POSITIVE
                C    WRITE CORRECTED AND RETRANSFORMED PARAMETERS AND THEIR ERRORS
0062                    DO 31 I=1,9
0063                    P(I)=DABS(P(I))
0064                 31 SIGP(I)=SIGP(I)*F
0065                    WRITE(6,12)
0066                 12 FORMAT('0 RATE CONSTANTS - REACTANTS IN ROWS, PRODUCTS IN COLUMNS'
                       *//' REACTANT',5X,3('K',12X,'ERROR',17X))
0067                    DO 30 I=1,3
0068                 30 WRITE(6,6)I,P(I),SIGP(I),P(I+3),SIGP(I+3),P(I+6),SIGP(I+6)
0069                    WRITE(6,29)
                C    WRITE THE CORRELATION MATRIX (LOWER TRIANGLE)
0070                 29 FORMAT(//'0CORRELATION MATRIX'///)
0071                    N3=0
0072                    DO 28 I=1,9
0073                    N1=N3+1
0074                    N3=N1+I-1
0075                 28 WRITE(6,27) I,(COR(J),J=N1,N3)
0076                 27 FORMAT(' ',I4,9F13.5)
                C    WRITE TABLE OF RESIDUALS
0077                    N2=NPTS(9)
0078                    DO 13 I=1,N2
0079                    W(I)=DSQRT(1.0/W(I))
0080                    OC(I)=Y(I)-YC(I)
0081                 13 OCW(I)=CC(I)/W(I)
0082                    WRITE(6,14) (I,Y(I),YC(I),OCW(I),OC(I),W(I),X(I),I=1,N2)
0083                 14 FORMAT('0',17X,'Y',19X,'YC',14X,'(O-C)/SIG',14X,'(O-C)',17X,'SIG',
                       *18X,'X'/(1X,I3,6F20.5))
0084                    N1=1
0085                    DO 197 I=1,9
0086                    WCORR=1.0

0087                    IF(NWT(I).NE.0) WCORR=1000.0
0088                    IF(I.NE.1) N1=NPTS(I-1)+1
0089                    N2=NPTS(I)
0090                    DO 196 J=N1,N2
0091                196 W(J)=WCORR/W(J)**2
0092                197 CONTINUE
0093                    GO TO 199
0094                100 CALL EXIT
0095                    END
```

The Computer Program

CALCEX

```
      SUBROUTINE CALCEX(RK,E)
      IMPLICIT REAL*8 (A-H,O-Z)
      LOGICAL*4 NX
      DIMENSION NPTS(9)
      DIMENSION COF(4),P(4),ROOTR(3),ROOTI(3),P(9)
C
C     THIS SUBROUTINE CALCULATES THE SUM OF SQUARES OF WEIGHTED RESIDUALS AND USES
C     SUBROUTINES EIGEN AND SIMQ FROM THE SCIENTIFIC SUBROUTINE PACKAGE (PUT IN
C     DOUBLE PRECISION FORM)
C       Y   - OBSERVATION
C       X   - INDEPENDENT VARIABLE
C       YC  - CALCULATED Y VALUE
C       W   - WEIGHT
C       C   - THREE SETS OF INITIAL CONCENTRATIONS
