ION EXCHANGE HPLC SEPARATION OF POLAR ORGANICS AND THE MASS SPECTROMETIC AND NUCLEAR MAGNETIC RESONANCE IDENTIFICATION OF A ^{13}C MIXTURE

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PREFACE

The objectives of this study were: (1) development of analytical methods for separating polar organic compounds, and (2) use of these and other techniques for analyzing a carbon-13 reaction mixture.

Special appreciation is due Dr. Louis P. Varga, who served as major adviser and made this study possible. The other members of the advisory committee were Dr. E. J. Eisenbraun, Dr. H. L. Gearhart, and Dr. S. L. Burks (who replaced Dr. A. F. Gaudy following his transfer). I would also like to thank Dr. G. R. Waller for allowing use of the LKB 9000 mass spectrometer.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTORY AND BACKGROUND INFORMATION OF THE OVERALL PROJECT

Stable Isotopes

Separated stable isotopes of numerous elements have been available in limited quantities for many years (1). Because they do not present the technical and political problems associated with radioactive isotopes, their use as tracers in the fields of medicine, agriculture, the environment, or other areas of interest is promising (2). However, despite this promise, the stable isotopes have not been fully utilized when compared to radioactive tracers (3). One reason for this is that only limited quantities of the isotopes were easily obtainable, causing them to be expensive to prepare and use. Another reason was the nonavailability of instruments and analytical techniques capable of detecting trace quantities of these stable isotopes (4).

One of these obstacles was partially overcome when, in 1972, Los Alamos Scientific Laboratories began making significant progress on their project to produce large quantities of stable isotopes of carbon, oxygen, nitrogen, and sulfur (ICONS), thus reducing their cost dramatically and increasing their availability. Thus progress has stimulated their application in many areas, including the production of labeled organic compounds to be used as standards and tracers.

The Problem and the Instrumentation

Once isotopic incorporation into compounds has been achieved, two important parameters need to be determined. These are: the extent of labeling in the molecule, and the locations of the isotopes in the molecule. Mass spectrometry has been conveniently used to determine the percentage of the isotopes present in the parent or fragment molecule and also to determine the molecular weight of the compounds present. However, tedious degration is often required to determine the exact labeled sight (5).

Within the last decade, the introduction of computerized fourier transform nuclear magnetic resonance (FT-NMR) has allowed the accumulation of multiple frequency scans of an organic compound, which has greatly aided in the determination of the exact location of the isotopes in the molecule (6). This increased sensitivity has produced a need for labeled organic compounds to be used as instrumental standards.

Polar Organics in Nature

In recent years there has been increased attention given to the problems and possible values associated with organic material in natural water. The presence of measurable concentrations of organic chemicals in lakes, rivers, and oceans has been well documented (7,8). The knowledge that some of the compounds are water soluble aids in an understanding of some of their major characteristics, namely hydrogen bonding, ionizability, and high polarity. The origin and fate of this organic material is dependent upon natural biological synthesis or degradation, and the various activities of man (9).

There are several possible approaches for the analysis of these organic compounds. One is attempted identification of the specific compounds which have either been added or are suspected to be present. Another is separation o- the mixture into groups and classes and identification of the individual compounds present in them. Combinations of several separation techniques may prove most advantageous (10). Whatever method is selected, it should reflect the characteristics and nature of the compounds to be isolated and identified (11).

In summary, when the problems of detecting and tracing of organic compounds in natural water is coupled with the increased production of ICONS, the need for labeled compounds of a similar nature has developed. Other important considerations are the methods for isolation and identification of labeled tracers and standards.

Objectives of the Study

The purpose of this study was the investigation of methods for separating polar organic compounds. The separation schemes will also be applied to resolve the products of a carbon-13 reaction mixture into polar labeled organic compounds. General and specific forms of mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), and other techniques will all be employed to help determine the identity and concentration of the isolated compounds. Analytical evaluations of the methods used will be presented, when pertinent.

CHAPTER II

THEORETICAL PRINCIPLES AND EQUATIONS OF THE INSTRUMENTAL METHODS PERFORMED

Mass Spectroscopy

Equations for calculating the exact mass, the theoretical isotopic abundance and corrections for the isotopic abundance at different masses are presented. Computer programming greatly facilitated what would otherwise have been very time consuming calculations.

Theoretical Isotopic Abundances

The relative isotopic abundances in a compound can be empirically calculated, assuming there is no fractionation of the isotopes during reaction and that the isotopic distributions of the product follow close to actual statistical behavior. The equation which describes this is a polynomial of the form:

$$(a+b)^n(c+d)^m \qquad (2\text{-}1)$$

where a,b,c, and d are the relative abundances of ^{12}C, ^{13}C, ^{16}O, and ^{18}O respectively (12,13). The number of atoms of each element in the molecule are denoted by nanal. The natural abundances of ^{2}H, ^{14}C, ^{17}O, and ^{15}N were neglected because they were small compared to that of the major isotopes.

As an example calculation of the equation, consider a molecule with six atoms of one element and six atoms of another element. An expansion of the equation will produce a 36 term polynomial. Each term represents a different possible combination of isotopes, and the relative percentage of terms can be calculated. For a compound that is 9.35% ^{12}C, 90.65% ^{13}C, 93.19% ^{16}O and 6.81% ^{18}O with 6 carbons and 6 oxygens, the predominant products are about 37% $^{13}C_6\ ^{16}O_6$, 23% $^{13}C_5\ ^{12}C\ ^{16}O_6$, 16% $^{13}C_6\ ^{16}O_5\ ^{18}O$, 10% $^{13}C_5\ ^{12}C\ ^{16}O_5\ ^{18}O$, 6% $^{13}C_4\ ^{12}C_2\ ^{16}O_6$, 3% $^{13}D_6\ ^{16}O_4\ ^{18}O_2$, and 2% $^{13}C_4\ ^{12}C_4\ ^{16}O_5\ ^{18}O$. Thus, if the compound is $C_6H_6O_6$, then the relative m/e intensities of the mass spectrum will be about 8% 178, 23% 179, 37% 180, 10% 181, 16% 182, and 3% 184. It is easy to see from this type of calculation that a group of peaks with a mounded distribution will be more prominent than single individual molecules, especially at higher masses.

Calculation of Exact Mass

The exact mass of a molecular or fragmented ion can be calculated from a high resolution mass spectrum of the parent compound (12,14). The distance between the lines (peaks) on the mass spectral photographic plates can then be read on a comparator and recorded. The knowledge that PFK (perfluorokersene) produces predictable mass deficient peaks allowed their easy identification. By using the equation:

$$M_x = \left[\frac{d_x - d_1}{d_2 - d_1}\left(\sqrt{M_2} - \sqrt{M_1} + \sqrt{M_2}\right)\right]^{\frac{1}{2}} \qquad (2\text{-}2)$$

where the distance to any line is d_x, the exact mass of that line (M_x) can be calculated. M_2 and M_1 are the exact masses, and d_2 and d_1 are

the distances of the higher and lower PFK peaks respectively. Manual error in reading the distances is generally greater than instrumental separation errors (15). Although the relative error varies with the mass, an error of about 5 millimass units is common. The photographic plate and comparator can be eliminated if sophisticated computer devices are interfaced with the mass spectrometer.

Mass Spectrometry Resolution of Isotopes

The resolution capabilities of a mass spectrometer are of particular importance when separating ions with the same m/e value (16,17). The resolution, R, is defined by the equation:

$$R = \frac{m_2}{m_2 - m_1} \qquad (2\text{-}3)$$

where m_2 is the mass of interest, and m_1 is the next closest mass. Higher resolution is required for separation as the m/e value increases. If the resolution is sufficiently high, separation of fragments at the same m/e value can be obtained.

Field Ionization

Field ionization is similar to low voltage ionization in that it produces principally molecular ions, with only about 5% fragmentation occurring (18). Field ionization generally produces molecular ions for most compounds, while low voltage ionization may have sufficient energy to ionize one molecule but not another (19).

Ideally, high resolution field ionization mass spectrometry could specify exactly which compounds are present. Again, however, there

exists a trade-off between resolution and low sensitivity. Higher resolution is obtained by reduction of the slit widths, but for complex mixtures, this results in a loss of the existing low peak intensities.

Nuclear Magnetic Resonance Spectroscopy

Carbon-13 Resonance

Direct determination of the types of ^{13}C nuclei in the molecule can generally be obtained from the position of the lines in the spectra (20). The chemical shifts for ^{13}C nuclei are large (δ up to 200 ppm) and generally produce resonance lines for each carbon. The characteristics of the surrounding molecules and solvents can shift the exact line position up or down field (21). The position of a carbonyl peak may vary from approximately 160 to 200 ppm depending upon its environment.

A combination of low natural abundance (1.1%), the adverse effect of the gyromagnetic ratio, and other parameters reduces the ^{13}C sensitivity to 6000 times less than that of ^{1}H resonance (22). The concentration of the compound can vary the time required to accumulate enough fourier transform NMR scans for analysis. Highly enriched compounds can be analyzed much faster than their natural abundance counterparts.

Quantitative analysis of the relative number of carbons at any site cannot be obtained by integration of the ^{13}C resonance peaks (23,24). This is largely because of the nuclear Overhauser effect and relaxation time, which are dependent upon the nature and environment of the specific carbons of interest. There are complex methods for obtaining quantitative spectral information, such as using the satellite peaks from ^{1}H NMR. This method will be discussed in later sections (25).

The ^{13}C-^{1}H coupling can cause difficulty in delineating the identity of the ^{13}C spectral lines when combined with the already present ^{13}C-^{13}C coupling. This difficulty can be remedied if the ^{13}C-^{1}H splitting is decoupled by means of irradiation in the proton region while obtaining the ^{13}C spectra (26). Accurate coupling constants for ^{13}C-^{13}C are still being measured and reported as more double and multiple labeled compounds are being synthesized. Paramagnetic shift reagents have been shown to be successful in separating overlapping multiplets but were not necessary for this study (27).

Proton Resonance

Although ^{13}C resonance spectra probably provide more valuable information for highly labeled compounds, ^{1}H NMR can also be useful for analysis purposes. Many of the ^{1}H-^{13}C coupling constants have been measured and recorded (6). The spectra can become complicated if the number of ^{1}H and ^{13}C becomes large. Spectral splitting from the directly bonded ^{13}C is easiest detected but coupling differences between vicinal and germinal ^{1}H and ^{13}C are much smaller and more equal in magnitude.

Liquid Chromatography

The theory of liquid chromatography varies only slightly in technique and physical properties from other chromatographic methods. Since authors may vary in the methods and terminology for calculating the same parameter, the following equations are presented as the ones used in this study (28). The degree to which two components are separated is defined as the resolution (R_s) and is determined by

$$R_s = 2\frac{(t_{R1} - t_{R2})}{(W_1 + W_2)} = \frac{2(V_1 - V_2)}{(W_1 + W_2)} \qquad (2\text{-}4)$$

where t_{R1} and t_{R2} are the retention times of two retained components, and W_1 and W_2 are the widths of the bases of their respective peaks as defined in Figure 1. If the flow rate is known, then R_s can be defined in terms of the retention volumes, V_1 and V_2.

For high speed liquid chromatography, if R_s is less than .8, separation is considered to be unsatisfactory. An R_s greater than 1 would represent baseline separation (29).

The number of theoretical plates (N) in a liquid chromatographic column is a measure of the column efficiency and can be calculated as:

$$N = 16\frac{(t_{R1})^2}{(W_1)^2} = 16\frac{(t_{R1})^2}{(2\ W_{h\frac{1}{2}})^2} = 16\left(\frac{V_1}{W_1}\right)^2 \qquad (2\text{-}5)$$

where $W_{h\frac{1}{2}}$ is the width at half height. The number of theoretical plates, N, is an indicator of the column efficiency. It is determined by the packing material and the solvent and can attain efficiencies of 10,000 theoretical plates/25 cm (30). The height equivalent to a theoretical plate (HETP) can then be found by dividing the length of the column by the number of theoretical plates as in Equation (2-6).

$$HETP = \frac{(L)}{(N)} \qquad (2\text{-}6)$$

The relative separation of different components is termed the column selectivity (a) and is defined as:

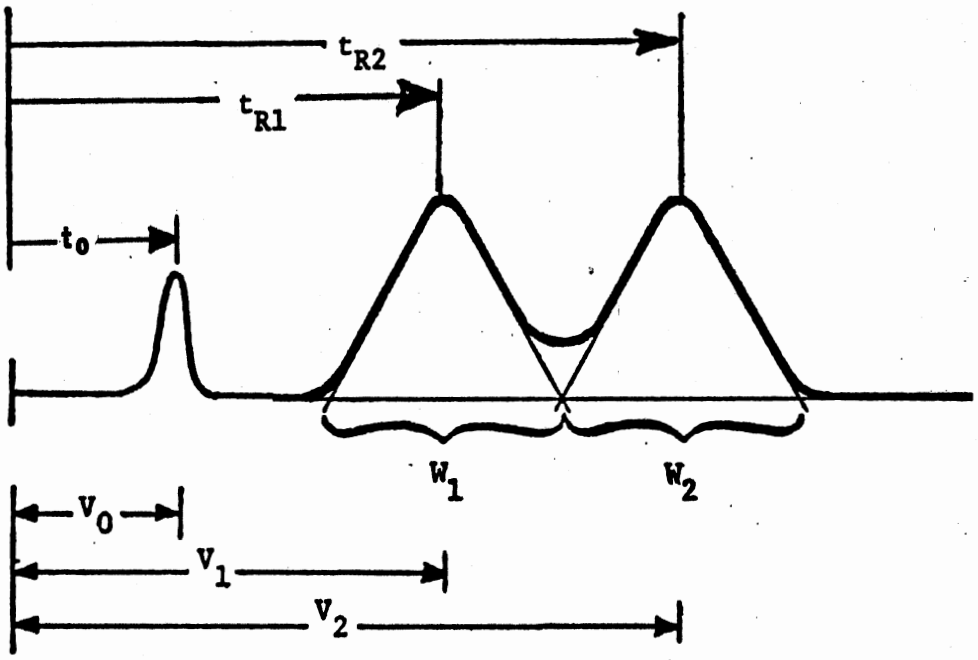


Figure 1. Example Chromatogram Showing Measured Parameters

$$a = \frac{(t_{R2} - t_o)}{(t_{R1} - t_o)} = \frac{V_2 - V_0}{V_1 - V_0} = \frac{(t'_{R2})}{(t'_{R1})} = \frac{K'_2}{K'_1} \qquad (2\text{-}7)$$

where t'_{R1} and t'_{R2} are the adjusted retention times of components 1 and 2 respectively, K'_1 and K'_2 the distribution coefficients of components 1 and 2 respectively, and t_o the retention time of an unretained component.

The retention volume of unretained components (V_0) and the volume of the stationary phase can be calculated from the flow velocity and other column parameters. The elution volume (V_R) can then be defined in terms of these two quantities, and the distribution coefficient (K) by Equation (2-8):

$$V_R = V_0 - (K)(V_s) \qquad (2\text{-}8)$$

Equation (2-9) defines the capacity factor k' and shows how it can be determined directly from the retention times or retention volumes:

$$k' = K\frac{(V_s)}{(V_0)} = \frac{(t_R - t_o)}{t_o} = \frac{(V_R - V_0)}{V_o} \qquad (2\text{-}9)$$

Peak areas are calculated by Equation (2-10):

$$A = (W_{h\frac{1}{2}})(h) \qquad (2\text{-}10)$$

where A is the area and h is the height.

Possible Liquid Chromatography Methods

The chromatographic separation of polar organic molecules can be achieved by implementation of both single and combination analytical techniques. One method uses the affinitive characteristics of the

organic molecules for the stationary phase (32). Separation can then be achieved by usin- common or reverse phase chromatography with an organic mobile phase (33). This method is best performed on compounds which are nonpolar and nonionic to the degree that they are readily soluble in organic solvents (34,35).

Another method involves some of the same column packing and mobile phases as the separations already described; however, in this technique, the ionic molecules are converted to nonionic molecules by reaction with a counter ion (36). The nonionic molecules are then separated by paired ion chromatography (PIC). Retention times can be varied by changing the length of the carbon chain on the PIC reagent (37). The extent to which the ion pairing occurs is important to consider if quantitative analytical data are to be obtained. Recovery of the original compound is carried out by shifting the equilibrium to favor the ionic form, and then separation of the PIC reagent. This method is not advisable for preparative separations (37).

Ion exchange is also capable of separating most polar organic compounds, especially those forming ions in solution (38,39). Cation and anion exchange columns are available for separation of acids, base, amprotic, and neutral compounds (40,41,42). Separation is generally achieved by either changing the column to a weaker or stronger exchanger, or altering the pH or ionic strength of the mobile buffer phase (43,44). One advantage of this technique over the others is the high solubility of most polar organic compounds in water, the mobile phase (45,46).

Some of the possible columns that could be used to separate different types of mixtures are shown in Figure 2. The flow diagram does not include all possibilities but only suggest a beginning point.

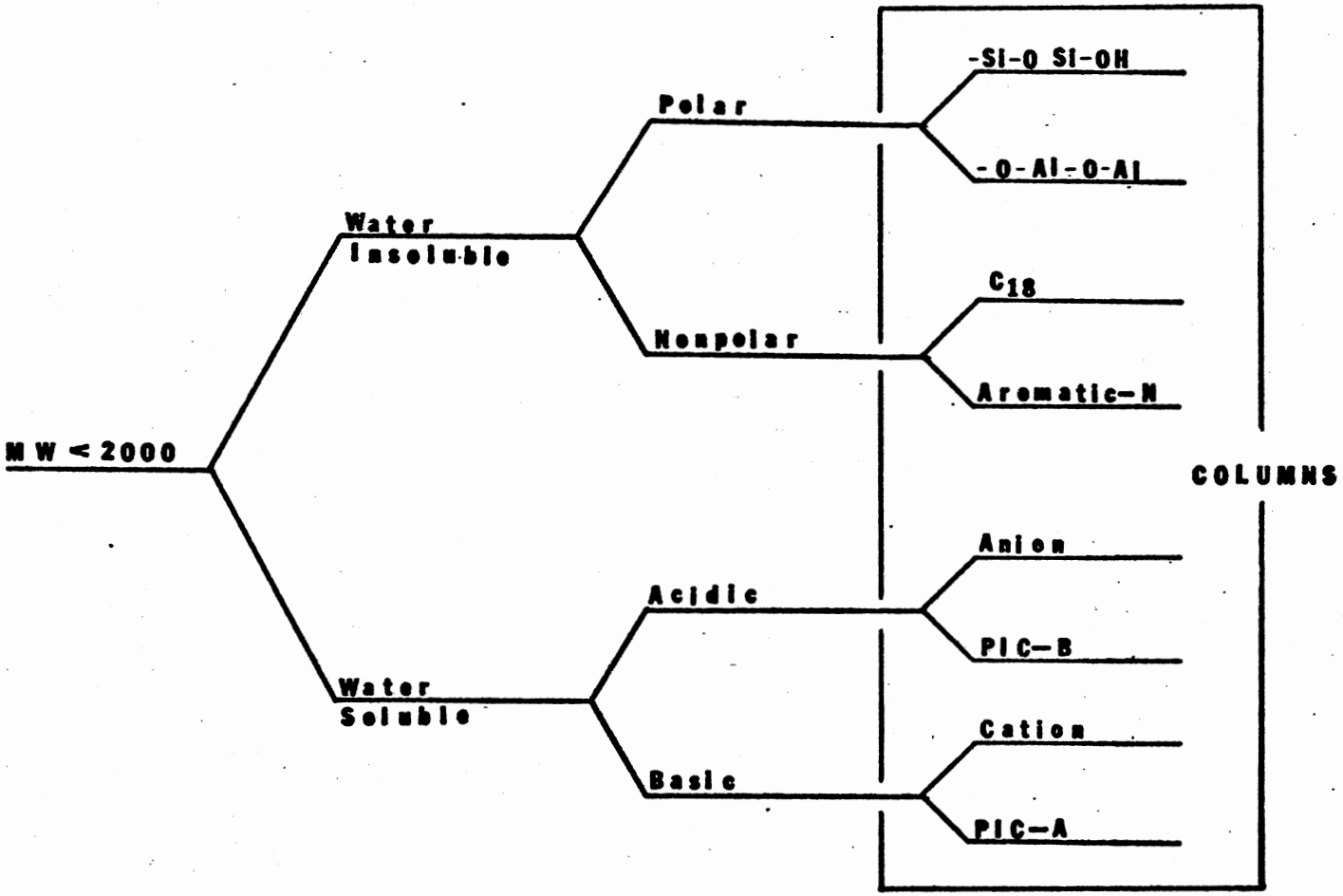


Figure 2. Possible Columns Selected for Different Types of Samples

Production of Enriched Carbon-13 Carbon Monoxide

A fractional distillation plant at Los Alamos Scientific Laboratories was able to separate natural abundance carbon-12 and carbon-13 isotopes of carbon monoxide by the difference in their vapor pressure (47). A .8% difference was sufficient to obtain about 20 l/day of 92% $^{13}C^{16}O$ while wasting 4000 k, but was unable to separate it from $^{12}C^{18}O$ which then also became enriched. The presence of other isotopes is important when considering isotope ratio calculations presented in this chapter. A review of some applications of these isotopes has been presented by Hammond (48).