C       N   -THREE SETS OF NUMBERS OF OBSERVATIONS FOR EACH PRODUCT
C       PDI - SYMMETRIC MATRIX FOR EIGEN
C     P,RK  - PARAMETERS, I.E. RATE CONSTANTS
C     EX,AE - EIGENVECTORS BEFORE NORMALIZATION USING INITIAL CONCENTRATIONS
C       RC  - TEMPORARY STORAGE FOR EX
C       B   - INITIAL CONCENTRATIONS FOR SIMQ INPUT AND THEN NORMALIZING CONSTANTS
C       W1,W2,W3 - EIGENVALUES RETURNED BY EIGEN
C
      EQUIVALENCE (EX,AE)
      DIMENSION X(200),Y(200),W(200),YC(200),C(3,3),A(3,3)
      DIMENSION PDI(6),RK(3,3),EX(9),RC(9),AE(3,3)
      COMMON /FUNC/ X,Y,W,YC,C,N,NM,NPTS
C     CONSTRUCT SYMMETRIC MATRIX
C     TREAT NEGATIVE RATE CONSTANTS AS THOUGH THEY ARE POSITIVE
      NX=0
      DO 5 IPROD=1,3
      DO 5 IREAC=1,3
      NX=NX+1
    9 P(NX)=DABS(RK(IREAC,IPROD))
      P(1)=-P(1)-P(4)-P(7)
      P(5)=-P(2)-P(5)-P(8)
      P(9)=-P(3)-P(6)-P(9)
C  FIND EIGENVALUES AND UNNORMALIZED EIGENVECTORS
      B(4)=1.0
      B(3)=-(P(1)+P(5)+P(9))
      B(2)=P(1)*P(5)+P(5)*P(9)+P(1)*P(9)-P(2)*P(4)-P(6)*P(8)-P(3)*P(7)
      B(1)=P(1)*P(6)*P(8)+P(5)*P(3)*P(7)+P(9)*P(2)*P(4)-P(4)*P(8)*P(3)
     *-P(7)*P(6)*P(2)-P(1)*P(5)*P(9)
      CALL DCLRT(B,COF,3,ROOTR,ROOTI,IER)
      IF(IER.NE.0) GO TO 10
      B1=P(2)-P(3)
      C1=P(3)
      A2=P(4)-P(6)
      C2=P(6)
      DO 11 I=1,3
      IF(ROOTI(I).GT.1.0D-12) GO TO 12
      WL=ROOTR(I)
      A1=P(1)-WL-P(3)
      B2=P(5)-WL-P(6)
      X2=B2*A1-B1*A2
      X1=(B1*C2-B2*C1)/X2
      X2=(A2*C1-A1*C2)/X2
      EX(3*(I-1)+1)=X1
      EX(3*(I-1)+2)=X2

   11 EX(3*(I-1)+3)=1.0-X1-X2
      W1=ROOTR(1)
      W2=ROOTR(2)
      W3=ROOTR(3)
C     CALCULATE SUM OF SQUARES - START OF LOOP
      E=0.0
      DO 6 IREAC=1,3
C     PREPARE TO FIND NORMALIZING CONSTANTS FROM INITIAL CONDITIONS
      I1=0
      I2=1
      IF(IREAC.EQ.1) I1=1
      I1=I1+1
      IF(IREAC.EQ.2) I2=2
      I2=I2+I1
      A1=AE(I1,2)*AE(I2,3)-AE(I1,3)*AE(I2,2)
      A2=AE(I1,3)*AE(I2,1)-AE(I1,1)*AE(I2,3)
      A3=AE(I1,1)*AE(I2,2)-AE(I1,2)*AE(I2,1)
      A1=A1/A3
      A2=A2/A3
      S1=0.0
      S2=0.0
      N2=0
      IF(IREAC.NE.1) N2=NPTS((IREAC-1)*3)
      DO 21 IPROD=1,3
      N1=N2+1
      N2=NPTS((IREAC-1)*3+IPROD)
      DO 20 NX=N1,N2
      A3=A1*AE(IPROD,1)*DEXP(W1*X(NX))+A2*AE(IPROD,2)*DEXP(W2*X(NX))+
     *AE(IPROD,3)*DEXP(W3*X(NX))
      S1=S1+A3*A3*W(NX)
      S2=S2+A3*W(NX)*Y(NX)
   20 CONTINUE
   21 CONTINUE
      A3=S2/S1
      B(1)=A3*A1
      B(2)=A3*A2
      B(3)=A3
      N2=0
      IF(IREAC.NE.1) N2=NPTS((IREAC-1)*3)
      DO 6 IPROD=1,3
C     FIND NORMALIZED COEFFICIENTS FOR EACH PRODUCT
      A1=AE(IPROD,1)*B(1)
      A2=AE(IPROD,2)*B(2)
      A3=AE(IPROD,3)*B(3)
C     PUT OUT EIGENVALUES AND NORMALIZED EIGENVECTORS WHEN NM IS TRUE
      IF(NM) WRITE(6,15) IPROD,A1,W1,A2,W2,A3,W3
   15 FORMAT('0CONCN. ',I3,' = ',F11.6,'  EXP(',F10.6,' * T) ',2('  +
     *',F11.6,'  EXP(',F10.6,' * T) '))
C     CALCULATE WEIGHTED SUMS OF SQUARES
      N1=N2+1
      N2=NPTS((IREAC-1)*3+IPROD)
      DO 6 NX=N1,N2
      YC(NX)=A1*DEXP(W1*X(NX))+A2*DEXP(W2*X(NX))+A3*DEXP(W3*X(NX))
      E=E+((Y(NX)-YC(NX))**2)*W(NX)
    6 CONTINUE
      RETURN
   10 WRITE(6,7) IER
    7 FORMAT('0PCLRT GIVES ERROR RETURN',I3)
      GO TO 13
   12 WRITE(6,8) ROOTR,ROOTI
    8 FORMAT('0NON REAL ROOT - '//(6X,3D20.5))
   13 CALL EXIT
      END
```

```
                                    VA04A
0001                SUBROUTINE VA04A(X,E,N,F,ESCALE,IPRINT,ICON,MAXIT,CALCFX)
0002                IMPLICIT REAL*8 (A-H,O-Z)
0003                DIMENSION X(10),E(10)
0004                COMMON W(130)
0005          1000  DDMAG=0.1*ESCALE
0006                SCER=0.05/ESCALE
0007                JJ=N*N+N
0008                JJJ=JJ+N
0009                K=N+1
0010                NFCC=1
0011                IND=1
0012                INN=1
0013                DO 1 I=1,N
0014                DO 2 J=1,N
0015                W(K)=0.
0016                IF(I-J)4,3,4
0017             3  W(K)=DABS(E(I))
0018                W(I)=ESCALE
0019             4  K=K+1
0020             2  CONTINUE
0021             1  CONTINUE
0022                ITERC=1
0023                ISGRAD=2
0024                CALL CALCFX(X,F)
0025                NFCC=NFCC+1
0026                FKEEP=DABS(F)+DABS(F)
0027             5  ITONE=1
0028                FP=F
0029                SUM=0.
0030                IXP=JJ
0031                DO 6 I=1,N
0032                IXP=IXP+1
0033                W(IXP)=X(I)
0034             6  CONTINUE
0035                IDIRN=N+1
0036                ILINE=1
0037             7  DMAX=W(ILINE)
0038                DACC=DMAX*SCER
0039                DMAG=DMIN1(DDMAG,0.1*DMAX)
0040                DMAG=DMAX1(DMAG,20.*DACC)
0041                DDMAX=10.*DMAG
0042                GO TO (70,70,71),ITONE
0043            70  DL=0.