Isotope Effects

The fractionation of isotopes of labeled organic compounds by gas chromatography has been demonstrated by several investigators (49,50). However, to date liquid chromatography (by ion exchange) has only enough resolving power to separate isotopes of simple organic compounds (51). Isotopic fractionation was not detected during this experiment and would have been difficult to determine because of the large number of isotopes present.

CHAPTER III

LITERATURE REVIEW OF THE LIGHT METAL CARBONYL REACTION

Reaction Importance

The preparation of large highly-labeled organic compounds generally is achieved by either a multiple step organic synthesis or biosynthetic production (52). Organic synthesis can produce small or specifically labeled compounds easily. However, one of the disadvantages of a long chain synthesis is that every reaction step increases the chances of product loss or the formation of impurities (53,54). Biosynthetic methods have been used for large scale production of labeled compounds, but prevention of dilution by carbon-12 is an added difficulty (55,56). The compounds produced by biosysthesis are highly specific but must be separated from the organisms and other products.

The most valuable asset of the alkali metal carbonyl reaction (AMCR) is that it allows the use of easily obtainable and highly isotopically-labeled carbon monoxide to produce organic compounds in a single reaction sequence (57). Its other important asset is that it produces water soluble polar organic compounds, which are of great interest to this study. However, identification of most of the major products and the elimination of bi-products are two obstacles that have not been overcome. Some of the identifiable products and reaction variables will be discussed in the following sections.

variables will be discussed in the following sections.

Reaction History

In 1825, Wohler and Berzeluis (58) attempted to produce potassium by the Brenner method. The reaction involves the decomposition of K_2CO_3 in the presence of carbon to yield potassium vapors, CO, and possibly CO_2. The potassium vapors from the heated iron reaction vessel were then condensed in a side arm cooling column. At temperatures below decomposition, CO was found to mix and condense with the metal in the column to form a peculiar grey-colored compound later named potassium carbonyl. The grey crystals tended to plug up the narrow sections of the column and cause explosions. The product could also detonate from the shock of being forceably removed from the tube.

After learning that exposure of the potassium carbonyl to water vapor or air could cause an explosion, Helles (59), two years later, was able to add water to a stabilized form of the grey material, and an acid was formed. He later named this new material rhodizonic acid (60).

Leibrg (61,62) later found the grey-colored compounds were also produced when dry CO was passed over molten potassium. By weighing the original K and calculating the increase in weight from CO, Brodie (63) in 1860, was able to determine the molecular formula of the product to be $(KCO)_2$. Carbonyls of Rb and Cs can also be prepared by this method, but molten Na fails to react.

The formation of $(KCO)_2$ from K and CO is quite interesting. Iridescent black crystals of the metal carbonyl form on the surface and along the edge of the molten pool. They appear to grow masses of crystals which seem at times to be coated with a thin layer of the metal.

The exact composition of the grey matter was greatly clarified when, in a similar experiment, Nietask (63) treated the product with acetic anhydride and isolated the hexa-acetyl derivative of hexahydroxybenzene. A possible reaction pathway for the hydrolysis, oxidation, and finally decomposition of hexahydroxybenzene to rhodozonic acid was also considered.

In 1893, Joannis (64) used a solution of liquid ammonia to dissolve sodium or potassium and then passed the CO through the metal-ammonia solution. The product had the same empirical formula as the earlier experiment; however, it was white instead of grey. Joannis stabilized the carbonyl by passing water vapor over the liquid ammonia solution, and was finally able to identify glycolic acid as one of the reaction products.

In subsequent years, the carbonyls of all of the common alkali metals and alkaline earth metals (with the exception of Be) have been prepared (65,66,67,68). Most of these polar metal carbonyls are reported to undergo thermal decomposition to yield carbon, plus the metal oxide and carbonates. The instability of the products has prevented their further investigation. The types of products obtained and their distribution has not been determined for most of the metal reactions.

Thermal measurements and magnetic susceptibility determinations have been made by Sager (69) on the reaction during introduction of CO. He proposes the formation of consecutive intermediates, $K_3C_2O_2$ and KCO, prior to the final product. He postulates a reaction scheme of:

$$K + CO \rightarrow (K_3C_2O_2)_x + CO \rightarrow (KCO)_x \rightarrow K_6C_6O_6$$

In 1948, some investigation began at Reed College directed mainly

toward studying the active carbonyl through its stabilized product. It was soon found by Scott (71) that any acid like substance (such as soluble salts, alcohols, etc.) in the liquid ammonia would stabilize the products. Over several years, and with varying levels of confidence, a variety of products were identified. These include Heller's rhodozonic acid, Joannis' glycolic acid, and a few new ones like glycolaldehyde, tartronic acid, and glycollic acid amide. When anhydrous reagents were used, glycolamide was identified as a major product.

Relevant Information About CO

Carbon monoxide is a very stable divalent compound of carbon, with a resonance energy of 58 kcal mole, compared to 39 for benzene (64). The interatomic bond distance of 1.13A is only slightly above nitrogen's 1.09A and well below the 1.22A for acetaldehyde.

Relevant Information About NH_3 Solutions

Mixing of liquid ammonia with any alkali or alkaline earth metals produces a blue solution if diluted, and a coppery metalic sheen if concentrated. The presence of "free", or at least solvated, electrons cause the solution to be an extraordinary electrical conductor.

Reaction Indicators

Some indication of the reaction process was gained by measuring the electrical conductivity of the liquid ammonia during the CO absorption. The conductivity began very high, corresponding to the metallic conductivity of a concentrated solution. As the CO was absorbed, the conductivity decreased lineally until the colored-point, where it rose slightly

and then leveled off. Magnetic susceptibility measurements indicated the electrons were paired.

Variable Reaction Parameters

The above information leads to the conclusion that the reaction is proportional to reaction temperature, stirring rate, and solubility of CO in liquid NH_3. Variations of the manner in which the metal is added, the amount of metal present, and the exclusion of O_2 and moisture, could change the product distribution. A minimum excess of the alkali metal decreased the chance of a base catalyzed polymerization product. An review of the AMCP reaction was presented by Ladenburg (71) in 1888.

CHAPTER IV

EXPERIMENTAL PROCEDURES AND SEPARATION SCHEMES

Introduction

The research project undertaken focused around the separation of two types of mixtures. Although both mixtures ultimately required similar procedures for the separation of their components, different methods were used in their identification. The first separation scheme was applied to a mixture of standard polar organic compounds dissolved in water. This procedure was used to evaluate the capabilities and effectiveness of various separation procedures. The second scheme was employed to resolve a mixture carbon-13 synthesis products whose preparation was discussed in Chapter III. The details of these procedures will be presented in the following sections. The types of instrumentation and methods of identifying the product will also be discussed.

Overall Separation Scheme

The complexity of the products from the synthesis dictated sample pretreatment in addition to separation by liquid chromatography. Therefore, a scheme involving both solvent extraction and liquid chromatography was initiated (71,72). A diagram of the procedure is shown in Figure 3. Not all of the steps were performed when sample quantities and time were limited.

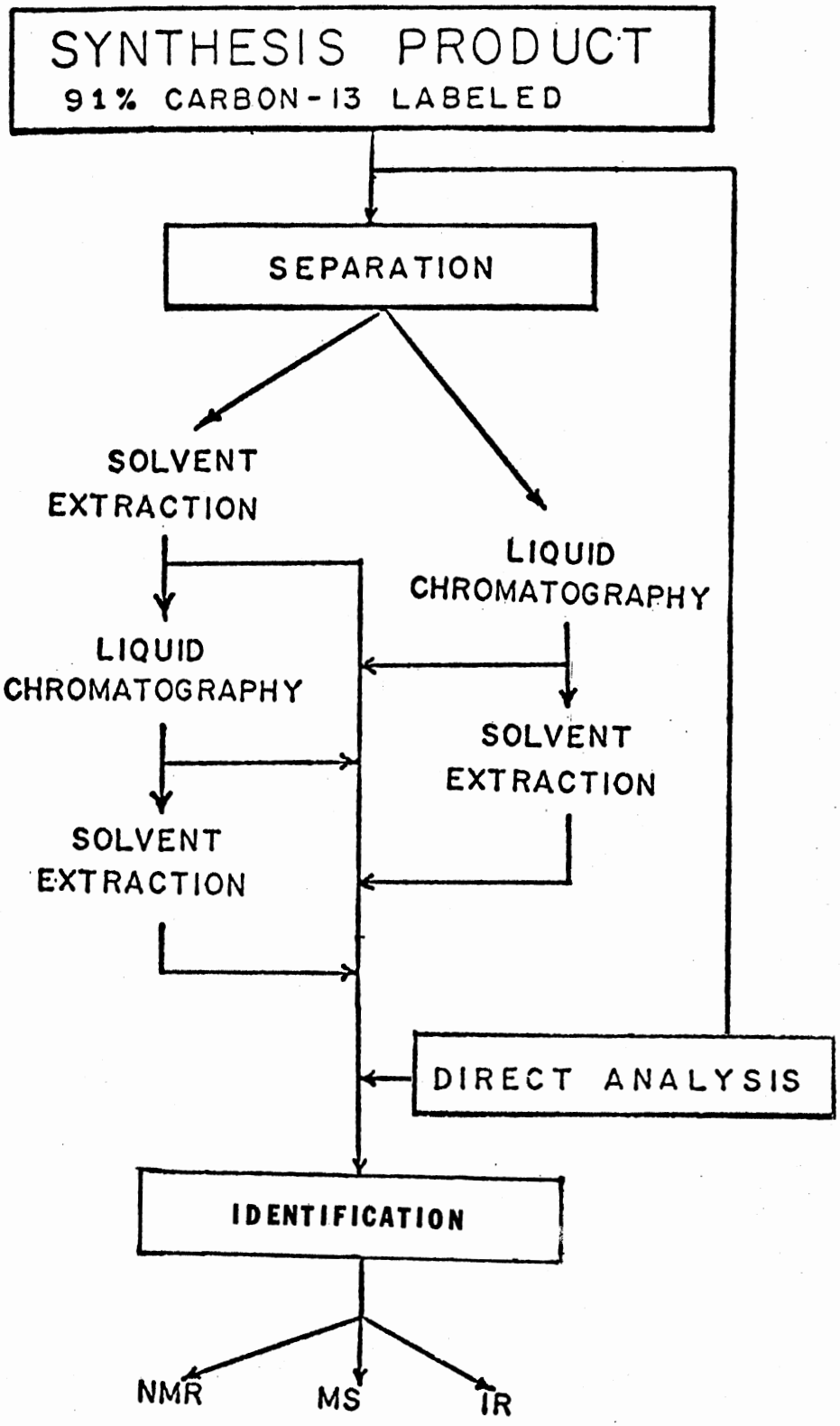


Figure 3. Separation Schemes Used on the AMCP

Solvent Extraction Procedures

The extraction process served two significant purposes. One of these was to remove the organics from the NH_4Cl used to quench the reaction. Solvent extraction of the reaction mixture, contained in a preweighed cup, was performed with increasing polarity solvents (73). Evaporation of the solvent, and then weighing of the compounds removed, allowed evaluation of extraction efficiencies. Table I is a summary of the amounts extracted. The solubility of NH_4Cl in most polar organic solvents presented quite a problem. Acetone and dioxane were the only solvents polar enough to dissolve most of the organics (but not the salt). However, stabilizers in dioxane eliminated it as a solvent.

After the correct solvent had been selected, the extraction was performed with three consecutive 10 ml portions of acetone per gram of mixture. Each extraction time, the sample was heated to $50^{o}C$ and ultrasonicated to dispense the solution. The sample was then centrifuged to remove the suspended solids.

Preparation of the Alkali Metal Carbonyl Product

With the aid of the Atomic Energy Commission's chemicals and facility at Los Alamos Scientific Laboratories, and using the method of Scott (70), the alkali metal carbonyl reaction discussed in Chapter III, was performed by Dr. L. P. Varga.

Sodium was the metal selected for the reaction and it was dissolved in the liquid ammonia. The carbon monoxide was bubbled through the ammonia solution. Care was taken to exclude air and water from the reaction until after quenching.

TABLE I

EXTRACTION EFFICIENCIES

Solvent*+	1.000g of Solid Percentage Extracted	.5000g of Solid Percentage Extracted	.5000g of Solid Percentage Extracted
Hexane	0.3		
Ether	-0.4		
THF	-0.2		
Acetone			4.8
Dioxane	0.1	1.9	
Acetronitrile	2.4	5.2	3.8
Propanol		14.0	
Ethanol	52.6	19.1	92.2
Methanol		12.7	
Water	44.0	1.0	4.0

*Extraction times varied from 6-17 hours.

+Extraction volumes were 100 ml.

The carbon monoxide used in this reaction was highly enriched in carbon-13 by the diffusion method described in Chapter II. Cylinder #30012, containing 300 ml of gas, was used for the reaction. Of the 8.87 grams of gas used from the cylinder, 6.99% was in the form of CH_4, and the remainder was assumed to be in the form of CO. The CO contents of the cylinder was comprised of 90.65% $^{13}C^{16}O$ and 6.81% $^{12}C^{18}O$. NH_4Cl was used to quench the reaction and stabilize the products.

The Alkali Metal Carbonyl Product (AMCP)

The sample was a grayish-black solid with an odor similar to that of quinones. Observance of the solid through a magnifying glass showed the presence of a mixture of white, gray, and black crystal. The white crystals are probably from excess NH_4Cl. The sample was shown to be capable of supporting bacterial growth. 58.5 grams of the solid was obtained.

A previous investigator at OSU had dissolved an unknown quantity of the material in water. During this investigation, the remaining sample was stored in a sealed glass container, which was kept in a desiccator. All liquid samples were stored in the refrigerator at $4^{\circ}C$ to reduce chances of biological degradation.

Liquid Chromatography Instrumentation

The liquid chromatograph was hand assembled from commercially available components. Only stainless steel, teflon, and glass were used to connect the mobile phase components. A diagram of the liquid chromatograph is displayed in Figure 4.

The pump was a Milton Roy pump with a 1000 psi maximum, containing

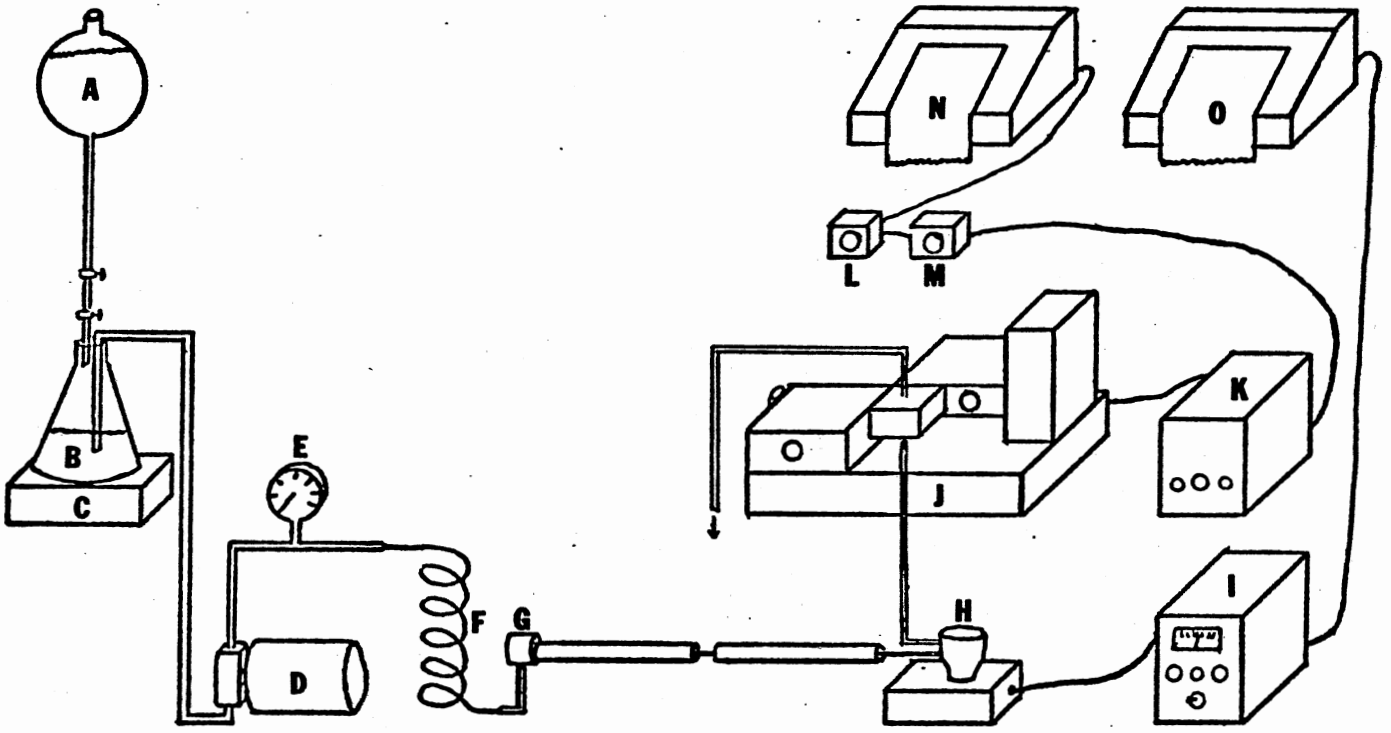


Figure 4. Block Diagram of the Liquid Chromatograph: A. Buffer Reservoir, B. Solvent Reservoir, C. Magnetic Stirrer, D. Pump, E. Pressure Gauge, F. Capillary Tubing, G. Injection Port, H. UV Detector, I. UV Monitor, J. Fluorescence Detector, K. Fluorescence Monitor, L. Attenuator, M. Bucking Voltage, N. Fluorescence Recorder, O. UV Recorder

a variable stroke length piston (74). This produced constant volume and variable pressure flow. The pressure generally remained between 500-600 psi unless blockage occurred. The pressure gauge and capillary tubing which followed, acted as a mechanical RC filter to reduce pressure fluctuations.

Dual analyzers were installed, the first being a Varian 254 mm single wavelength detector with a mercury vapor lamp (75,76). The cell had an internal volume of 8 microliters in a C configuration. This detector was relied upon as the much heavier of the two because of stability and versatility.

The other detector was a Farrand Series 190 fluorescent spectrophotometer, with variable excitation and emission monochromators (77). The cell was a 15 microliter linear quartz type. This single beam instrument required correction for baselines when scans were performed (78).

Types of Columns Attempted

Permaphase ETH. Separation on a 3 ft. moderately polar permaphase ETH column was attempted early in the experimentation. Mixtures of ethanol-water were first used as the mobile phase for this reverse phase separation. Acetone was also tried later. The components appeared too soluble in the mobile phase for separation. The limited solubility of large amounts of the mixture in more nonpolar solvents prohibited the use of this type of preparative separation.

Octadodecialsilane. Normal phase separation was attempted, using a nonpolar C_{18} column with a gradient of 100% ethanol to water. Minimal

separation was obtained, but most components were unretained and eluted with the solvent front.

Strong Anion Exchange. Connection of two (250 mm x 4.6 mm ID) columns (PXS-1025) in series gave good performance. This system was just large enough to allow small preparative scale work, without overloading the column. The packings were 10 micron strong anion exchange (SAX), prepacked by Whatman, Inc. The columns were used over a period of approximately six months with no noticeable loss in efficiency. A 2 cm pre-column, packed with a Zipax 30 micron bead, which was changed periodically, was used to protect the columns.

Variation of the Mobile Phase

Optimizing pH and Ionic Strength. The SAX columns produced some separation from the first time they were employed. The second step was an adjustment of the pH of the buffered mobile phase to produce an optimum separation. This occurred at a pH between 3.5-4.4 depending upon which peaks were of most interest. However, the separation was still moderate at best. This was improved by testing a stepwise increase of the buffer ionic strength from 0.002 M to 0.020 M. As the strength increased more and more peaks appeared that had not been eluted at lower ionic strength. A gradient was then attempted and is described in the following section.

Gradient Elution Conditions. Figure 4 depicts the manner in which the gradient elution was employed. The solvent reservoir contained 400 ml of distilled water, which was treated with 0.50 ml of the buffering solution. The buffering solution was prepared by dissolving 200 g of

KH_2PO_4 in enough water to make 1.0 ℓ (a 1.47 M solution).