0044                D=DMAG
0045                FPREV=F
0046                IS=5
0047                FA=F
0048                CA=DL
0049             8  DD=D-DL
0050                DL=D
0051            58  K=IDIRN
0052                DO 9 I=1,N
0053                X(I)=X(I)+DD*W(K)
0054                K=K+1
0055             9  CONTINUE
0056                CALL CALCFX(X,F)
0057                NFCC=NFCC+1
0058                GO TO (10,11,12,13,14,96),IS

0059            14  IF(F-FA)15,16,24
0060            16  IF (DABS(D)-DMAX) 17,17,18
0061            17  D=D+D
0062                GO TO 8
0063            18  PRINT 19
0064            19  FORMAT(5X,44HVA04A MAXIMUM CHANGE DOES NOT ALTER FUNCTION)
0065                GO TO 20
0066            15  FB=F
0067                DB=D
0068                GO TO 21
0069            24  FB=FA
0070                DB=DA
0071                FA=F
0072                CA=0
0073            21  GO TO (83,23),ISGRAD
0074            23  D=DB+DB-DA
0075                IS=1
0076                GO TO 8
0077            83  D=0.5*(DA+DB-(FA-FB)/(DA-DB))
0078                IS=4
0079                IF((DA-D)*(D-DB))25,8,8
0080            25  IS=1
0081                IF(DABS(D-CB)-DDMAX)8,8,26
0082            26  D=DB+DSIGN(DDMAX,DB-DA)
0083                IS=1
0084                DDMAX=DDMAX+DDMAX
0085                DDMAG=DDMAG+DDMAG
0086                IF(DDMAX-DMAX)8,8,27
0087            27  DDMAX=DMAX
0088                GO TO 8
0089            13  IF(F-FA)28,23,23
0090            28  FC=FB
0091                DC=DB
0092            29  FB=F
0093                DB=D
0094                GO TO 30
0095            12  IF(F-FB)28,28,31
0096            31  FA=F
0097                DA=D
0098                GO TO 30
0099            11  IF(F-FB)32,10,10
0100            32  FA=FB
0101                DA=DB
0102                GO TO 29
0103            71  DL=1.
0104                DDMAX=5.
0105                FA=FP
0106                DA=-1.
0107                FB=FHOLD
0108                DB=0.
0109                D=1.
0110            10  FC=F
0111                DC=D
0112            30  A=(DB-DC)*(FA-FC)
0113                B=(DC-DA)*(FB-FC)
0114                IF((A+B)*(DA-DC))33,33,34
0115            33  FA=FB
0116                DA=DB

0117                FB=FC
0118                DB=DC
0119                GO TO 26
0120            34  D=0.5*(A*(DB+DC)+B*(DA+DC))/(A+B)
0121                DI=DB
0122                FI=FB
0123                IF(FB-FC)44,44,43
0124            43  DI=DC
0125                FI=FC
0126            44  GO TO (86,86,85),ITONE
0127            85  ITONE=2
0128                GO TO 45
0129            86      DUMMY=DABS(D-DI)
0130                    DUM=0.03*DABS(D)
```

```
0121              IF (DUMMY-DACC) 41,41,93
0122           93 IF (DUMMY-DUM) 41,41,45
0123           45 IF ((DA-DC)*(DC-D)) 47,46,46
0124           46 FA=FB
0125              DA=DB
0126              FB=FC
0127              DB=DC
0128              GO TO 25
0129           47 IS=2
0130              IF ((DB-D)*(D-DC)) 48,8,8
0131           48 IS=3
0132              GO TO 8
0133           41 F=FI
0134              D=DI-DL
0135              DD=DSQRT((DC-DB)*(DC-DA)*(DA-DB)/(A+B))
0136              DO 49 I=1,N
0137              X(I)=X(I)+DD*W(IDIRN)
0138              W(IDIRN)=DD*W(IDIRN)
0139              IDIRN=IDIRN+1
0140           49 CONTINUE
0141              W(ILINE)=W(ILINE)/DD
0142              ILINE=ILINE+1
0143              IF(IPRINT-1)51,50,51
0144           50 PRINT 52,ITERC,NFCC,F,(X(I),I=1,N)
0145           52 FORMAT (/1X,9HITERATION,I5,I15,16H FUNCTION VALUES,
                 110X,3HF =,E21.14/(5E24.14))