The buffering solution was allowed to drop at a constant rate into the solvent reservoir, while being continuously stirred. The buffer reservoir was large compared to the volume of buffer removed, thus producing a constant flow rate. The rate of addition of buffer could be adjusted with stopcocks to produce slower or faster flow rates. By experimentation, separation was optimumized between time and resolution, when the flow rate was adjusted to 0.42 ml/min.

The flow rate out of the solvent reservoir was a constant 0.40 ml/min. at a normal pump setting of 15. Thus, the net change in volume of the reservoir was negligible for a typical chromatogram. This allows approximate calculation of KH_2PO_4 molarity in the reservoir at any given time. Knowing that the dead volume between the solvent reservoir was 5.2 ml, the molarity of the solvent in the column was then determined.

Three consecutive chromatograms of the same mixture were run to check the validity of the gradient. The flow rate from the buffer solution was changed each time. Analysis of the recorder response proved the same peaks were present, but their retention times had changed in direct correlation to the buffer flow.

Cerate Oxidation System

The ultraviolet detector provided a very versatile monitor for organic compounds which contain at least a conjugated double bond system. The fluorometer serves as a specific detector because of the combination of excitation and emission. However, for the unknown compounds in the reaction, it became difficult to find a suitable pair of wave-

lengths to monitor the reaction mixture. Scans were performed by hand, but still proved inefficient when the mixture was chromatographed. In addition, neither detector could detect saturated compounds if they were present.

To remedy this situation, a cerate oxidation system modeled after that described by Katz (79) was installed between the UV and fluorescent detector. A diagram of the oxidative system utilized is shown in Figure 5. The flow of the reagent from the reservoir was adjusted by changing its height. The mixing chamber was a 2 mm Y-shaped glass tubing, as was the gas separator. Degassing is almost impossible to prevent in the reaction chamber unless a back pressure can be obtained. The bubble separator was used instead of back pressure, but only with limited success. The reaction chamber contained 15 feet of .25 mm teflon tubing that was kept at 75 C. The mixture spent about 5.3 minutes in the reaction chamber.

The advantages of this system over other oxidation systems are that it offers sensitivity, versatility, and simplicity (80). The oxidation of the organic molecules causes the reduction of Cerium IV to Cerium III, which is fluorescent. The Ce IV was dissolved in 2N $H_2 \leftrightarrow SO_4$ to buffer and aid oxidation. The Ce III was monitored at its maximum excitation and emission wavelengths of 260 nm and 350 nm respectively (81).

Mass Spectral Analysis

Positive identification of the LC column effluents can only be obtained by a combination of methods as described earlier in this chapter (10). However, sufficient quantities of many of the components needed to perform all of the analysis were not available. Therefore, many of

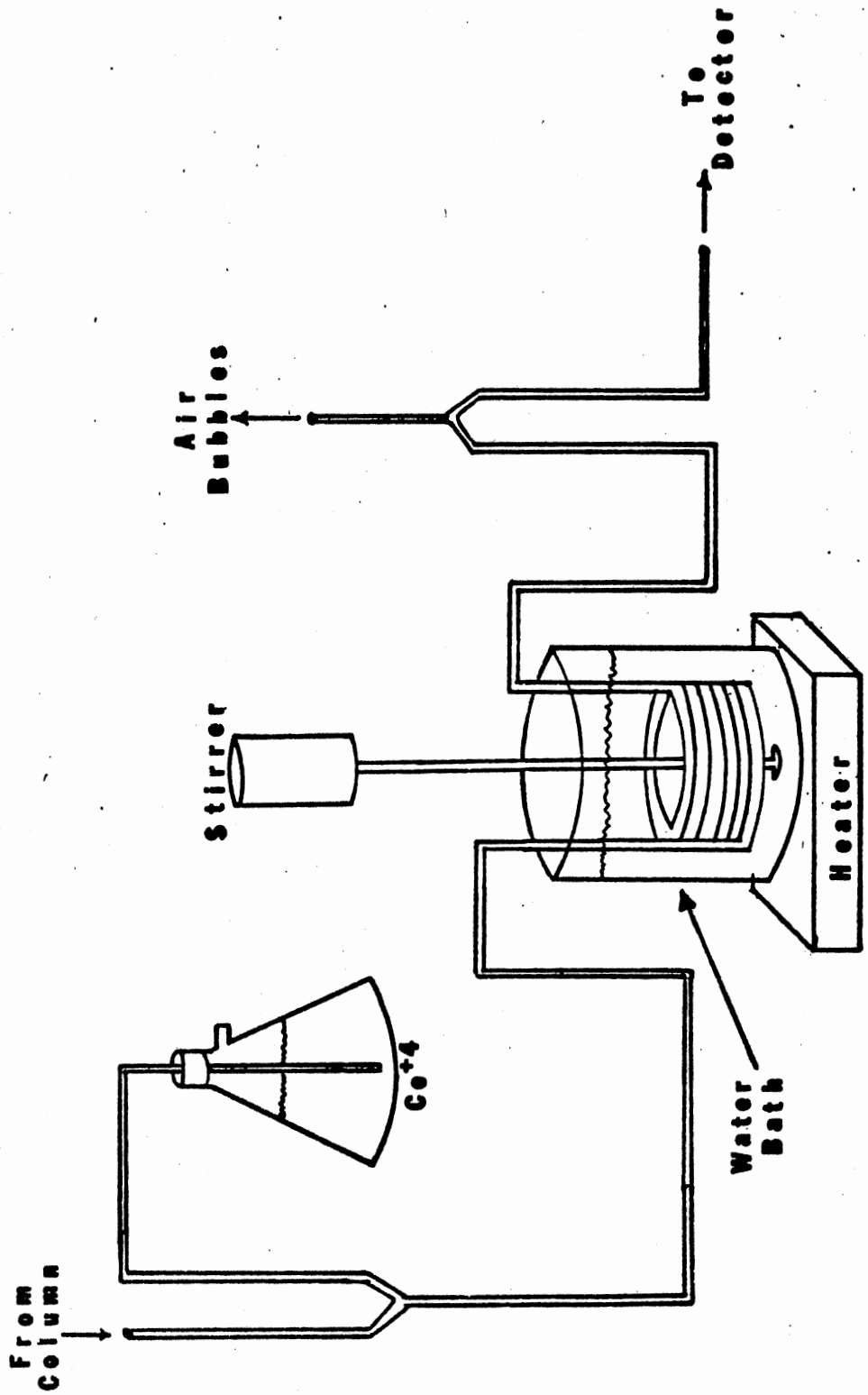


Figure 5. Diagram of the Ce^{+4} Oxidation System

the identifications were assigned by mass spectral patterns only. Molecular ions were generally not obtained, or were very weak for the polar compounds of interest. This is common but increased the difficulty in positive identification of unknown compounds (82,83).

The Synthesis Mixture

Early in the expoeriment, small portions of the original reaction product were introduced into the mass spectrometer to obtain data about the type of function groups which were present and the kind of compounds that had been produced (84,85). These data were first used primarily to aid in selection of the type of separation scheme to be used.

As peaks from the liquid chromatograph appeared, the column eluent containing the standards was collected in individually numbered vials. They were evaporated to dryness by heating at about 40°C in a stream dry nitrogen to avoid air oxidation. The amount of buffering salt remaining after evaporation was dependent upon the amount of effluent collected and when the peak excited the column. The weight of salt increased as the retention time increased. The salt was scratched from the sides and the bottom of the vial, and a small amount was placed in a mass spectrometer direct probe tube.

Many of the organics required enrichment by extraction into about 0.1 ml of either acetone, methanol, or both. Part of the solvent extract was then placed in a mass spectrometer direct probe tube and again evaporated to dryness under nitrogen with heat. Organic extraction of the evaporated effluent or raw product was also important with these compounds because of the presence of NH_4Cl which, unlike the mobile phase buffer, sublimes in the mass spectrometer to produce a high ion

current. Most direct probe analyses were performed on the LKB 9000 at Oklahoma State University by the principal investigator. The extraction efficiencies can be evaluated from the early steps in Figure 2.

The probes were then slowly introduced into the mass spectrometer and heated slowly to evolve each of the individual compounds. The total ion current was monitored as the temperature increased, thus producing a recorder response as the compound or impurities were vaporized. A typical ion current versus temperature scan for a liquid chromatogram peak is shown in Figure 6. Mass spectrums were run at variable intervals to determine the identities of the compounds being evolved. Each mass spectrum and the temperature at which it was taken were indicated on the total ion current scan for later cross-referencing.

High voltage (70 EV) scans were taken routinely, but low voltage (8-17 EV) scans were made at ion current maxima to obtain molecular ions. The low voltage setting was optimized to produce the best ionization and fewer fragments multiple low voltage scans could be obtained. Low voltage scans are far less sensitive than their high voltage counterparts. The scan can be obtained in about 1½ second, up to a mass of about 300.

The mass spectrometer is sensitive to about 5 ng of compound; however, this is still too high for many of the samples because of the large amount of salt present. Also, the small sample sizes and high sensitivity made contamination from outside sources a major problem. Much of the contamination problem was solved by thoroughly cleaning all glassware and then firing it in an oven at 940^{o}C.

High Resolution and Field Ionization. The complexity and nature of the reaction products required the use of more elaborate mass spectro-

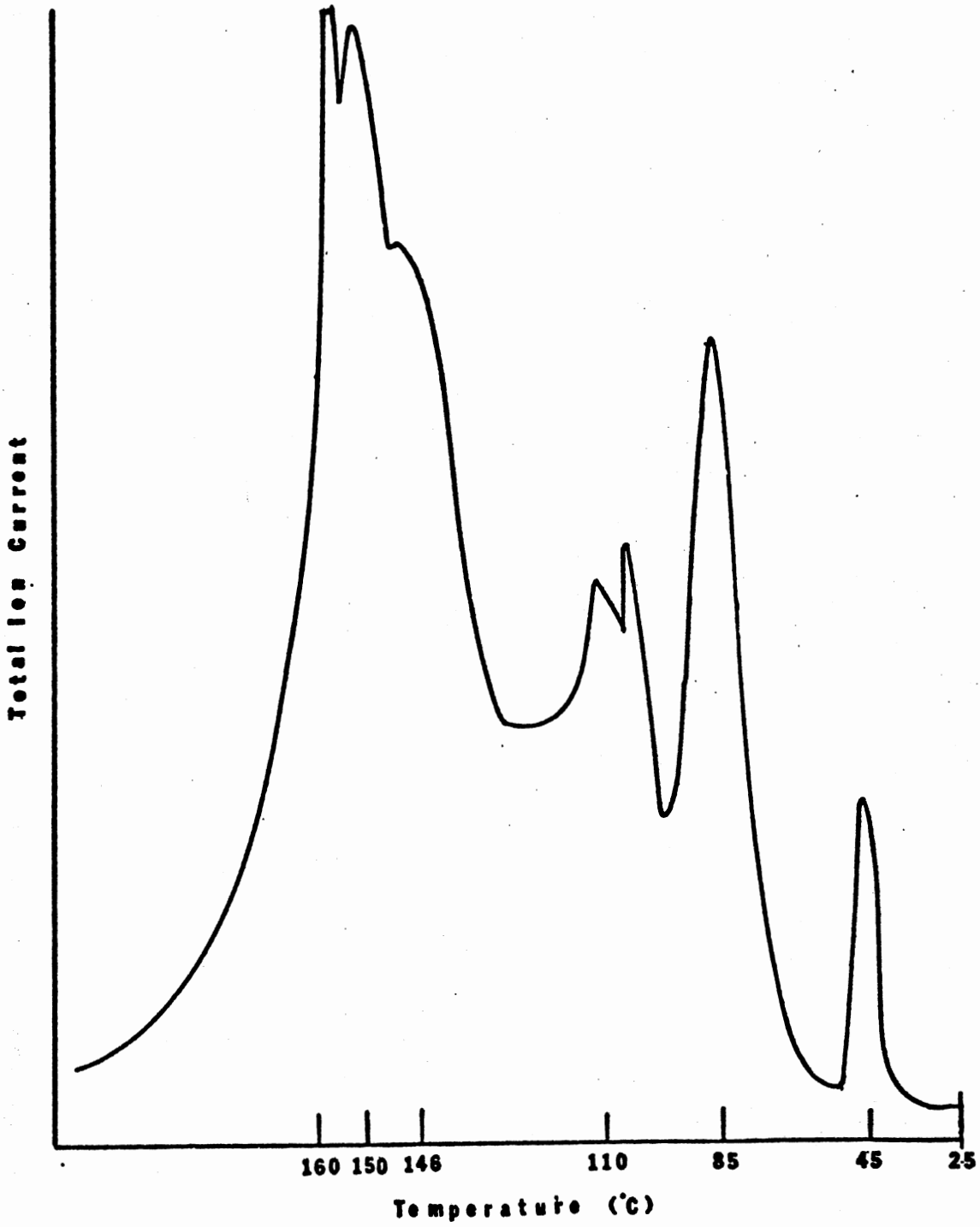


Figure 6. Recorder Trace of the Mass Spectrometer Ion Current Versus Temperature

metry techniques (86). Field ionization and high resolution electron impact both provided valuable information about characteristics of the compounds (87). Both the field ionization and high resolution data were obtained from the departmental mass spectroscopy group using the CEC-110-21B high resolution mass spectrometer at Oklahoma State University, and Phillips Petroleum (Bartlesville) and the MS30 at the University of Nebraska.

The high resolution data (x20,000) was recorded on a photographic plate, using PFK (perfluorkerosene) as a reference. After reading the plate, the exact masses were calculated from Equation (2-3), using the distances between peaks on the plate. The program in Appendix B was used to execute the calculation. High resolution-field ionization was not obtained because of the low sensitivity.

Comparison of this data with that of a library containing the total exact mass of compounds with ^{13}C, ^{14}N, ^{1}H, and ^{16}O was the next step. This library was unavailable due to the unusually high concentration of carbon-13 and had to be generated by a simplified form of the program in Appendix A. Limitations were placed on the contents of possible ions to omit improbable ions, and to reduce the size of the library. A sample of the output is also given in the Appendix. Modifications to that program were then made so that it would compare the theoretical and experimental masses and print out any matches. This program is presented in Appendix A.

Nuclear Magnetic Resonance

No NMR data was taken on the standards because of the ease of analysis by mass spectrometry and the samll sample sizes. Both the pro-

ton and carbon-13 NMR were obtained from a Varian XL-100 at Oklahoma State University.

Proton

Proton NMR spectra of the solid and acetone extracts of AMCP were recorded. Most of the samples were dissolved in 0.5 ml of acetone-d_6 (99.96%D) and run immediately. D_2O was also used as a solvent for some early samples. Deuterium exchange occurred reasonably fast in both solvents, especially the samples that were allowed to remain at room temperature for very long. The disappearance of the large peak produced from the acid protons ($\delta = 7$) occurred in the span of one day, while some of the others disappeared after longer times.

Carbon-13

Most of the Carbon-13 spectra were taken with D_2O or H_2O as the solvent and a capillary tube of TMS and D_2O as a lock. FT was required on all samples, with pulse times for most samples running about two hours. Both gated normal and proton decoupled BC NMR were performed on the extracts to determine the H-C splitting.

Infrared Spectroscopy

A KBr pellet was made of the AMCP before extraction with any solvent, and the pellet was analyzed with a Beckman IR 5. The red-brown oil remaining after extraction was smeared between two NaCl plates and the IR spectrum taken. One sample of extract was scanned with an IR 7 in an attempt to resolve some of the broad overlapping bands. The resolution improved only slightly, and no new peaks appeared. The wide

peaks were attributed to intermolecular and intramolecular hydrogen bonding. A fourier transform NMR of the acetone extract was also performed on the neat oil. The background was subtracted by the FT computer.

CHAPTER V

RESULTS AND CONCLUSIONS

Liquid Chromatography

Standard Organic Compounds

Fifteen organic compounds which have characteristics similar to the types of compounds which might be present in the reaction mixture were selected as standards. Three of them were not strong enough UV absorbers to be monitored and were eliminated. A list of the standards and their concentrations is given in Table II. A mixture standard was found to be best separated using the two SAX columns and pH discussed in Chapter II. The buffer gradient was initially allowed to flow into the solvent reservoir at a rate of .41 ml/min. This rate was finally reduced to .27 ml/min. to optimize time and separation of most of the peaks.

A typical UV recorder trace for the HPLC chromatography of the standards is displayed in Figure 7, with the peak number corresponding to the standdards in Table II. The peaks were identified by comparing the relative retention times and relative detector response with standards injected individually and in different mixtures. Catechol and hydroquinone were the only compounds that were not at least partially separated. The pyrogallol absorption was obscured by the acetophenone and diethyl phthalate doublet. Several unidentified peaks appeared and can probably be attributed to degradation of the standards in the mixture,

TABLE II

SEPARATION OF TWELVE ORGANIC STANDARDS

Peak Number	Identity
1	m-Cresol
2	Phenol
3	Catechol and Hydroquoinone
4	Unidentified
5	p-Chlorophenol
6	Benzoquinone
7	Unidentified
8	Diethylphthalate-Pyrogallol
9	Acetophenone-Pyrogallol
10	Unidentified
11	Unidentified
12	m-Aminobenzoic Acid
13	Barbarturic Acid
14	Gallic Acid
15	Unidentified

Standards not detected: Glyoxalic Acid, Citric Acid, and Tartaric Acid.

All individual standards were made with a concentration of 1 mg/ml, 25% of which was injected.

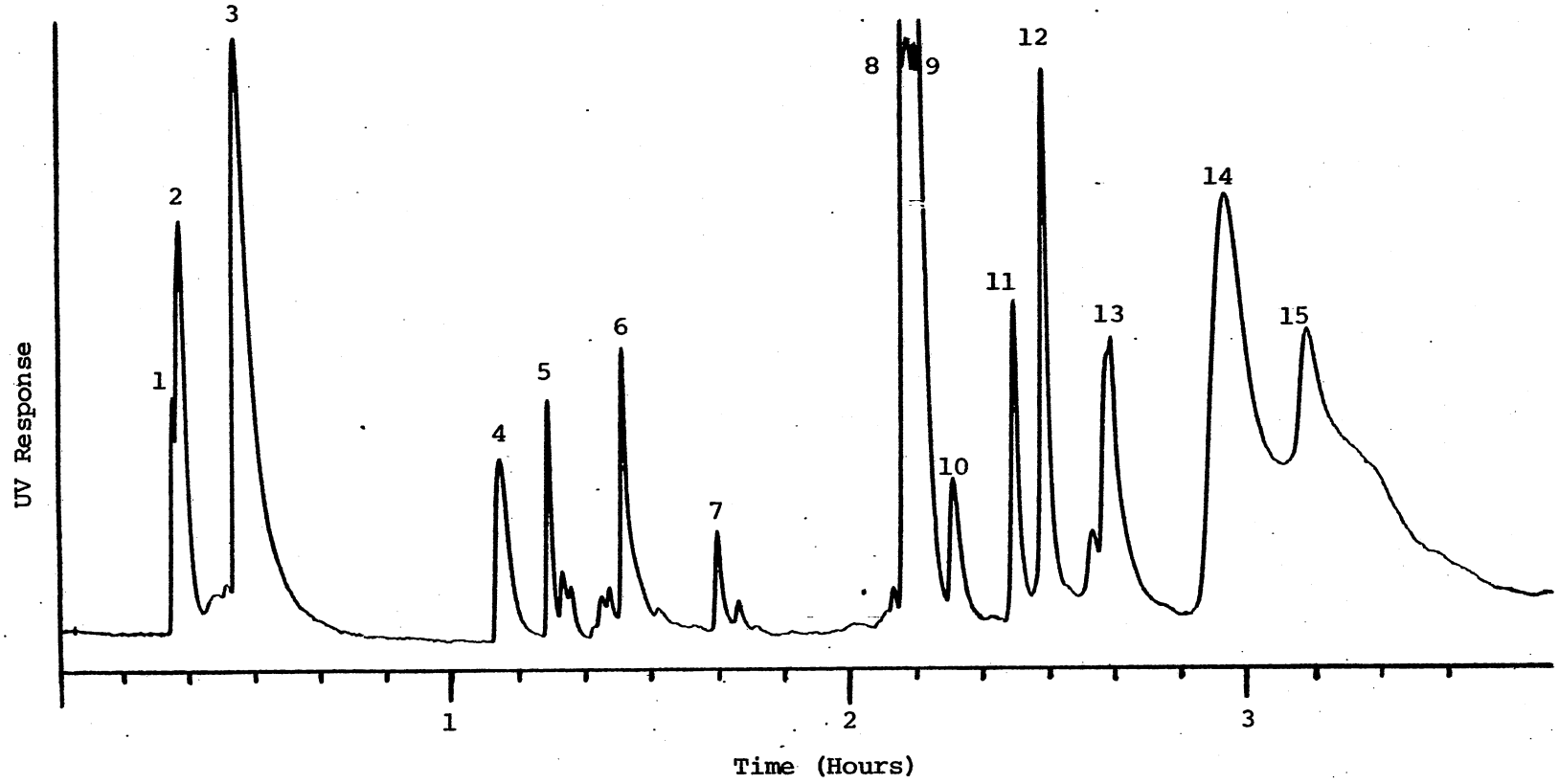


Figure 7. Chromatogram of the Standards in Table II

or reaction with each other. The retention times have been found to change between the mixture and the individual injections. This can partially be explained by considering the effect of the other compounds on the active sites of the stationary phase, the pH and ionic strength of the mobile phase, and the synergistic effects interaction of each compounds with the eleven others (88,89,90). Although twelve compounds is a small sample, it appears as if the compounds with -OH groups were eluded first, the more neutral compounds second, and the acids retained the longest. This is reasonably consistent with some other work by Pitt (91) in which the general elution order was sugars, nitrogen compounds, and then acids.

Several different combinations of excitation and emission wavelengths of the fluorometer were selected using standard fluorescence tables for the compounds. Two very intense emissions (350 nm) corresponding to m-cresol-phenol peak and the catechol-hydroquinone peak were detected with an excitation wavelength of 280 nm (92). These were the only fluorescence peaks observed at any of the variations of wavelengths attempted. However, three small negative peaks resulting from absorption of the background were observed. These corresponded to the 6, 8, 9 and 12 peaks of the UV chromatogram, respectively.

The ^{13}C Reaction Mixture

The AMCR products were also resolved by gradient elution SAX chromatography, and a sample UV chromatogram is shown in Figure 8. Two major peaks were observed, each partially separated into two peaks. Several other smaller peaks were also obtained, but the entire sample was eluted in approximately two and one half hours.

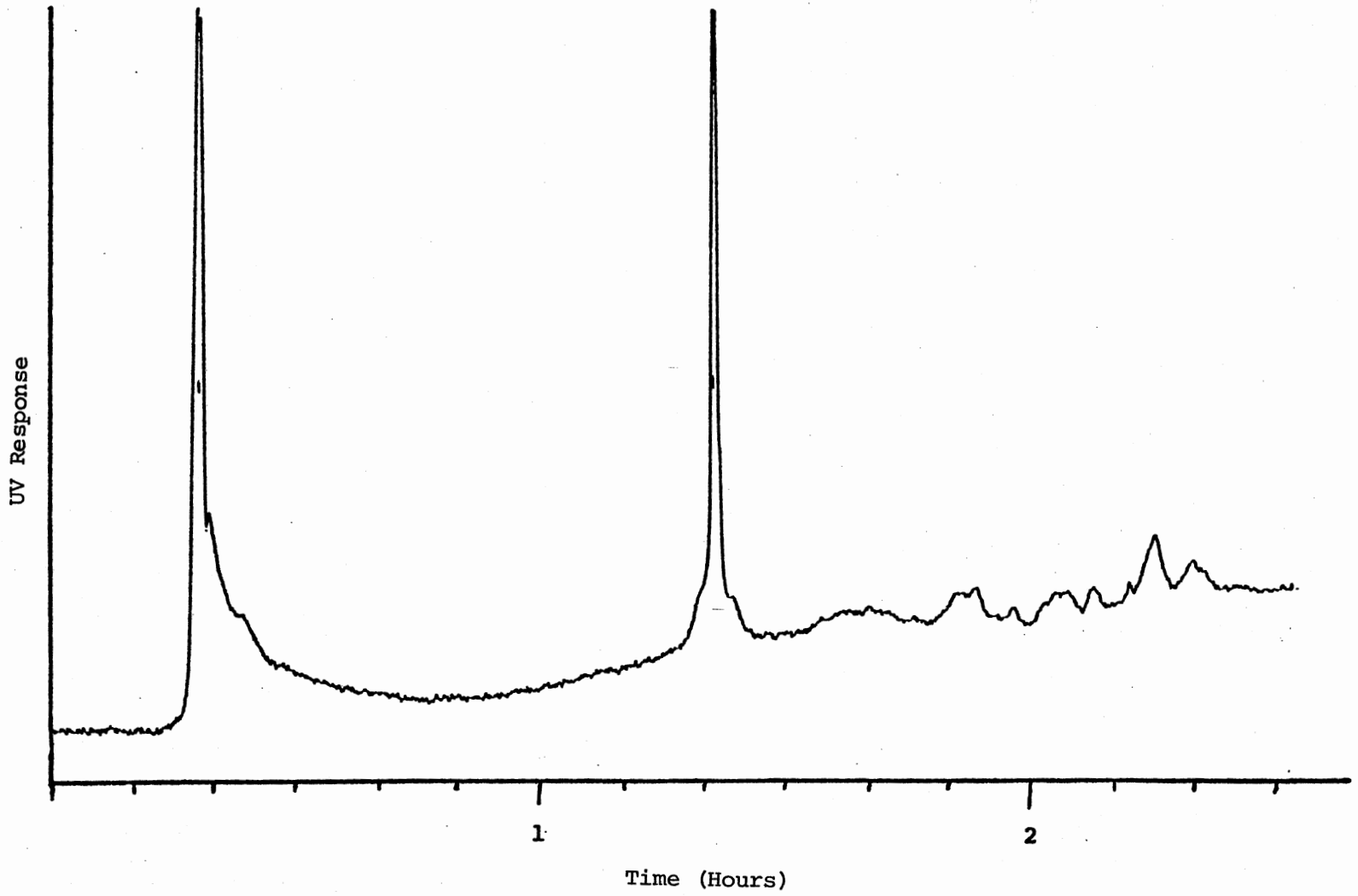


Figure 8. Chromatogram of Alkali Metal Carbonyl Reaction (Fresh)

Selection of a fluorescence excitation and emission wavelength for the unknown mixture was difficult even with knowledge of the structures. The unresolved mixture was placed in a cell and both monochromators were manually adjusted while fluorescence readings were taken. The solvent background was subtracted to produce a fluorescence maximum at 375-455 nm. This setting and several others were tried but the chromatographed sampled failed to produce any peaks at any combination of settings.

Using the UV retention times of the standards as general references, the first major peaks can be produced by hydroxyl type compounds while the second would correspond more closely to neutral esters. The small steady rise in the baseline was sometimes produced by the gradient buffer.

When the sample in water was allowed to remain at room temperature for approximately 48 hours, the chromatogram in Figure 9 was then produced. Twenty-six separate measurable peaks were obtained, some of which were not present in the original sample. About one fourth of the total detector response was calculated to be in peak number two. Many of the new peaks can possibly be attributed to biodegradation or air oxidation of the sample. The presence of acidic organics is suggested by the longer retention times of some of the new peaks.

Liquid chromatographic separation of AMCP contained in the acetone extract is displayed in Figure 10. Over thirty peaks are easily discernible, while others appear on the shoulders of the off-scale peaks. The two intense peaks (at 20 and 30 minutes) and the two large peaks (at 75 and 85 minutes) correspond to the large peaks in the unextracted sample. However, the later peak appears to be concentrated by extraction. Their retention times occur in the area the acid standards were present.

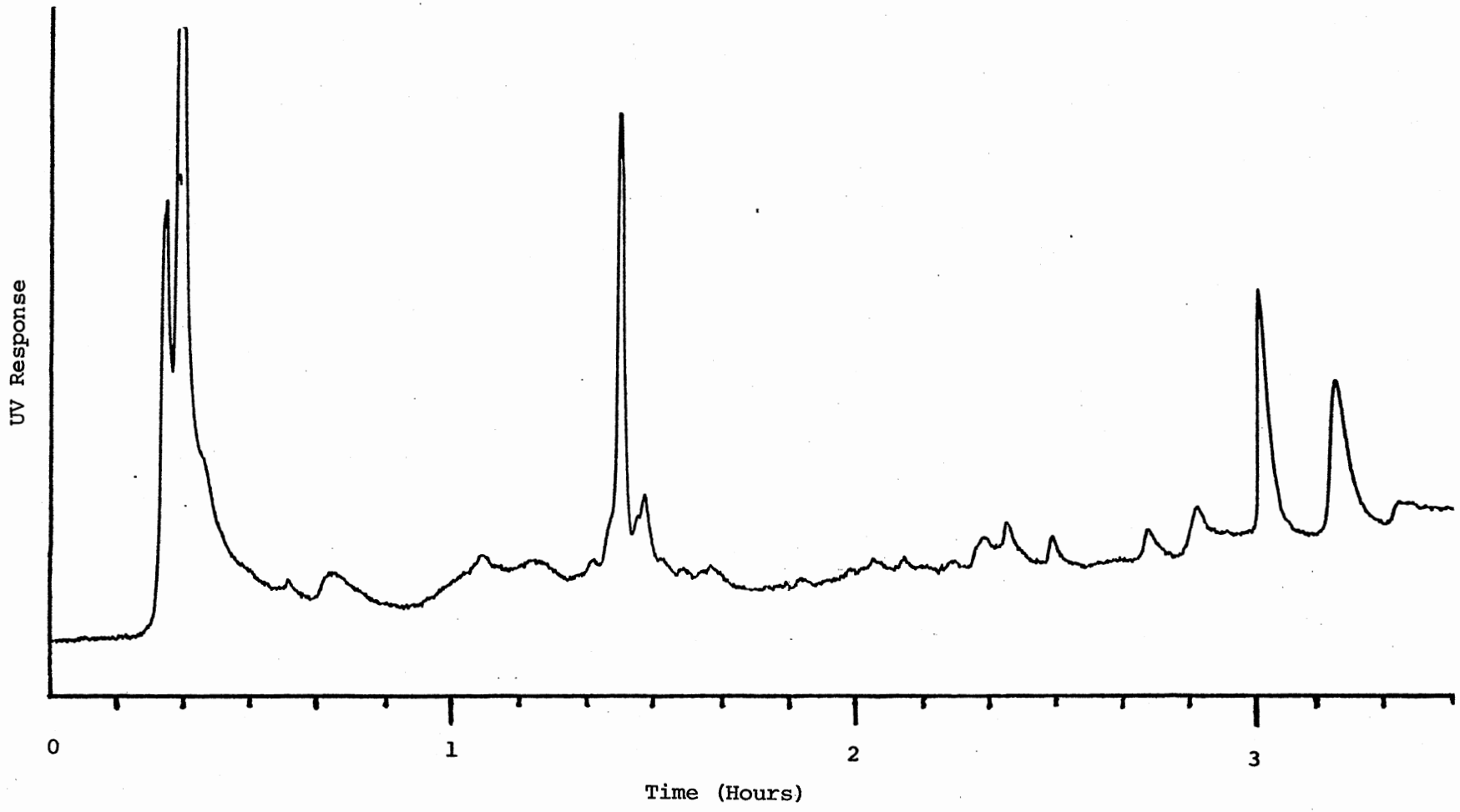


Figure 9. Chromatogram of Alkali Metal Carbonyl Reaction (After Standing)

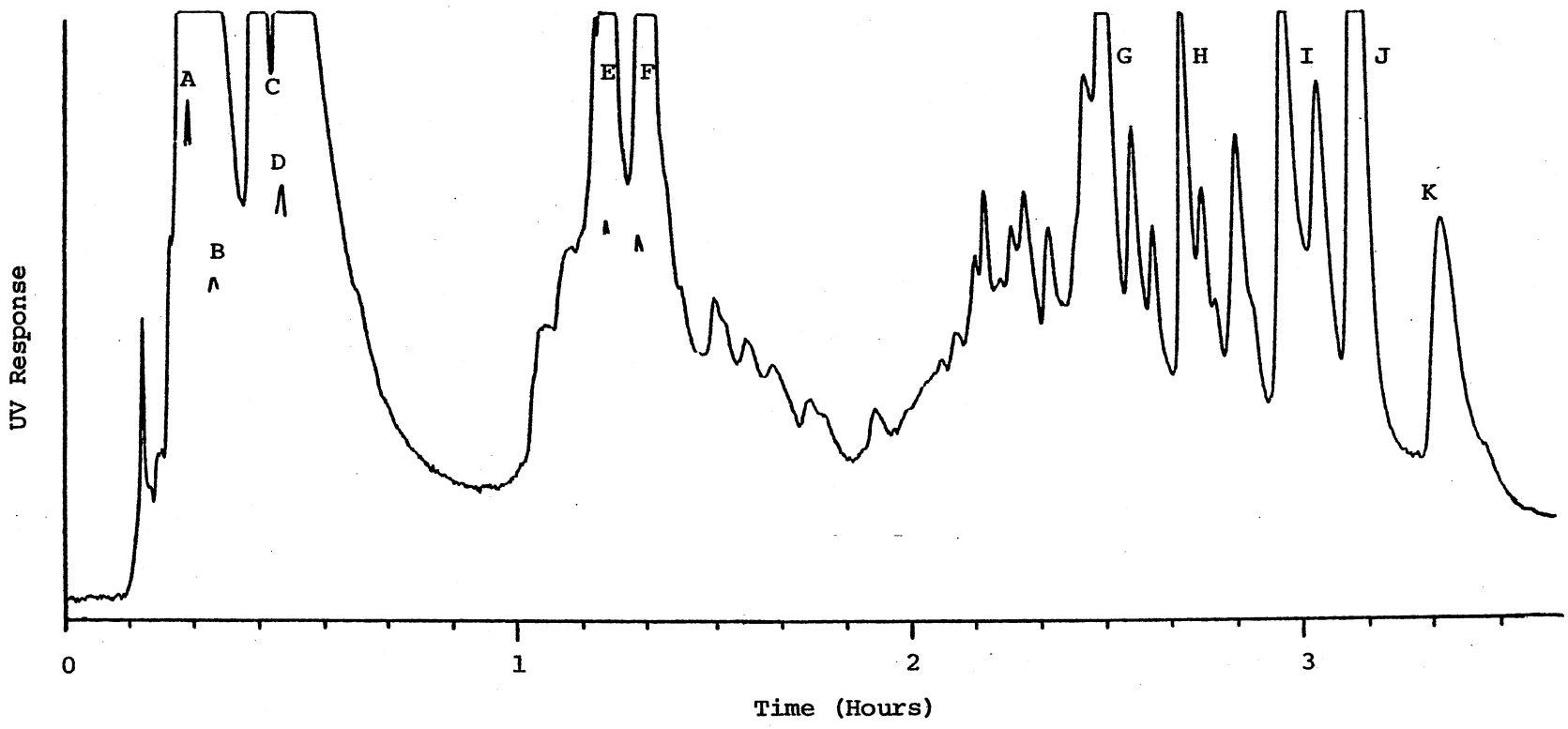


Figure 10. Chromatogram of the Alkali Metal Carbonyl Reaction Acetone Extract

Ce^{+4} Oxidation Chromatogram. The Ce^{+4} oxidation system was installed to determine if measurable amounts of compounds were produced from the reaction that could not be detected by the UV or fluorescent detector (80,81). The elimination of gas bubbles present in the solvent, or produced later in the hating bath, was a difficult problem to overcome. Small corrections were made to the baseline shifts to obtain the chromatogram in Figure 11. The absence of any peaks that were not also observed by the UV detector allowed the conclusion that all of the separated products possessed sufficient conjugation to absorb the 254 nm radiation. The presence of some of these nonabsorbing compounds is confirmed by FI-MS; however, they appear to be eluted with other UV absorbant compounds.

SAX Column Effectiveness

The liquid chromatograph ion exchange columns were evaluated by calculation of the height of the theoretical plate, the column selectivity, the capacity factor, and the resolution. Using the equations in Chapter II, the calculations were performed on the fifteen major peaks in the chromatogram. A complete summary of these calculations is given in Table III.

The resolution of many peaks is near the baseline, as can be seen in Figure 7. Althoug the average number of plates measured 44,800, the individual numbers varied greatly with the compound and its retention time. Some of the later peaks had over 150,000 plates per meter, which is excellent for ion exchange chromatography. The average HETP was .23 mm, which is below the 1.0-0.30 mm requirement for high performance.

Similar theoretical analysis of the ^{13}C reaction mixture peaks pro-

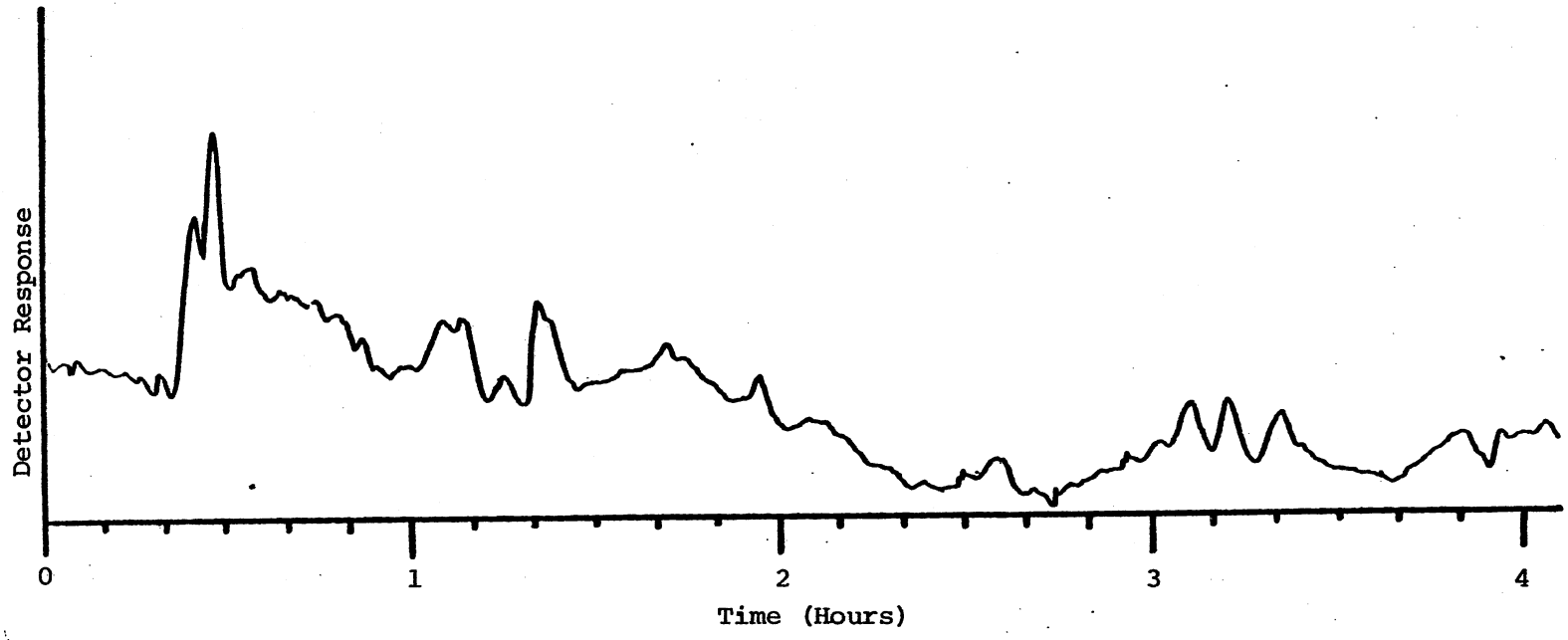


Figure 11. Chromatogram of the Alkali Metal Carbonyl Reaction Cerium Oxidation (Fluorescence)

TABLE III

SAX COLUMN EFFICIENCIES

Peak	h (cm)	w (cm)	A (cm^3)	tr (cm)	N	α	K^1	Rs	HETP (mm)
1	9.0	.41	1.8	4.4	1,843			.49	.27
2	15.2	.82	6.2	4.7	526	8.0	.07	1.90	.97
3	27.9	1.39	19.4	6.8	384	5.3	.55	8.79	1.30
4	6.9	.93	3.2	17.0	5,346	1.14	2.86	2.68	.09
5	9.2	.41	1.9	18.8	33,641	1.13	3.27	4.17	.015
6	11.0	.50	2.8	20.7	27,423	1.27	3.70	8.57	.018
7	4.0	.55	1.1	25.2	33,589	1.35	4.73	5.08	.015
8	60.9	2.32	70.0	32.5	3,140	1.01	6.39	.17	.16
9	66.0	2.32	75.9	33.9	3,218	1.05	6.48	.92	.16
10	5.6	.72	2.0	34.3	36,311	1.08	6.80	3.77	.014
11	12.1	.50	3.0	36.6	85,732	1.09	7.32	2.10	.006
12	20.9	.55	5.7	37.7	75,175	1.08	7.57	3.13	.007
13	10.6	1.05	5.6	40.2	23,452	1.08	8.14	2.16	.021
14	15.7	3.10	24.3	44.7	3,328	1.07	9.16	.88	.15
15	10.7	3.65	19.5	47.7	2,733		9.84	.18	

duced similar results. The number of plates averaged approximately 21,000 and the mean HETP was .27 mm. Overall, better separation was produced with 50 cm of this 10 micron packing than with 180 cm of a 37 micron packing. The larger column diameter also allowed increased sample size (93).