0146              GO TO(51,53),IPRINT
0147           51 GO TO (55,38),ITONE
0148           55 IF (FPREV-F-SUM) 94,95,95
0149           95 SUM=FPREV-F
0150              JIL=ILINE
0151           94 IF ((IDIRN-JJ) 7,7,84
0152           84 GO TO (92,72),IND
0153           92 FHOLD=F
0154              IS=6
0155              IXP=JJ
0156              DO 59 I=1,N
0157              IXP=IXP+1
0158              W(IXP)=X(I)-W(IXP)
0159           59 CONTINUE
0160              DD=1.
0161              GO TO 58
0162           96 GO TO (112,87),IND
0163          112 IF (FP-F) 37,91,91
0164           91 D=2.*(FP+F-2.*FHOLD)/(FP-F)**2
0165              IF (D*(FP-FHOLD-SUM)**2-SUM) 87,37,37
0166           87 J=JIL*N+1
0167              IF (J-JJ) 60,60,61
0168           60 DO 62 I=J,JJ
0169              K=I-N
0170              W(K)=W(I)
0171           62 CONTINUE
0172              DO 97 I=JIL,N
0173              W(I-1)=W(I)
0174           97 CONTINUE
0175           61 IDIRN=IDIRN-N
0176              ITONE=3
0177              K=IDIRN
0178              IXP=JJ
0179              AAA=0.
0180              DO 65 I=1,N
0181              IXP=IXP+1
0182              W(K)=W(IXP)
0183              IF (AAA-DABS(W(K)/E(I))) 66,67,67
0184           66 AAA=DABS(W(K)/E(I))
0185           67 K=K+1
0186           65 CONTINUE
0187              DDMAG=1.
0188              W(N)=ESCALF/AAA
0189              ILINE=N
0190              GO TO 7
0191           37 IXP=JJ
0192              AAA=0.
0193              F=FHOLD
0194              DO 99 I=1,N
0195              IXP=IXP+1
0196              X(I)=X(I)-W(IXP)
0197              IF (AAA*DABS(E(I))-DABS(W(IXP))) 98,99,99
0198           98 AAA=DABS(W(IXP)/E(I))
0199           99 CONTINUE
0200              GO TO 72
0201           38 AAA=AAA*(1.+DI)
0202              GO TO (72,106),IND
0203           72 IF (IPRINT-2) 53,50,50
0204           53 GO TO (109,88),IND
0205          109 IF (AAA-0.1) 89,89,76
0206           89 GO TO (20,116),ICON
0207          116 IND=2
0208              GO TO (100,101),INN
0209          100 INN=2
0210              K=JJJ
0211              DO 102 I=1,N
0212              K=K+1
0213              W(K)=X(I)
0214              X(I)=X(I)+10.*E(I)
0215              FKEEP=F
0216          102 CONTINUE
0217              CALL CALCFX (X,F)
0218              NFCC=NFCC+1
0219              DDMAG=0.
0220              GO TO 108
0221           76 IF (F-FP) 35,78,78
0222           78 PRINT 80
0223           80 FORMAT (5X,37HVA04A ACCURACY LIMITED BY ERRORS IN F)
0224              GO TO 20
0225           88 IND=1
0226           35 DDMAG=0.4*DSQRT(FP-F)
0227              ISGRAD=1
0228          108 ITERC=ITERC+1
0229              IF (ITERC-MAXIT) 5,5,81
0230           81 PRINT 82,MAXIT
0231           82 FORMAT(I5,30H ITERATIONS COMPLETED BY VA04A)
0232              IF (F-FKEEP) 20,20,110
0233          110 F=FKEEP
0234              DO 111 I=1,N
0235              JJJ=JJJ+1
0236              X(I)=W(JJJ)
0237          111 CONTINUE
0238              GO TO 20
0239          101 JIL=1
0240              FP=FKEEP
0241              IF (F-FKEEP) 105,78,104
0242          104 JIL=2
0243              FP=F
0244              F=FKEEP
0245          105 IXP=JJ
0246              DO 113 I=1,N
0247              IXP=IXP+1
0248              K=IXP+N
0249              GO TO (114,115),JIL
0250          114 W(IXP)=W(K)
0251              GO TO 113
0252          115 W(IXP)=X(I)
0253              X(I)=W(K)
0254          113 CONTINUE
0255              JIL=2
0256              GO TO 92
0257          106 IF (AAA-0.1) 20,20,107
0258           20 RETURN
0259          107 INN=1
0260         1167 GO TO 35
0261              END
```

```
                                    POLRT
0001                SUBROUTINE POLRT(XCOF,COF,M,RCOTR,RCOTI,IER)                    PRT 0450
                    DIMENSION XCOF(1),COF(1),RCOTR(1),RCOTI(1)                      PRT 0460
0002                REAL*8 DABS
0003                DOUBLE PRECISION XCOF(4),COF(4),RCOTR(3),RCOTI(3)
0004                DOUBLE PRECISION XC,YC,X,Y,XPR,YPR,UX,UY,V,VT,XT,U,XT2,YT2,SUMSQ,  PRT 0470
                   1 DX,DY,TEMP,ALPHA                                               PRT 0480
                C                                                                   PRT 0670
0005                IFIT=0                                                          PRT 0680
0006                N=M                                                             PRT 0690
0007                IER=0                                                           PRT 0700
0008                IF(XCOF(N+1))10,25,10                                           PRT 0710