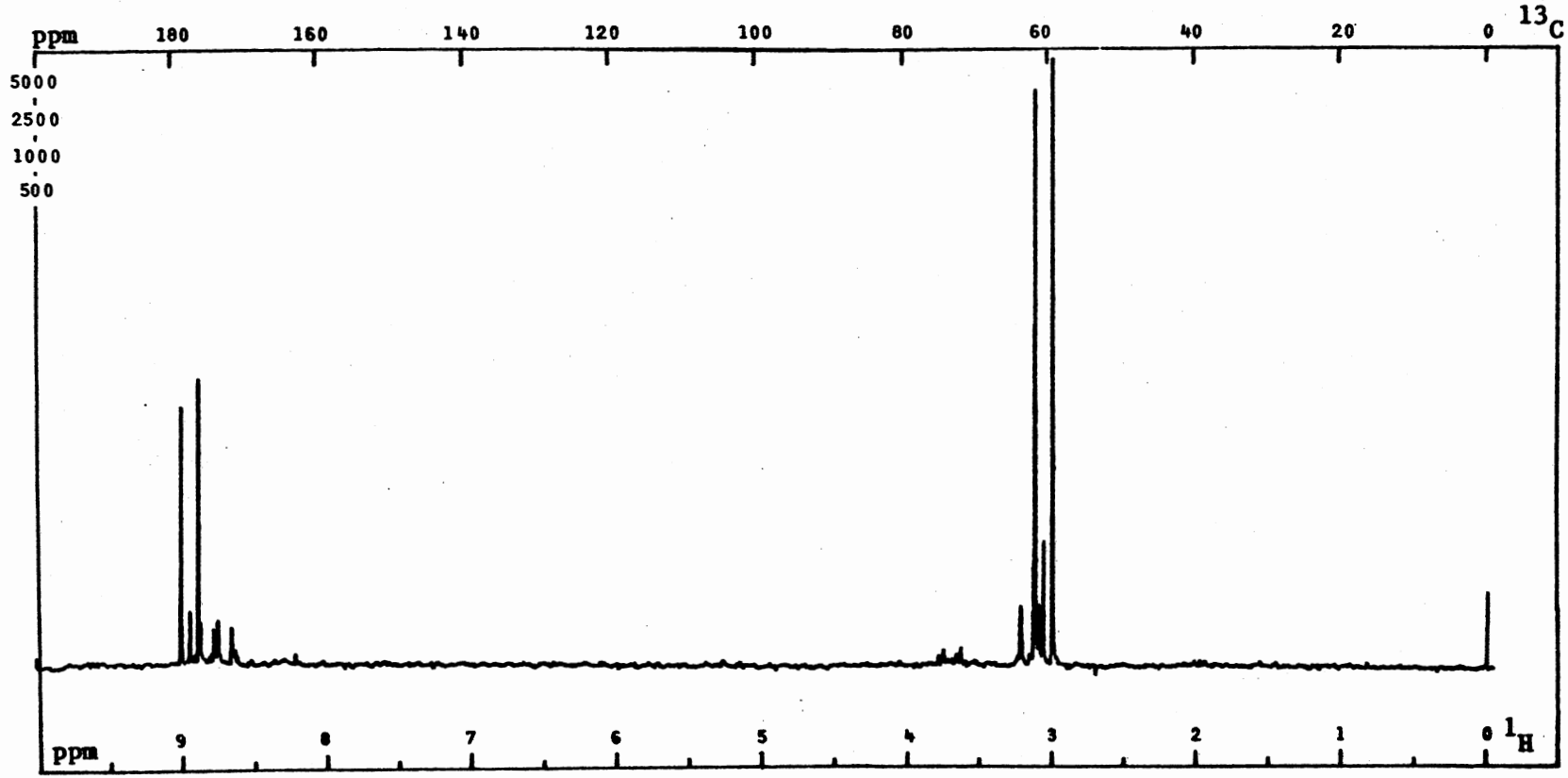
Nuclear Magnetic Resonance Analysis of the Carbon-13 Mixture

Carbon-13

A proton band decoupled ^{13}C NMR spectrum from the acetone extract of the AMCP is displayed in Figure 12. Instrumental and experimental conditions of the analysis are given below the spectrum. The value of this and the other NMRs was to identify the types of functional groups and possible bonding. The simplicity of the spectrum was difficult to understand, considering that it was an unresolved mixture. The absence of any CH_3 or CH_2 means that a high degree of unsaturation must have been present.

Four large resonance bands in two doublets (δ = 178.3, 176.9 and 61.5, 59.2) were directly observed. In each case, the spin of the neighboring ^{13}C atoms split what would normally be a large, single line into an intense doublet. A small peak, corresponding to the nonsplitting 10% ^{12}C in the molecules, occurs exactly halfway between (δ = 177.1, 60.3) each of the two intense doublets.

Approximately nine smaller resonance bands were also found. The high ^{13}C percentage had caused splitting of a triplet centered at δ = 175.8 and δ = 173.3. The multiplets at δ = 171.4 and δ = 173.8 were



PFT X CW _ ; Solvent. . D_2O ; SO. . 35001 Hz; PW. . 500 Hz; T. . 25 °C; Acq/SA. . NA

Size. . 5 K; P2/RF. . 0.10 µs/dB; SF. . 25.2 Hz; FB. . NA Hz; Lock. . 2H ; D5/ST. . 1.3 s

DC. . NA; Gated Off. . NA ; Offset. . 45308 Hz; RF. . 900 W/dB; NBW. . 100 Hz

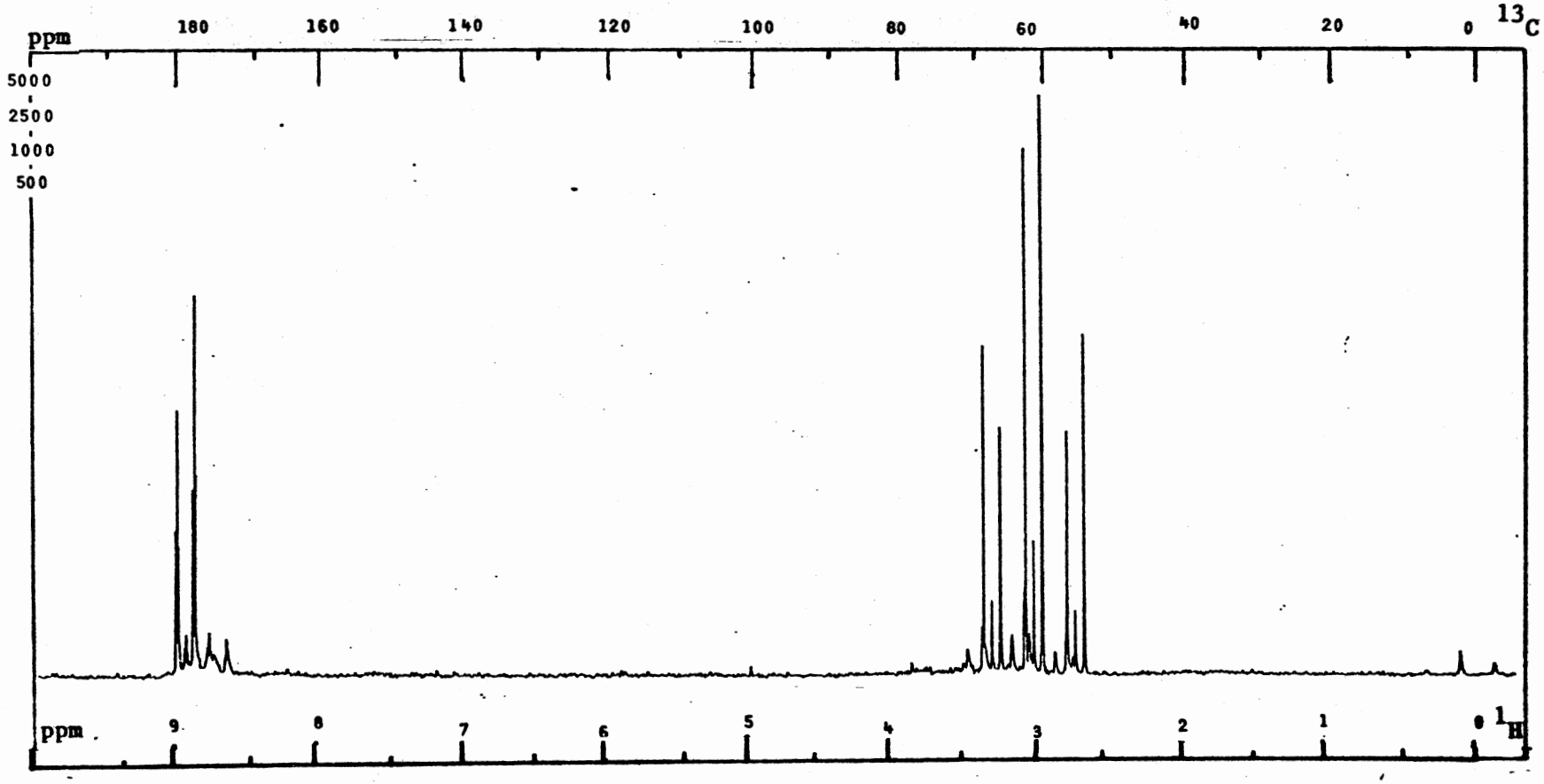
Figure 12. Carbon-13 NMR of AMCP (Proton Developed)

also produced by similar splitting. There appeared to be no correlation of the lines at δ = 61.1, δ = 61.7, and δ = 63.5 with any other peaks.

The doublet centered at δ = 177.1 corresponded closely to a carboxylic acid carbon with either a methyl or methylene group attached to the acid carbon (93). Secondary alcohol carbons have shielding constants approximately equal to the δ = 60.8 midpoint of the other two large doublets (94). A few esters and ethers also have chemical shifts in this same range (96). The less intense lines in the spectrum were produced from minor products of the reaction and appear to be close in nature to the major products.

The coupling constant Jcc between the two large doublets is 58.5 Hz. This constant corresponds to a compound of the type CH_3-$COOC_2H_5$, where the coupling is located along the indicated band (95). The triplets (δ = 175.8 and δ = 173.3), and the multiplets (δ = 171.4 and δ = 173.8) each have a coupling constant of 62.7 and 61.1 Hz, respectively. These two constants were close enough to the 58.5 Hz of the large doublets to be considered as similar in character. By considering both the coupling constant and the shielding constant, the carbons at approximately 60 Hz were best identified as alcohol carbon bonded to a carboxylic carbon.

When the identical mixture was re-analyzed while allowing proton coupling, the spectrum (Figure 13) appeared remarkably different. The doublet, centered about 60.8, was split into a triplet of doublets by two hydrogens. No additional splitting of the carboxylic carbon was noted, as expected. The combination of this information, then, gave the predominate structure of $H_2C(OH)COO$-.



PFT _ CW _ ; Solvent. . H_2O ; SO. . 35001 Hz; PW. . 5000 Hz; T. . 25 °C; Acq/SA. . NA

Size. . 5 K; P2/RF. . 0.10 µs/dB; SF. . 25.2 Hz; FB. . NA Hz; Lock. . ^2H ; D5/ST. . 1.3 s

DC. . NA; Gated Off. . ON ; Offset. . 45308 Hz; RF. . 900 W/dB; NBW. . 100 Hz

Figure 13. Carbon-13 NMR of the AMCP

Proton NMR

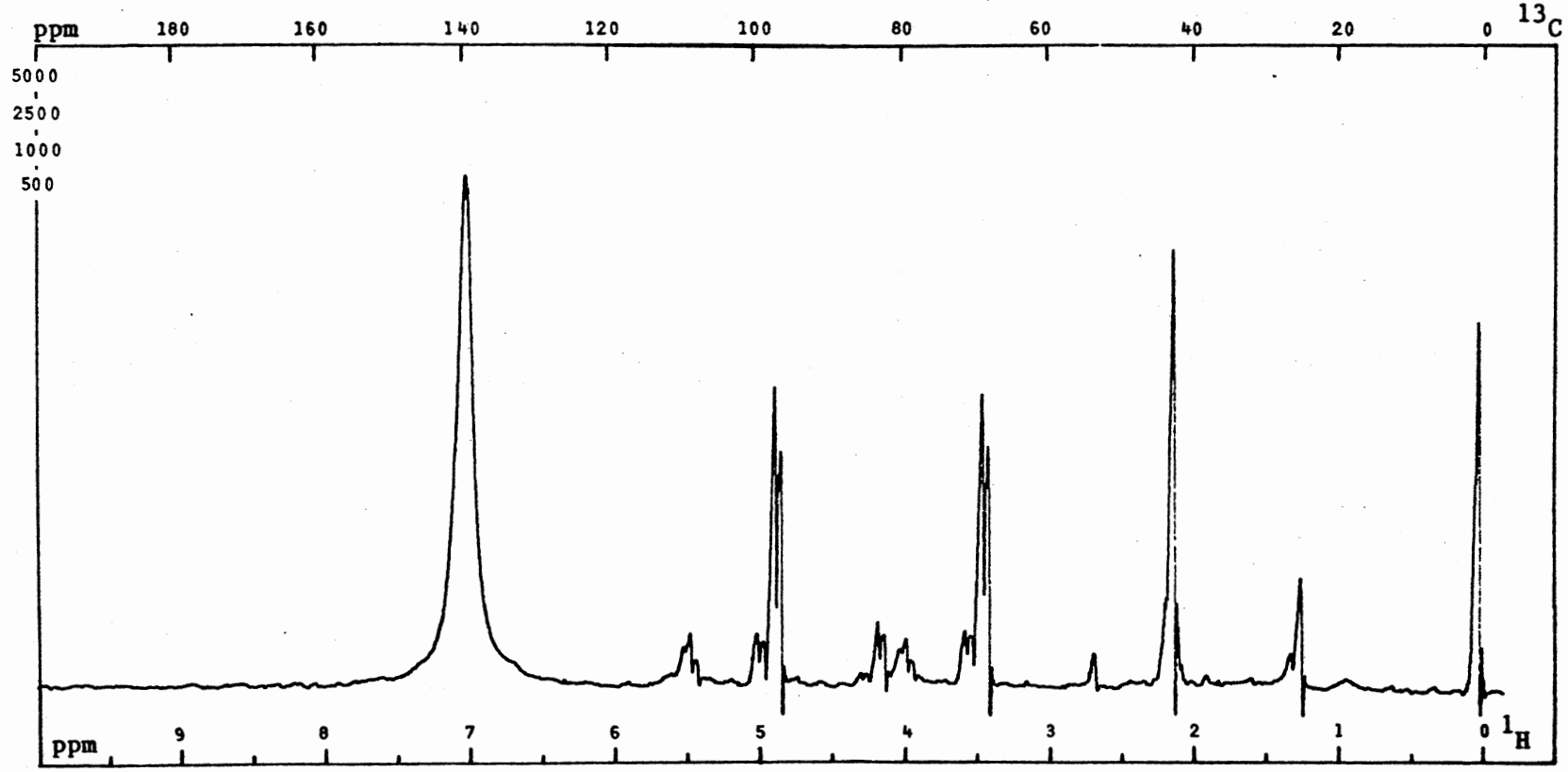
The acetone extract of the AMCP was further investigated by performing a proton NMR, which is shown in Figure 14. Deteurium exchange from the solvent occurred rapidly with the proton at $\delta = 6.98$, and it disappeared if not analyzed readily. Those protons corresponded closely to vinyl, pyridine, or hydroxyl hydrogens.

The large peak at $\delta = 2.10$ was caused by the acetone-d_6 solvent. The two intense doublets centered at $\delta = 3.37$ and $\delta = 4.80$, and the weak isotope doublet at approximately $\delta = 4.85$ were all produced from identical protons which are ^{13}C-H coupled. The triplets at $\delta = 3.94$ and $\delta = 5.43$ are related, as are the two doublets equally spaced about $\delta = 3.48$ and $\delta = 4.91$.

The percentage of ^{13}C at any position in the molecule can be calculated by dividing the height of the split lines by the total height of all the lines. This calculation gave a 91.4% carbon-13 for the hydrogens at $\delta = 4.85$, which is within 1% of the 90.65 value of the ^{13}CO gas used.

Infared Spectroscopic Analysis of the Carbon-13 Mixture

An IR spectrum of the unextracted AMCP in a KBr pellet is depicted in Figure 15. The strong and wide IR absorption at 3120 cm^{-1} was characteristic of carboxylic acids dimer O-H stretching (97). The 1660 cm^{-1} band was accounted for as C=O stretching vibrations of α-β, unsaturated acids. The other strong absorption at 1400 cm^{-1} involved O-H bonding. Small chemical shift differences in ^{12}C and ^{13}C molecules have been detected in some compounds (98).



PFT _ CW _ ; Solvent.Acetone-d_6 ; SO. . 86289 Hz; PW. . NA Hz; T. . 25 °C; Acq/SA. . NA

Size. . 5 K; P2/RF. . 0.014µs/dB; SF. . NA Hz; FB. . NA Hz; Lock. . ; D5/ST. . 250 s

DC. . NA; Gated Off. . NA ; Offset. . NA Hz; RF. . NA W/dB; NBW. . NA Hz

Figure 14. Proton NMR of the AMCP Acetone Extract

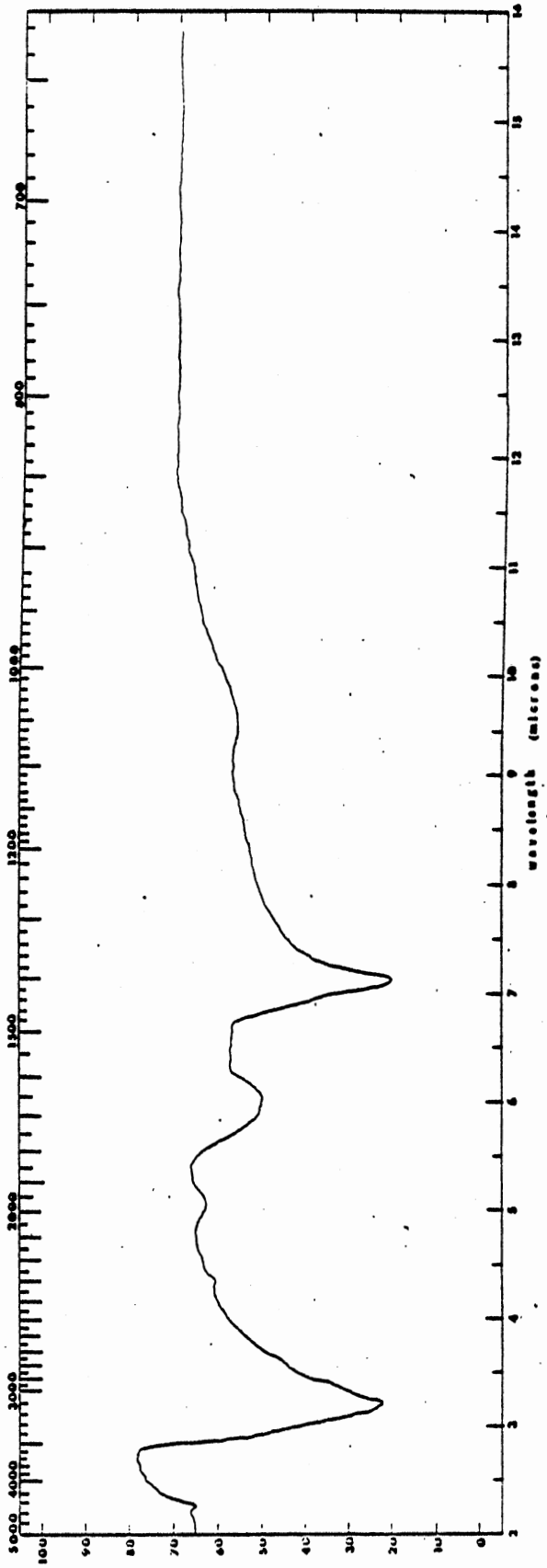


Figure 15. Infared Spectrum of the AMCP in a KBr Pellet

The oil remaining from evaporation of the acetone extract of the AMCP produced the IR spectrum (smear) in Figure 16. The absorption band was still broad due to hydrogen bonding and shifted to produce intense bands at 3370, 1670, 1630, 1215, and 1063 cm^{-1} and weaker absorptions at 1430 and 1380 cm^{-1}. The bands at 3370, 1670 cm^{-1} and (1430, 1480) cm^{-1} represent the O-H stretching, C=O stretching, and O-H bending, respectively of carboxylic acids. The 1630 cm^{-1} band was produced by C-O stretching of alcohols in solution. The presence of conjugated double bonds is more probable with the appearance of absorption in the 500 to 700 cm^{-1} range.