0009             10 IF(N) 15,15,32                                                  PRT 0720
                C                                                                   PRT 0730
                C     SET ERROR CODE TO 1                                           PRT 0740
0010             15 IER=1                                                           PRT 0760
0011             20 RETURN                                                          PRT 0770
                C                                                                   PRT 0780
                C     SET ERROR CODE TO 4                                           PRT 0790
0012             25 IER=4                                                           PRT 0810
0013                GO TO 20                                                        PRT 0820
                C     SET ERROR CODE TO 2                                           PRT 0840
0014             30 IER=2                                                           PRT 0860
0015                GO TO 20                                                        PRT 0870
0016             32 IF(N-36) 35,35,30                                               PRT 0880
0017             35 NX=N                                                            PRT 0890
0018                NXX=N+1                                                         PRT 0900
0019                N2=1                                                            PRT 0910
0020                KJ1 = N+1                                                       PRT 0920
0021                DO 40 L=1,KJ1                                                   PRT 0930
0022                MT=KJ1-L+1                                                      PRT 0940
0023             40 COF(MT)=XCOF(L)                                                 PRT 0950
                C                                                                   PRT 0960
                C     SET INITIAL VALUES                                            PRT 0970
                C                                                                   PRT 0980
0024             45 XC=.005D0101                                                    PRT 0990
0025                YC=0.01D0101                                                    PRT 1000
                C     ZERO INITIAL VALUE COUNTER                                    PRT 1020
0026                IN=0                                                            PRT 1040
0027             50 X=XC                                                            PRT 1050
                C                                                                   PRT 1060
                C     INCREMENT INITIAL VALUES AND COUNTER                          PRT 1070
                C                                                                   PRT 1080
0028                XC=-10.0*YC                                                     PRT 1090
0029                YC=-10.0*X                                                      PRT 1100
                C                                                                   PRT 1110
                C     SET X AND Y TO CURRENT VALUE                                  PRT 1120
                C                                                                   PRT 1130
0030                X=XC                                                            PRT 1140
0031                Y=YC                                                            PRT 1150
0032                IN=IN+1                                                         PRT 1160
0033                GO TO 55                                                        PRT 1170
0034             55 IFIT=1                                                          PRT 1180
0035                XPR=X                                                           PRT 1190
0036                YPR=Y                                                           PRT 1200
                C     EVALUATE POLYNOMIAL AND DERIVATIVES                           PRT 1220
0037             59 ICT=0                                                           PRT 1240
0038             60 UX=0.0                                                          PRT 1250
0039                UY=0.0                                                          PRT 1260

0040                V =0.0                                                          PRT 1270
0041                VT=0.0                                                          PRT 1280
0042                XT=1.0                                                          PRT 1290
0043                U=COF(N+1)                                                      PRT 1300
0044                IF(L) 65,130,65                                                 PRT 1310
0045             65 DO 70 I=1,N                                                     PRT 1320
0046                L =N-I+1                                                        PRT 1330
0047                TEMP=COF(L)                                                     PRT 1340
0048                XT2=X*XT-Y*YT                                                   PRT 1350
0049                YT2=X*YT+Y*XT                                                   PRT 1360
0050                U=U+TEMP*XT2                                                    PRT 1370
0051                V=V+TEMP*YT2                                                    PRT 1380
0052                FI=I                                                            PRT 1390
0053                UX=UX+FI*XT*TEMP                                                PRT 1400
0054                UY=UY-FI*YT*TEMP                                                PRT 1410
0055                XT=XT2                                                          PRT 1420
0056             70 YT=YT2                                                          PRT 1430
0057                SUMSQ=UX*UX+UY*UY                                               PRT 1440
0058                IF(SUMSQ) 75,110,75                                             PRT 1450
0059             75 DX=(V*UY-U*UX)/SUMSQ                                            PRT 1460
0060                X=X+DX                                                          PRT 1470
0061                DY=-(U*UY+V*UX)/SUMSQ                                           PRT 1480
0062                Y=Y+DY                                                          PRT 1490
0063             78 IF(DABS(DY)+DABS(DX)-1.0D-10) 100,80,80                         PRT 1510
                C                                                                   PRT 1520
                C     STEP ITERATION COUNTER                                        PRT 1530
                C                                                                   PRT 1540
0064             80 ICT=ICT+1                                                       PRT 1550
0065                IF(ICT-500) 60,85,85                                            PRT 1560
0066             85 IF(IFIT)100,90,100                                              PRT 1570
0067             90 IF(IN-5) 50,95,95                                               PRT 1590
                C     SET ERROR CODE TO 3                                           PRT 1610
0068             95 IER=3                                                           PRT 1620
0069                GO TO 20                                                        PRT 1630
0070            100 DO 105 L=1,NXX                                                  PRT 1640
0071                MT=KJ1-L+1                                                      PRT 1650
0072                TEMP=XCOF(MT)                                                   PRT 1660
0073                XCOF(MT)=COF(L)                                                 PRT 1670
0074            105 COF(L)=TEMP                                                     PRT 1680
0075                ITEMP=N                                                         PRT 1690
0076                N=NX                                                            PRT 1700
0077                NX=ITEMP                                                        PRT 1710
0078                IF(IFIT) 120,55,120                                             PRT 1720
0079            110 IF(IFIT) 115,50,115                                             PRT 1730
0080            115 X=XPR                                                           PRT 1740
0081                Y=YPR                                                           PRT 1750
0082            120 IFIT=0                                                          PRT 1760
0083            122 IF(DABS(Y)-1.0D-3*DABS(X)) 135,125,125                          PRT 1770
0084            125 ALPHA=X+X                                                       PRT 1780
0085                SUMSQ=X*X+Y*Y                                                   PRT 1790
0086                N=N-2                                                           PRT 1800
0087                GO TO 140                                                       PRT 1810