A clearer picture of the absorption was seen by the FT-IR spectrum of Figure 17. The wide absorption at 3300, 1700, 1440, and 1380 cm^{-1} again resembles -C-O-H vibration modes. The absorptions at 1190 cm^{-1} and 1070 cm^{-1} suggest the C-C-O stretching of unsaturated acetates and the C-O stretching of primary alcohol acetates, respectively.

Evaluation of all three spectra showed the presence of acid, carbonyl, and hydroxyl carbons. The extract spectra contained all the absorptions of the KBr pellet, which suggested an efficient extraction.

Mass Spectrometric Analysis of the Carbon-13 Mixture

Field Ionization

The acetone extract of the AMCP produced the field ionization mass spectra depicted in Figure 18. The spectra shown is a composite of two sets of data taken at different times. This allowed reduction of extraneous ions that might have been produced during sample handling. Both

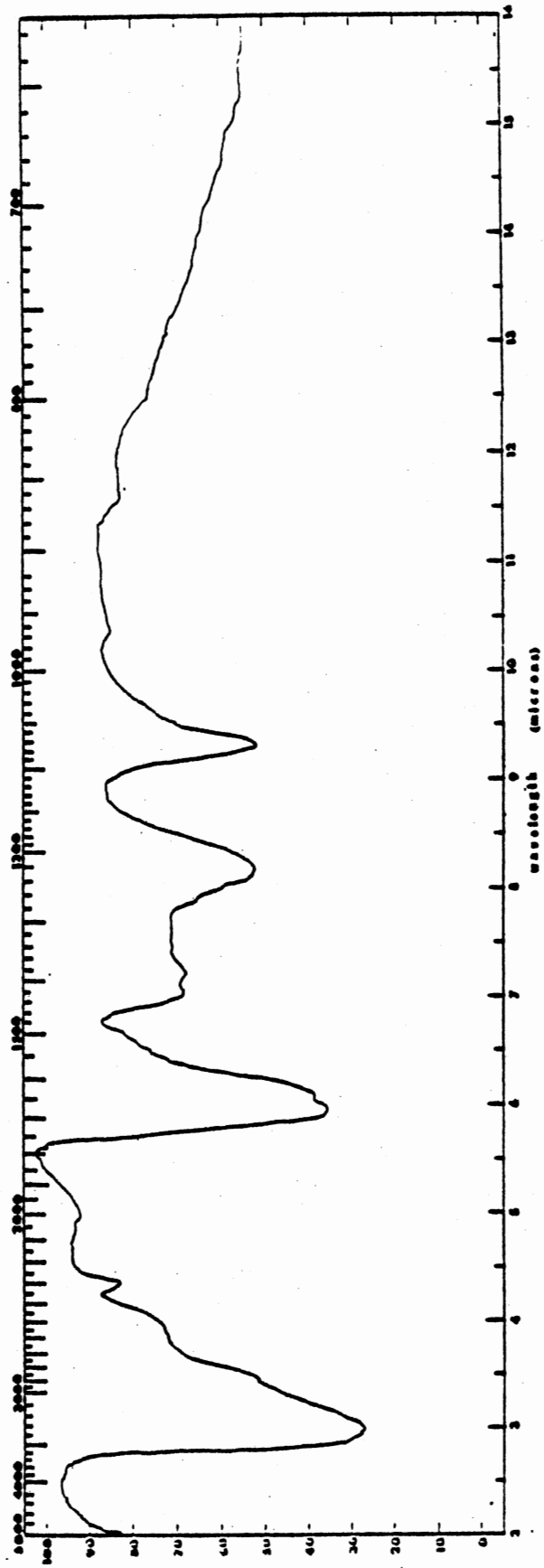


Figure 16. Infared Spectrum of the AMCP Acetone Extract

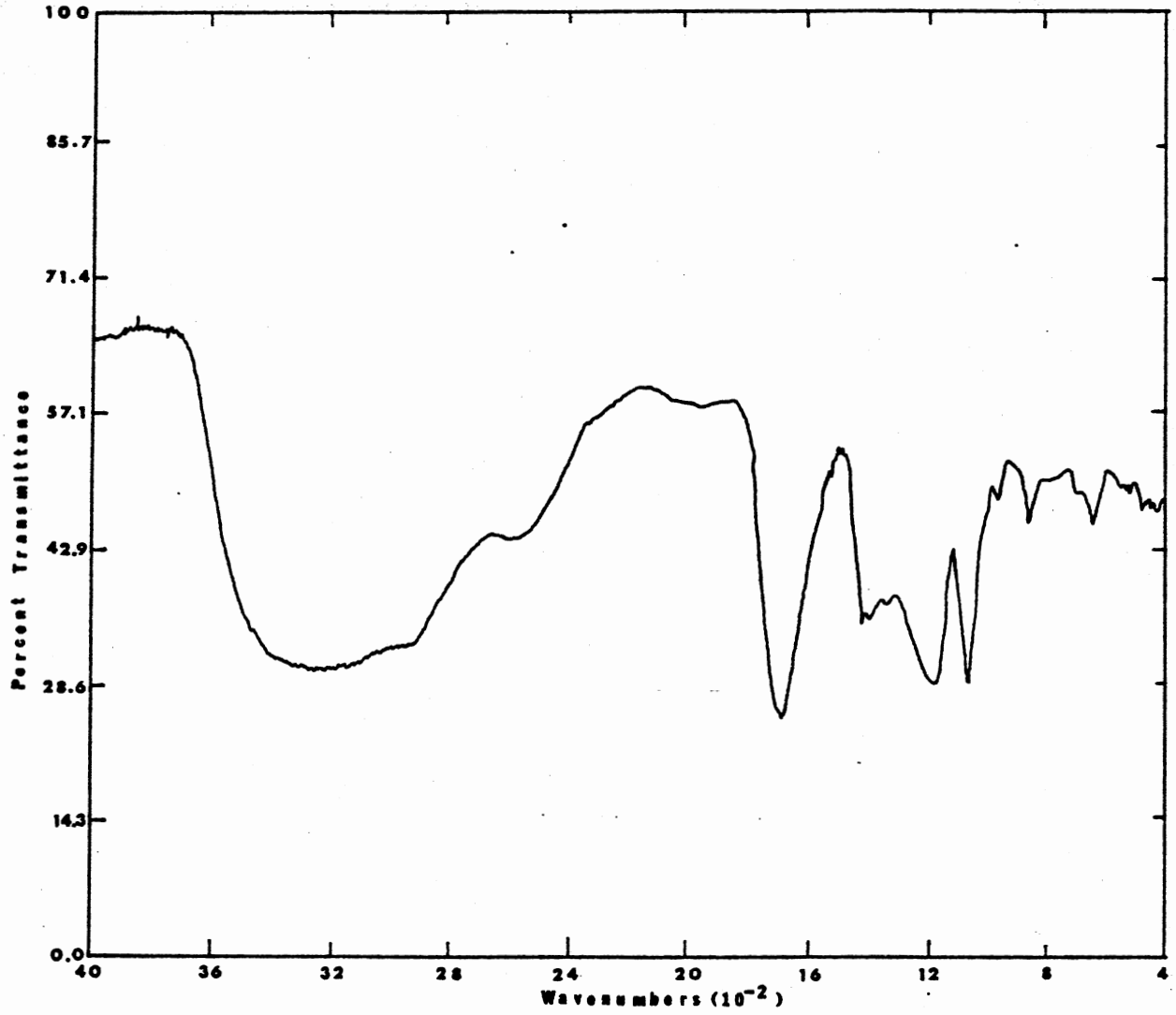


Figure 17. FT-IR of the AMCP Extract

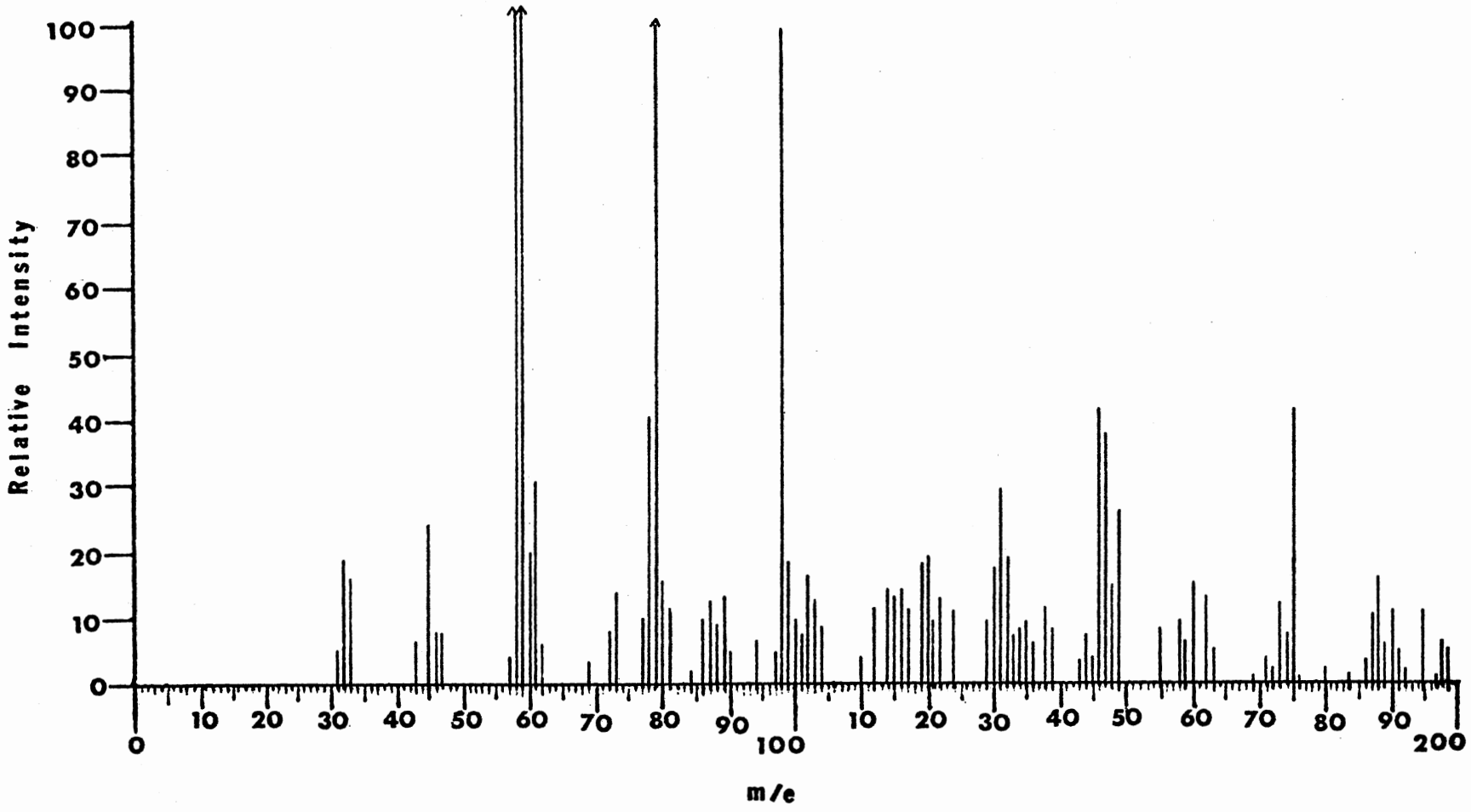


Figure 18. Field Ionization Mass Spectrometry of the AMCP Acetone Extract

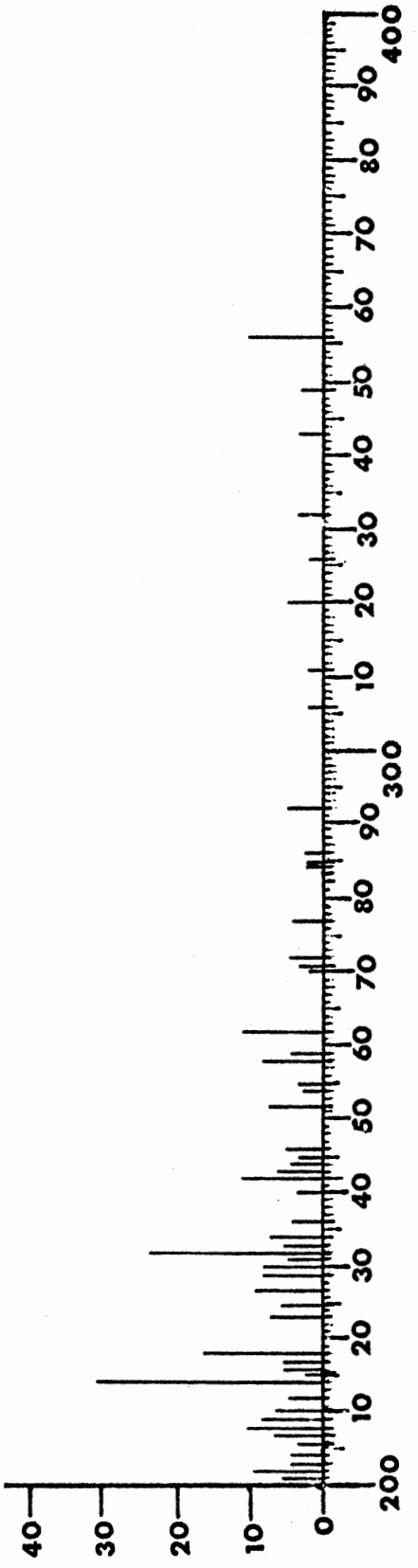


Figure 18 (Continued)

sets of data were very similar, with an especially good match of the more intense mass ions. The data were computer collected and analyzed, but many of the peaks were too small in relation to the residual acetone base peak to be detected.

The most intense peak in the spectrum was 58, with another large peak at 79. Most of the intensity of the off scale peaks at 58 and 59 is from the residual acetone (P and P+1), but the 70% ratio between the masses indicated the presence of ^{13}C compounds at one or both masses. The peak at 98 was used for normalization and is also a prominent peak in many of the mass spectra of the LC collected fractions. The types of compounds in this mixture were extremely insensitive to field ionization when compared to normal aromatics or saturates. Calibration of the instrument was more difficult in FI because of the inavailability of a satisfactory standard mixture to produce calibrating masses, as PFK does similary in the EI mode.

Remembering each peak in the spectrum represents either a different compound or an isotopic peak of the same compound, the large number of peaks was expected (74). Realizing that isotopic peaks will be tightly grouped, the field ionization spectra depicted the presence of a very complex mixture. Analysis of all the products from the reaction would have been very difficult; however, several methods were used to identify the major products.

High Resolution Mass Spectrometry Data

Two high resolution mass spectra were obtained from the acetone extracts of the alkali metal carbonyl products. Each plate was read once and the computer data converted to exact masses. A second reading of the

first plate was performed, and it was determined that error in reading the same plate was approximately equal to the error in different plates. Instrumental conditions (such as resolving power, background, and inlet temperature) and sample preparation can produce variation in the individual intensities.

The two computer outputs were then collated with each other and the average exact mass of common ions determined. Possible molecular formulas were then proposed for the individual exact masses which matched the theoretical formulas. If more than one formula was viable, eliminations were made on the basis of neighboring ions formed and any other relevant information. Most ligitimate masses will have another exact mass, either one higher or one lower, corresponding exactly to the mass of one more or one less hydrogen. The structure of some of the molecular formulas was determined when there was only one possibility. A summary of this data and its interpretation is tabulated in Table IV.

Resolution of about 13,000 was necessary to separate most C, H, N, and O compounds, but this caused the intensity of the masses above 100 to be very low. For this reason, another set of high resolution data was gathered by Gil Greenwood, Phillips Petroleum Company, using a computer system instead of plates, and this data is displayed in Table V. However, even this data was unable to give accurate molecular ions for masses above 100.

Some of the exact masses failed to match any library formulas of common carbon-12 or carbon-13 compounds, probably a result of weak peaks produced from the background. Low mass carbon-12 ions were also detected, which were also a result of sample impurity or instrument background. A few of the low mass fragments arose from the 10% ^{12}C in the reaction

TABLE IV

EXACT MASS DATA AND PROBABLE STRUCTURE INTERPRETATION

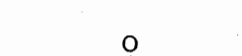
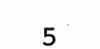
Exact Mass	Molecular Formula ^{1}H	^{13}C	^{14}N	^{16}O	Probable Structure	Other Isotope Structure
13.0114						$-{}^{12}CH$
14.0095	0	0	1	0	N	
.0152	1	1	0	0	-CH	$-{}^{12}CH_2$
15.0242	2	1	0	0	$-CH_2$	$-{}^{12}CH_3$
.9982	0	0	1	0	O	
16.0285	3	1	0	0	$-CH_3$	
17.0049	1	0	0	1	-OH	
.0690	3	0	1	0	$-NH_3$	
18.0105	2	0	0	1	H_2O	
19.0081						$-{}^{18}_{2}OH$
.0165						HHO
.0246					weak	
20.0155						$H_2{}^{18}O$
21.0209					weak	
24.0001						C_2^{12}
25.0025					weak	
26.0100	0	2	0	0	C_2	
27.0110	0	1	1	0	-CN	
.0216	0	2	0	0	HC_2	
.9721					weak	
.9940	0	0	2	0	N_2	
28.0016						
.0132	1	1	1	0	HCN	
.0260	2	2	0	0	HC≡CH	
.9834						
.9920	0	1	0	1	C≡O	
29.0205	2	1	1	0	HC≡NH	
.0353	3	2	0	0	$H_2C{=}CH_2$	
.9951	0	0	1	1	NO	
30.0255	3	1	1	0	H_2CNH	
.0353	4	2	0	0	$H_2C{=}CH_2$	
31.0116						$H_2{}^{12}COH$
.0254						
.0406	5	2	0	0	C_2H_5	
.9850	0	0	0	2	O_2	
32.0074						
.0169	2	0	1	1	H_2NO	
33.0142	3	0	1	1	H_3NO	
34.0187						
.9587						^{35}Cl
35.9700						$H^{35}Cl$
36.9585						^{37}Cl
37.0015						$HC^{12}_{3}{}^{37}$
.9661	1	0	0	1		HCl
39.0088	0	3	0	0	C_3	
.0154						
.9583						Ar
40.0160	1	3	0	0	C_3H	
.9968					weak	
41.0254	2	3	0	0	C_3H_2	

TABLE IV (Continued)

Exact Mass	Molecular Formula ^{1}H	^{13}C	^{14}N	^{16}O	Probable Structure	Other Isotope Structure
42.0020	0	2	0	1	$\diagdown C=C=O$	
.0300	3	3	0	0	C_3H_3	
.0405					weak	
.9977	0	1	1	1	$H\diagdown CNO$	
43.0100	1	2	0	1	$-C=C=O$	
.0382					C_3H_4	
.0500						
.9875						$C^{12}O_2$
					$H\diagdown C=C=O$	
44.0133	2	2	0	1	H	
.0380	4	2	1	0	$CH_2-C=NH$	
.0480						
.9793						
.9872	0	1	0	2	CO_2	
					O	
45.0195	2	1	1	1	$-C-NH_2$	
.0481	5	2	1	0	C_2H_5N	
.9752					weak	
					O	
.9998	1	1	0	2	$-C-OH$	
					O	
46.0286	4	2	0	1	$H-C-CH_3$	
.0508					weak	
.8002						
.8318						

Exact Mass	Molecular Formula ^{1}H	^{13}C	^{14}N	^{16}O	Probable Structure	Other Isotope Structure
.9752					weak	
.9930					HNO2	
					O	
47.0034	2	1	0	2	$H-C-OH$	
.0357	5	2	0	1	H	
.9637					$H_3C-C-OH$	
.9994						
48.0139	3	1	0	2	H	C_4^{12}
49.0048	4	1	0	2	$HO-C-OH$	
50.0093					$H_2-C-(OH)_2$	
.0170						$C_4^{12}H_2$
51.0154						
.0226						$C_4^{12}H_3$
52.0052						
.0171						
.0285					C_4	$C_4^{12}H_4$
.7293						
.9834						
.9971						
53.0255	1	4	0	0	C_4H	
.0340						$^{12}C_4H_5$
54.0067						
.0305	2	4	0	0	C_4H_2	
.0440						$^{12}C_4H_6$
55.0160						
.0527					O	$^{12}C_4H_7$
56.0142	1	3	0	1	$-C-C\equiv CH$	
.0465	4	4	0	0	C_4H_4	

TABLE IV (Continued)

Exact Mass	Molecular Formula ^{1}H	^{13}C	^{14}N	^{16}O	Probable Structure	Other Isotope Structure
.0598						$^{12}C_4H_8$
57.0219	2	3	0	1		
.0672						$^{12}C_4H_9$
.9966	0	2	0	2		
58.0265	3	3	0	1		
.0360						
.0689						weak
.9944	0	1	1	2		
59.0318	4	3	0	1		
.-420						weak
60.0183	2	2	0	2		
.0414	5	3	0	1		
61.0137	2	1	1	2		
.0230						weak
62.0251	4	2	0	2		
63.0241	4	1	1	2		
.9259						weak
.9590						weak
64.0130	3	1	0	3		
65.0222	4	1	0	3		
.0388						weak

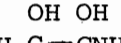
Exact Mass	Molecular Formula ^{1}H	^{13}C	^{14}N	^{16}O	Probable Structure	Other Isotope Structure
69.0647						$^{12}C_5H_9$
70.0043	0	2	2	1	C_2N_2O	
.0396	3	4	1	0	H_3C_4N	
.0752						$^{12}C_5H_{10}$
71.0469	4	4	1	0	H_4C_4N	
.0842						$^{12}C_5H_{11}$
73.0499	5	4	0	1	H_5C_4O	
74.0000						weak
.0071						weak
.0126	2	2	1	2		
.0496	5	3	1	1		
75.0123						
.0254	3	2	1	2		
.0353	3	4	0	2		
76.0306	4	2	1	2		
77.0072	2	1	1	3		
.0180						
.0363	5	2	1	2		
78.0472	6	2	1	2		
79.0003						
.0030						
.0518	7	2	1	2		
88.0318	4	3	0	2		
	4	3	1	2		
89.0397	5	4	0	2		
90.0069						weak
.0366						weak
91.0531	7	4	0	2		

TABLE IV (Continued)

Exact Mass	Molecular Formula ^{1}H	^{13}C	^{14}N	^{16}O	Probable Structure	Other Isotope Structure	Exact Mass	Molecular Formula ^{1}H	^{13}C	^{14}N	^{16}O	Probable Structure	Other Isotope Structure
	6	3	1	2									
92.0555	7	3	1	2									
93.0670	8	3	1	2									

mixture or from the residual acetone fragments. The presence of strange ions such as C_4^+ is not uncommon but has very little significance.