0088            130 X=0.0                                                           PRT 1820
0089                NX=NX-1                                                         PRT 1830
0090                NXX=NXX-1                                                       PRT 1840
0091            135 Y=0.0                                                           PRT 1850
0092                SUMSQ=0.0                                                       PRT 1860
0093                ALPHA=X

0094                N=N-1
0095            140 COF(2)=COF(2)+ALPHA*COF(1)                                      PRT 1880
0096                IF(N.LE.2) GO TO 170                                            PRT 1890
0097            145 DO 150 L=2,N                                                    PRT 1900
0098            150 COF(L+1)=COF(L+1)+ALPHA*COF(L)-SUMSQ*COF(L-1)                   PRT 1910
0099            155 RCOTI(N2)=Y                                                     PRT 1920
0100                RCOTR(N2)=X                                                     PRT 1930
0101                N2=N2+1                                                         PRT 1940
0102                IF(SUMSQ) 160,165,160                                           PRT 1950
0103            160 Y=-Y                                                            PRT 1960
0104                SUMSQ=0.0                                                       PRT 1970
0105                GO TO 155                                                       PRT 1980
0106            165 IF(N) 20,20,45                                                  PRT 1990
0107            170 COF(3)=COF(3)+ALPHA*COF(2)-SUMSQ*COF(1)
0108                GO TO 155
0109                END                                                             PRT 1990
```

```
0001                SUBROUTINE CORREL(P,E,F)
0002                IMPLICIT REAL*8 (A-H,O-Z)
0003                COMMON C(45),D(9)
0004                DIMENSION P(9),E(9)
0005                IX=0
0006                INC=10
0007                DO 3 I=1,9
0008                EI=INC*CABS(E(I))
0009                P(I)=P(I)-EI
0010                CALL CALCFX(P,FN)
0011                P(I)=P(I)+2*EI
0012                CALL CALCFX(P,FP)
0013                FN=(FP+FN-2.0*F)/(EI*EI)
0014                D(I)=FP
0015                DO 1 J=1,I
0016                IX=IX+1
0017                IF(I.EQ.J) GO TO 2
0018                EJ=INC*CABS(E(J))
0019                P(J)=P(J)+EJ
0020                CALL CALCFX(P,FC)
0021                C(IX)=(F+FC-FP-D(J))/(EI*EJ)
0022                P(J)=P(J)-EJ
0023              1 CONTINUE
0024              2 P(I)=P(I)-EI
0025              3 C(IX)=FN
0026                CALL DSINV(C,9,1.0E-12,IER)
0027                IF(IER.NE.0) GO TO 4
0028                IX=0
0029                DO 7 I=1,9
0030                IF(C(IX+I).GT.0) GO TO 9
0031                C(IX+I)=1.0
0032                WRITE(6,8) I
0033              8 FORMAT('0WARNING-DIAGONAL ELEMENT NO',I3,' WAS NEGATIVE OR ZERO.')
0034              9 D(I)=DSQRT(C(IX+I))
0035                DO 7 J=1,I
0036                IX=IX+1
0037              7 C(IX)=C(IX)/(D(I)*D(J))
0038                RETURN
0039              4 DO 5 I=1,9
0040              5 D(I)=0.0
0041                DO 6 I=1,45
0042              6 C(I)=0.0
0043                RETURN
0044                END
```

VITA

Surname: HARVIE Given Names: GORDON ARNOLD

Place of Birth: EDMONTON, ALBERTA Date of Birth: JANUARY 28, 1947

Educational Institutions Attended, with Dates of Entering and Leaving:

UNIVERSITY OF ALBERTA	1964 to 1967
UNIVERSITY OF VICTORIA	1968 to 1971

Degrees, Diplomas, Etc., Awarded, with Dates and Names of Institutions:

B.Sc.	1967	University of Alberta Edmonton, Alberta

THE UNIVERSITY OF VICTORIA LIBRARY

MANUSCRIPT THESIS

AUTHORITY TO DISTRIBUTE

AUTHOR: This thesis may be lent or microfilm copies made available:

(signature of the author in one of the spaces below)

(a) Without restriction

(b) With the restriction that, for a period of five years (until _____) the
Date
written approval of the following is required:

(1) The Chairman, School of Graduate Studies _____

(2) The Author _____

(3) Both the Chairman, School of Graduate Studies and the Author _____

BORROWERS: The borrower undertakes, by signing below, to give proper credit for any use made of the thesis, and to obtain the consent of the author if it is proposed to make extensive quotations, or to reproduce the thesis in whole or in part.

Signature of Borrower	Address	Date