Structure Interpretation From the FI and High Resolution Data

The advantage of identification of these low molecular weight compounds before separation into fractions is that they may be lost under a fragment peak at the same mass. Also, these compounds will not, in general, absorb UV and therefore will probably be collected with other compounds. Some of them can also be generated in the EI mode by rearrangement of larger organic molecules.

The first peak in Figure 20 is at mass 31. Table V shows a molecular ion formula at mass 31 of $^{13}CH_2O$ which means the structure is that of formaldehyde. The structure of mass 32 (from the FI spectra) was not identified because the exact mass tables did not contain ions at mass 32 which could also be neutral molecules. The major portion of the 33 peak is composed of $^{13}CH_4O$, as can be observed in Table IV. Methanol is the only possible structure for this formula. Another part of peak 33 is comprised of an H_3NO ion according to Table IV. Hydroxylamine has this exact mass formula and is a very likely candidate.

The next series of masses contained a large peak at m/e 45 which corresponds with the $^{13}CO_2$ of the exact mass tables and was identified as carbon dioxide (82). Almost all spectra contained considerable m/e 45, even some spectra taken at $-80^{\circ}C$.

This was a very intense peak even on the plates. The peak at m/e 46 could not be assigned a molecular structure, but the ion at m/e 47 was identified as formic acid, as was confirmed by both sets of exact

TABLE V

EXACT MASS DATA AND PROBABLE INTERPRETATION

Exact Mass	Molecular Formula ^{1}H	^{13}C	^{14}N	^{16}O	Probable Structure	Other Isotope Structure
31.0057					HNO	
.0097						
.0113	2	1	0	1	H_2CO	
.9848					O_2	
32.0107					H_2NO	
.0209	3	1	0	1	H_3CO	
.0226						$H_3{}^{12}COH$
33.0192					H_3NO	
.0267	4	1	0	1	H_3COH	
34.0261						
.0301						
35.0360						
.9795						^{35}Cl
37.9776						^{37}Cl
39.0269						$^{12}C_3H_3$
41.0428						$^{12}C_3H_5$
.0446						
42.0058	0	2	0	1	C_2O	
.0111						$^{12}C_2H_2O$
.0366	3	3	0	0	H_3C_3	
.0513						$^{12}C_3H_6$
43.0160						
.0213						
.0233	2	1	2	0	HC=NH	
.0575						
.0597						
.9940						
44.0212						
.0302	3	1	2	0	H_3CN_2	
.9962	0	1	0	2	CO_2	
45.0009						
.0167	2	1	1	1	$-C(=O)-NH_2$	
.0292					C_2H_3	
46.0035	1	1	0	1	-COOH	
47.0113	2	1	0	2		
.0423	5	2	0	1	C_2H_5O	
48.0171	3	1	0	2	H_2COH	
49.0189					H_3NO_2	
50.0326						
						$H_3{}^{12}C{}^{12}C(=O)-$
						$^{12}C_3H_7$
						$^{12}CO_2$
						$^{12}C_2H_4O$
						$^{12}C_2H_5O$
						$^{12}CH_2O_2$

TABLE V (Continued)

Exact Mass	Molecular Formula ^{1}H	^{13}C	^{14}N	^{16}O	Probable Structure	Other Isotope Structure
53.0406						$^{12}C_4H_5$
54.0499						$^{12}C_4H_6$
55.0206	2	2	2	0	$C_2H_2N_2$	$^{12}C_3H_3O$
.0556						$^{12}C_4H_7$
56.0168	3	1	0	1	CH_3O	$^{12}C_2H_2NO$
.0645						$^{12}C_4H_8$
57.0218	2	3	0	1	C_3H_2O	$^{12}CH_2NO$
.0558	5	4	0	0	C_4H_5	$^{12}C_3H_7N$
.0714						$^{12}C_4H_9$
.9969	0	2	0	2	C_2O_2	
58.0418					$C_2H_4N_2$	$^{12}C_3H_6O$
.9968	0	1	1	2	CNO_2	
59.0051	1	2	0	2	HC_2O_2	$^{12}C_2H_3O_2$
.0100						
.0694	7	4	0	0	C_4H_7	
60.0125	2	2	0	2	HCOHCO	
.0204						$^{12}C_2H_4O_2$
.0196	3	2	0	2	$C_2H_3O_2$	
.0278						$^{12}C_2H_5O_2$
62.0156	3	1	1	2	CH_3NO_2	$^{12}C_5H_2$
.0281	4	2	0	2	$C_2H_4O_2$	
63.0352	5	2	0	2	$C_2H_5O_2$	
64.0375	5	1	1	2	CH_5NO_2	
65.0223	4	1	0	3	CH_4O_3	
.0390						$^{12}C_5H_5$
67.0508						$^{12}C_5H_7$
.0538						
68.0621						$^{12}C_5H_8$
69.0057	3	0	1	1	CNO	
.0693	3	3	2	0	$C_3H_3N_2$	$^{12}C_5H_9$
70.0735						
.0787						$^{12}C_5H_5$
71.0490	4	3	2	0	$C_3H_4N_2$	$^{12}C_4H_7O$
71.0847						$^{12}C_5H_{11}$
73.0269	2	1	3	1	CH_2N_3O	$^{12}C_3H_5O_2$
	3	1	2	1	CH_3N_2O	

TABLE V (Continued)

Exact Mass	Molecular Formula ^{1}H	^{13}C	^{14}N	^{16}O	Probable Structure	Other Isotope Structure
.0451	5	1	0	1	CH_5O	
.0601	6	3	2	0	$C_3H_6N_2$	
74.0282	3	3	0	2	$C_3H_3O_2$	$^{12}C_2H_4NO_2$
.0348	4	2	2	1	$C_2H_4N_2O$	$^{12}C_6H_3O_2$
.9985	7	4	0	1	C_4H_6O	
77.0335	4	1	2	2	$CH_4N_2O_2$	$^{12}CH_5N_2O_2$
.0379	5	2	1	2	CH_5NO_2	$^{12}C_6H_5$
78.0318						$^{12}C_2H_6O_3$
.0320						$^{12}C_5H_4N$
79.0523	7	2	1	2	$C_2H_7NO_2$	$^{12}C_6H_7$
80.0626	8	2	1	2	$C_2H_8NO_2$	$^{12}C_6H_8$
81.0270	1	4	2	0	C_4HN_2	
.0695						$^{12}C_6H_9$
82.0780						$^{12}C_6H_{10}$
83.0529	5	4	1	0	C_5H_4N	
.0857						$^{12}C_6H_{11}$
84.0554	5	5	1	0	C_5H_5N	$^{12}C_5H_5O$
.0949						$^{12}C_6H_{12}$
93.0716						$^{12}C_7H_9$
.0792	9	3	1	1	C_3H_9NO	

mass data, producing a formula of $^{13}CH_2O$.

Residual acetone accounted for the off scale m/e 58, plus an isotope peak at 59. According to its exact mass, the remaining composition of m/e 59 was $^{13}C_3H_4O$ which would yield a $H^{13}CO^{13}CH=^{13}CH_2$ structure. Its actual peak height was masked by the acetone isotope peak. The ion at m/e 60 and m/e 62 were related in that they differ by exactly two hydrogens and thus had the formulas of $^{13}C_2H_2C_2O$ and $^{13}C_2H_4O_2$, respectively. Since $^{13}CHO^{13}CHO$ is the only possible structure for m/e 60 the m/e 62 logically had the structure of $^{13}HCO^{13}CH_2OH$, hydroxyacetaldehyde; however, acetic acid, $^{13}CH_3{}^{13}COOH$ was also a possibility. Exact mass data predicted an empirical formula of $H_5{}^{13}C_4O$ for the field ionization at m/e 73 which has a cyclic structure of $H^{13}C=^{13}CH^{13}C(OH)=^{13}CH$ or the ketone form of the same structure.

The next group of identifiable mass are at m/e 77-81. The identity of the large 79 has a surprising molecular formula of $^{13}C_2H_7NO_2$. α-Amino glycol is a more likely structure for this formula than $(HO)_2{}^{13}CH^{13}CH_2(NH_2)$. The exact mass data also showed a formula of two less hydrogens, $H_5{}^{13}C_2NO_2$, for the first m/e at 77. Although aminoacetic acid was possible, hydroacetamide was more probable, and its presence has been suggested by an earlier investigator (70). The reaction sequence should favor structures with the oxygens distributed throughout the carbon chain rather than all at one carbon. Dehydrogenation is a common mass spectrometric process and may have been occurring to some extent (12). Dehydration is also possible, especially with these high oxygenated compounds, but it is impossible since the FI spectra were only taken down to mass 30.

Another ion determined from its exact mass was m/e 94, which has a

molecular formula of $^{13}C_3H_9NO_2$. The structural isomers of $H_2{}^{13}C(OH){}^{13}CH(OH){}^{13}CH_2NH_2$ were more consistent with the previous ions described than any other structure.

As discussed earlier, low sensitivity and cumulative background effect of these oxygenated compounds did not allow for acquisition of accurate exact masses for much above m/e 100. However, the field ionization peak at m/e 120 is moderately intense in both sets of exact mass data. The average exact mass is 120.0030, which fits the formula $^{13}C_4H_4O_4$. Sager (69) has also predicted this formula as the potassium salt. The structure is either $H{}^{13}CO{}^{13}C(OH)={}^{13}C(OH){}^{13}COH$ or $H{}^{13}CO{}^{13}CO{}^{13}C(OH)={}^{13}C(OH)H$ or a combination of both.

Mass Spectrometry of the LC Fractions

Fractions of liquid chromatographic effluents were collected in individual vials as the peaks exited the UV detector. Further handling of the collected samples was discussed in detail in Chapter IV. It was found that small amounts of the solid buffer residue failed to contain sufficient amounts of the organics to produce readable mass spectra. This problem was overcome by extraction of the residue with acetone and evaporation of it in the direct probe tube.

Some of the extracted fractions produced superior mass spectra compared to the raw samples because of the absence of minor products which increased the inlet pressure. Figures 19 through 24 are the mass spectra of fractions A, B, C, D, F, and J, as are indicated on Figure 10. These were the only peak fractions containing sufficient quantities of organics to obtain positive qualitative data. This involved injection of the largest possible sample (200 mg) without swamping the detector. Multiple

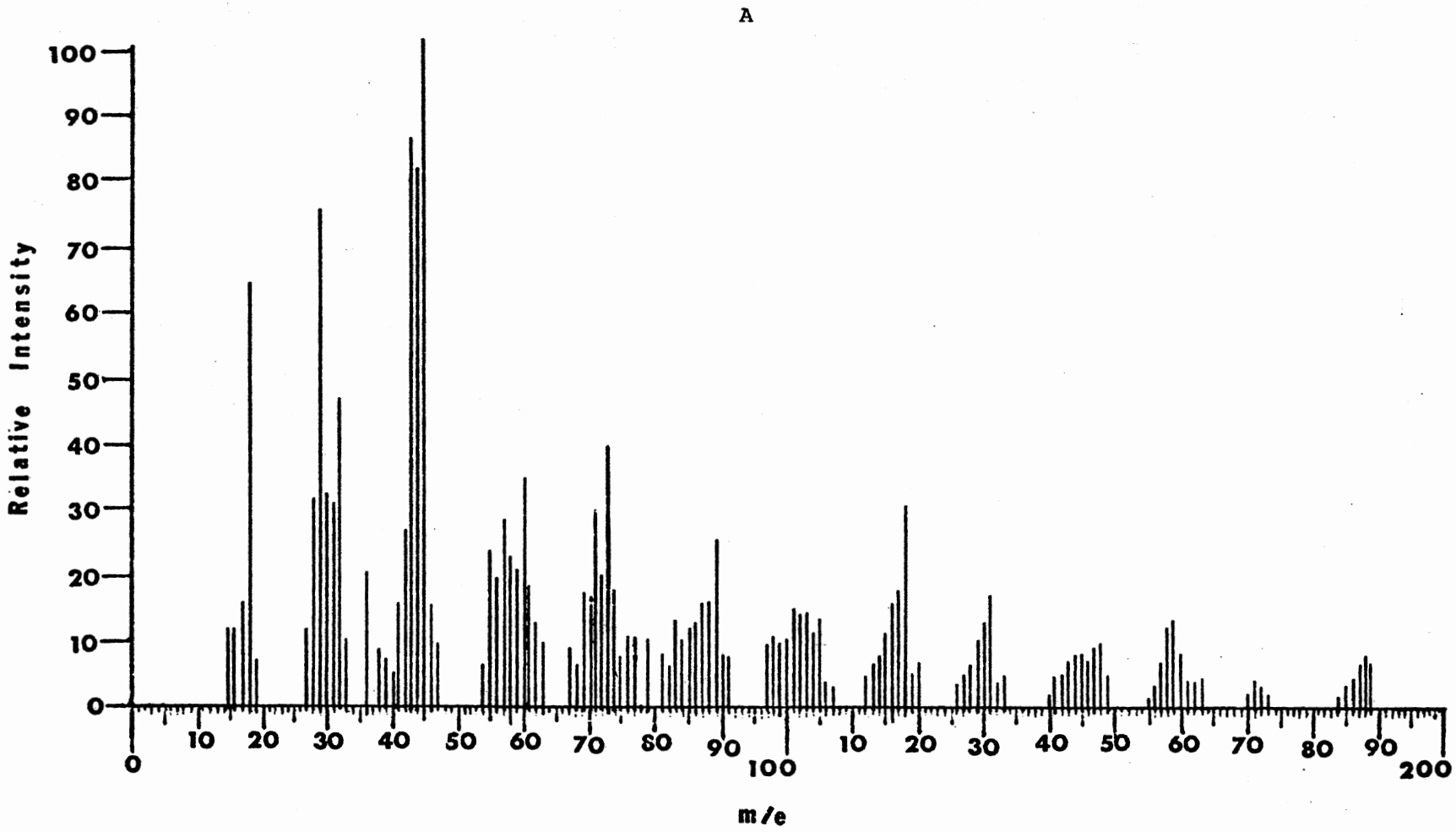


Figure 19. Mass Spectrum of Fraction A

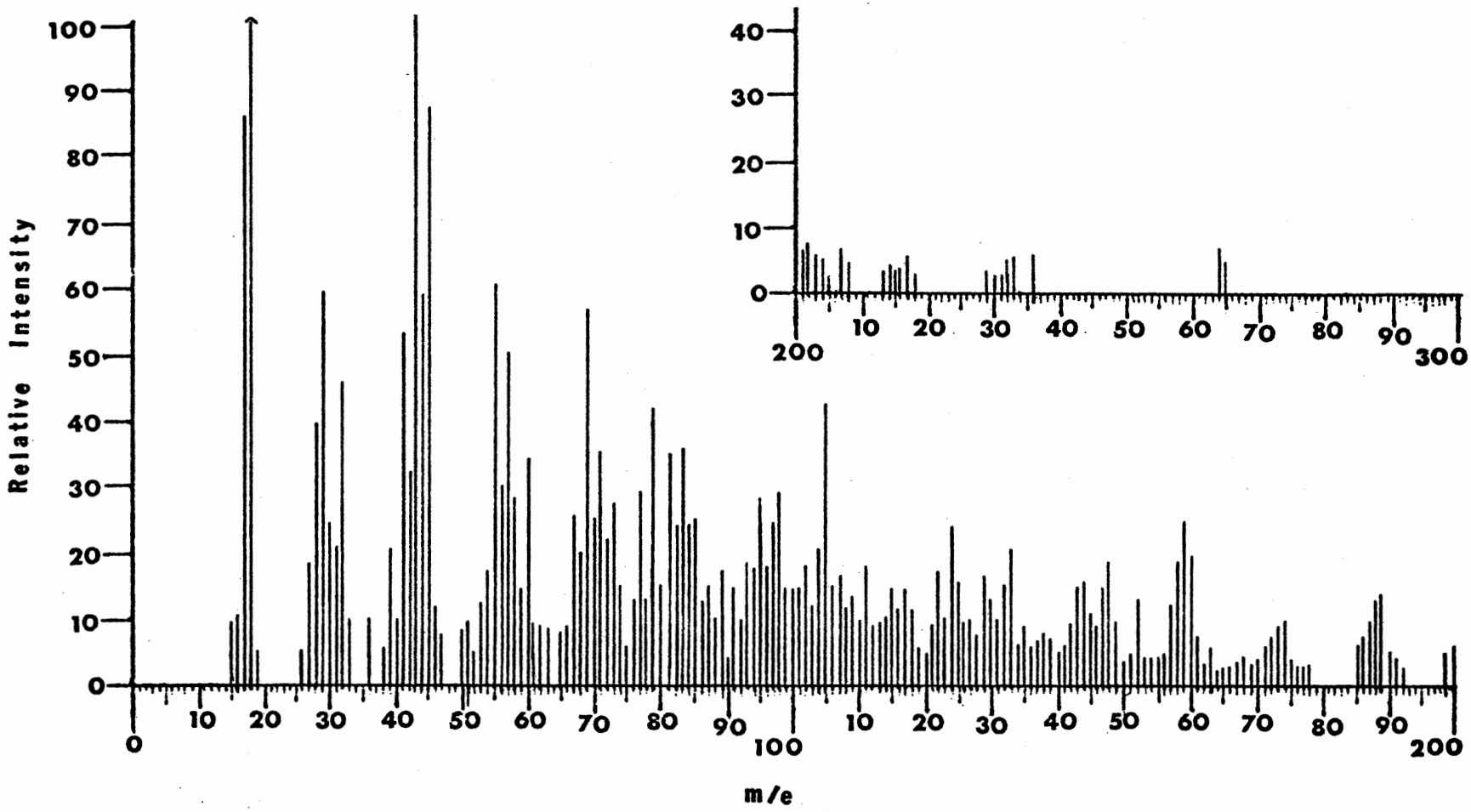
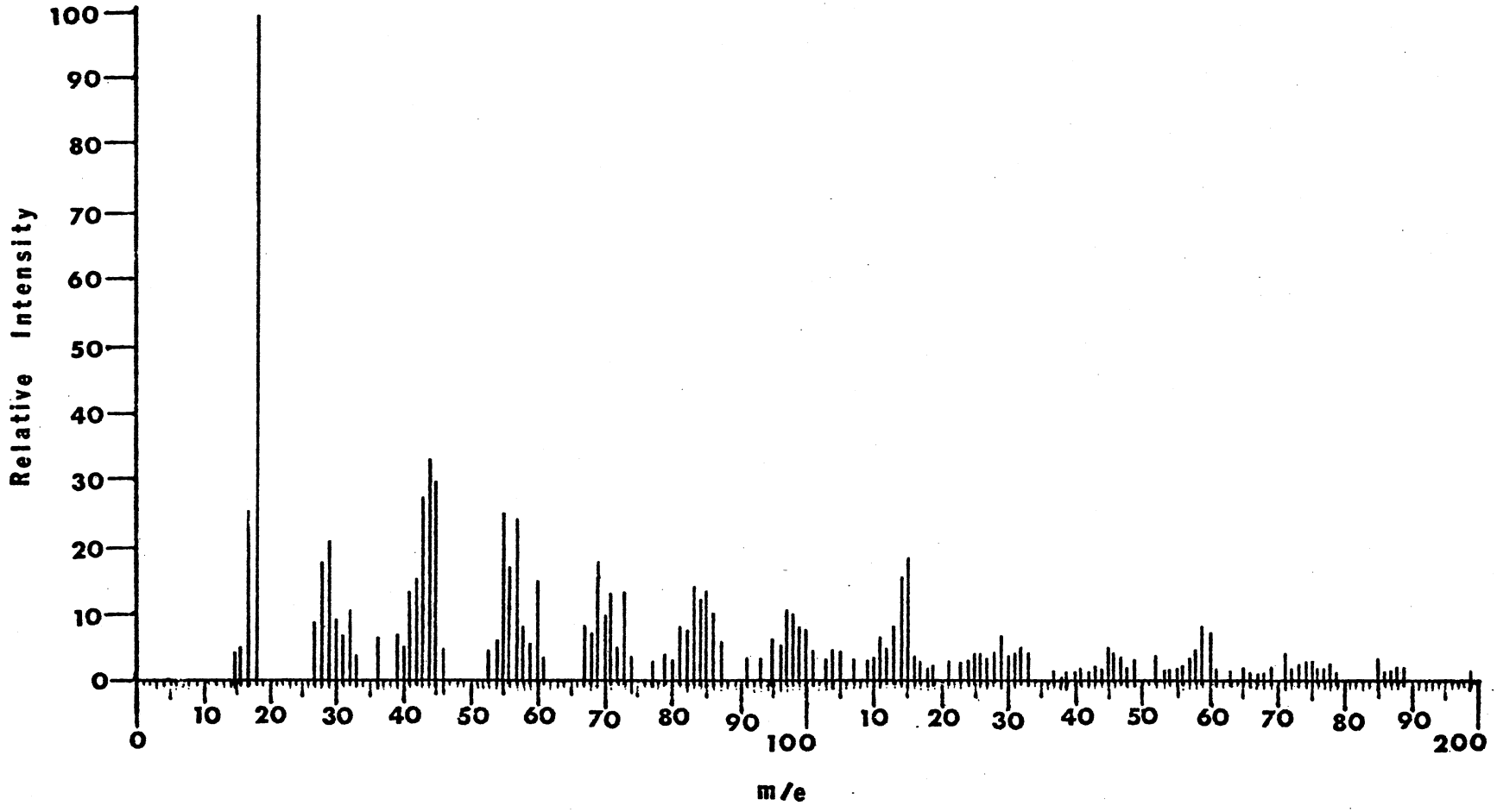


Figure 20. Mass Spectrum of Fraction B



Fraction 21. Mass Spectrum of Fraction C

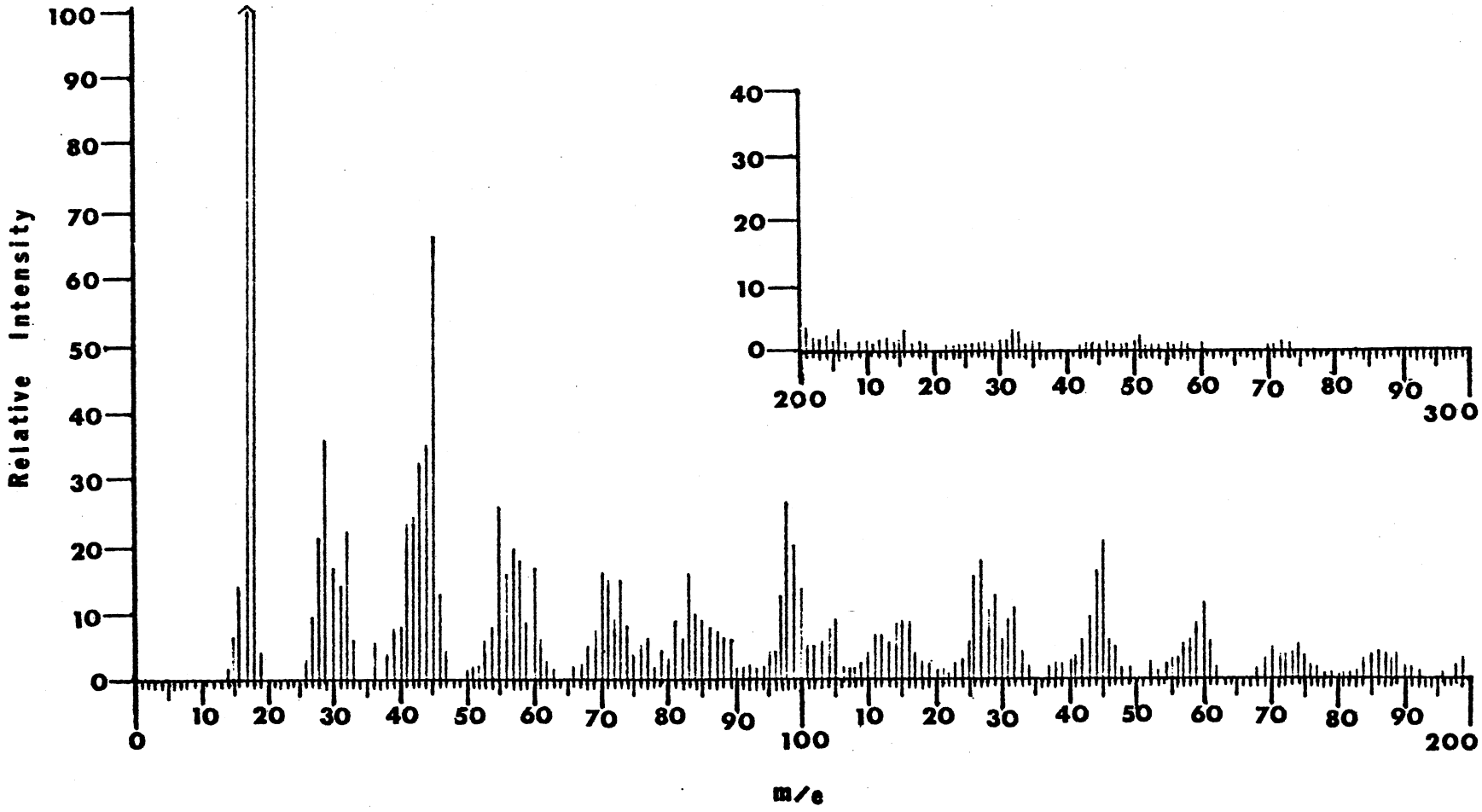


Figure 22. Mass Spectrum of Fraction D

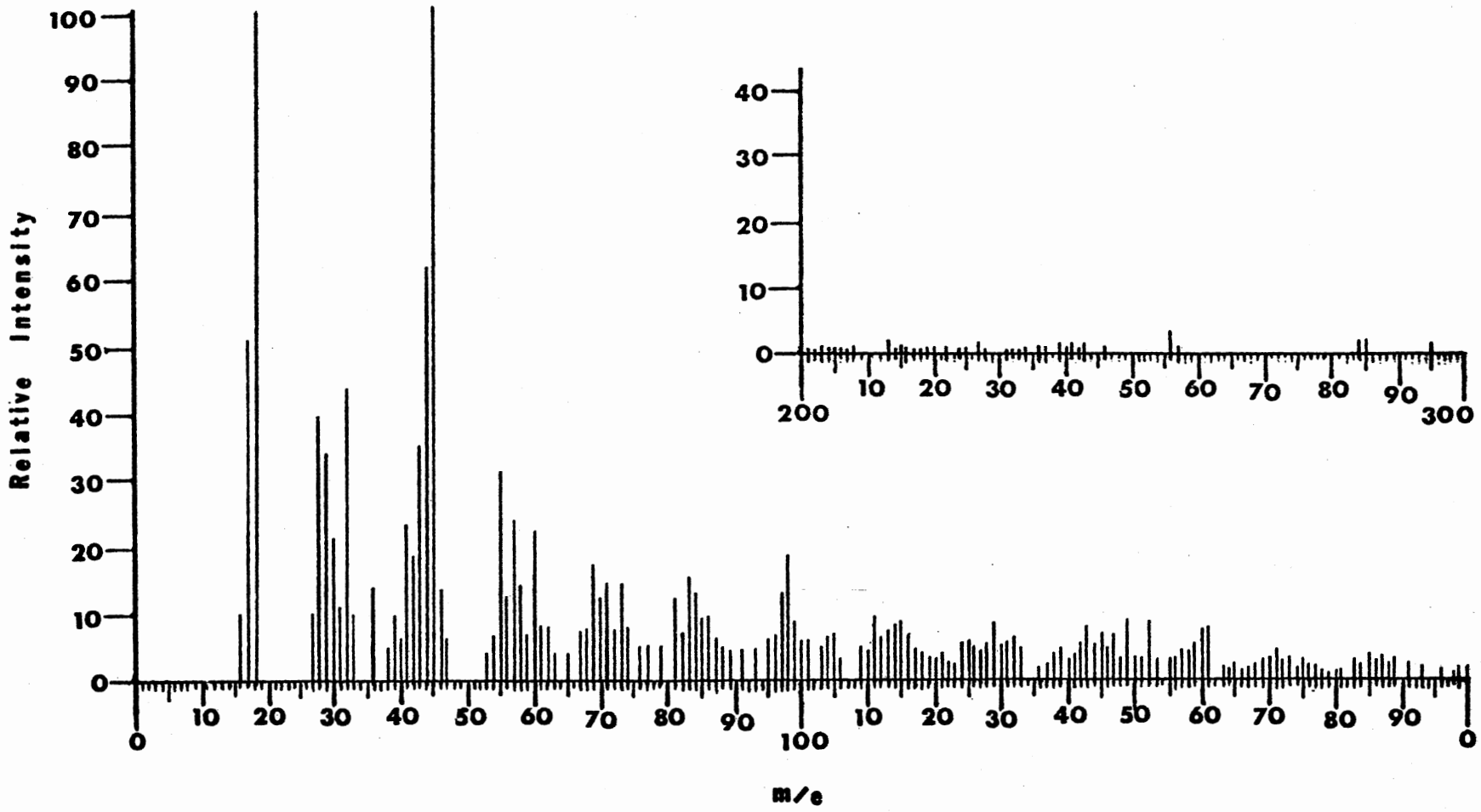


Figure 23. Mass Spectrum of Fraction F

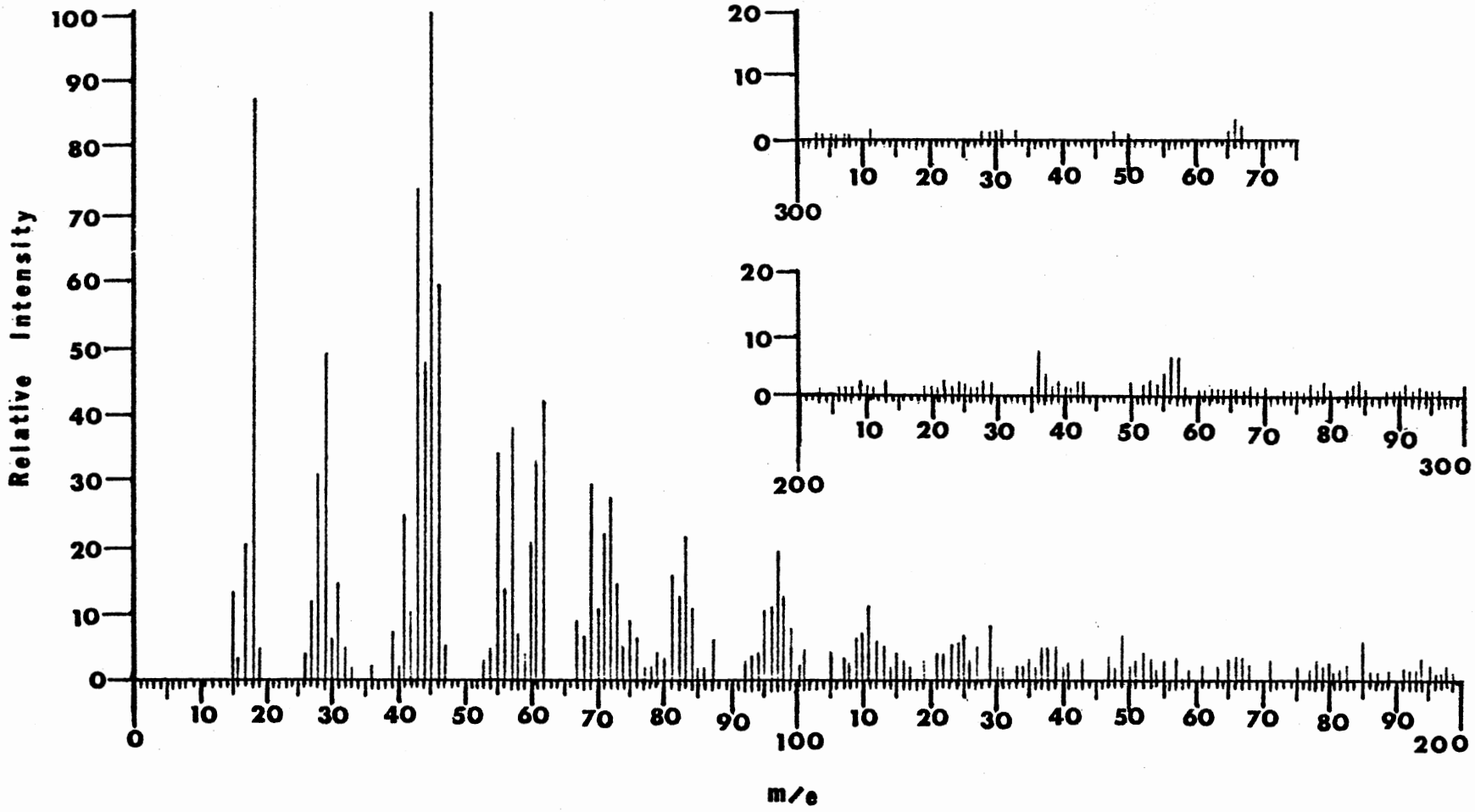


Figure 24. Mass Spectrum of Fraction J

injection increased the buffer residue as well as the amount of samples collected. The problem involved with obtaining very reproducible chromatograms arose when making multiple injections and collections. The high concentration of some fractions allowed the collection of low voltage analysis like that of fraction D displayed in Figure 25. Major low voltage peaks from the other fractions will be introduced to the text, but their spectra will not be presented.

Fraction A. The mass spectrum of Fraction A contained two series of compounds. One group came out at a lower temperature than the scan shown in Figure 19. The spectrum changed considerably between the two scans.

Of the first group of compounds to be distilled off, three were identifiable. Although not shown, the first scan contained intense m/e values of 148, 118, 103, and 87, plus the still present peak of 89, 73, 59, 45, 44, 43, 32, and 29. A low voltage scan gave ions at m/e 148*, 126, 118, 110, and 87*, where the ions which also appeared in the field ionization spectrum are indicated by an asterick. The m/e at 118 was produced from the parent m/e 148 by P-30 (P-^{13}COH) and the 103 is generated by a P-45 (P-^{13}CH$_2$^{13}COH) loss. The m/e 59 was probably composed of H^{13}CO^{13}CO-, which when removed from 148, left an 89 fragment. The difference in m/e 89 and m/e 45 is m/e 44 which should have the composition of the keto-enol forms of -^{13}COH=^{13}CH-. The composite of this data gave an H^{13}CO^{13}CH$_2$^{13}C(OH)=^{13}CH^{13}CO^{13}COH; however, several different keto-enol forms are feasible and were probably all present. The enol form is suggested by Sager (99) to be the preferred structure for a similar 6-carbon structure.

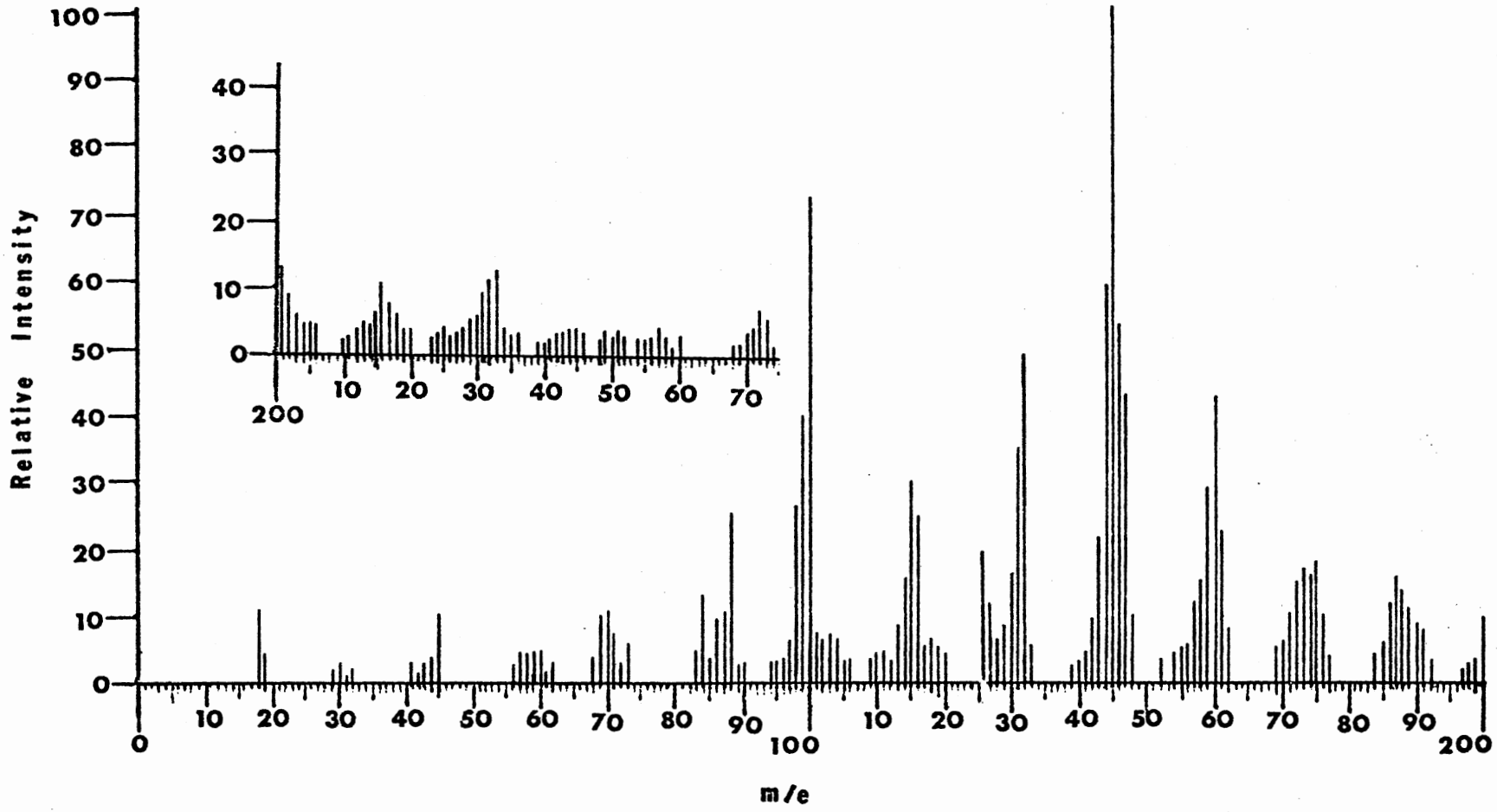


Figure 25. Mass Spectrum of Fraction D (Low Voltage)

The m/e at 60 in fraction A is partially produced from the $H^{13}CO^{13}OH$, identified from the exact mass data, because it is present in both the low and high voltage mass spectra. Many of the other fractions also show an intense m/e 60, but only in the 70 EV spectra. These other m/e 60's must have been produced by rearrangement of larger ions.

The structure of mass 120 ($^{13}C_4H_4O_4$) had already been determined from exact mass and FI data. The fragments at m/e 90($^{13}C_3H_3O_3$) and 30(^{13}CHO) were consistent with the unsaturated 2-3 dihydroxy structure. The fragments at 59 and 61 could easily have been generated from the same structure but in the ketone from $H^{13}CO^{13}CO^{13}CH(OH)^{13}COH$.

The structures of the other compounds were much more difficult to determine than the first three because of overlapping masses. The low voltage scan yielded 7 masses (189, 159, 147, 146, 131, 116, and 101) which matched the field ionization masses. Removal of water from the molecular ion 189 gave the 171 fragment ion shown.

The structure of the m/e 147 was of interst because of the moderate field ionization intensity and occurrence in fractions A and B. Studies by earlier investigators have predicted a cyclic structure of $\underbrace{^{13}CO^{13}CO^{13}C(OH)=}{}^{13}C(OH)^{13}C$ which would have a 147 molecular weight. All fragments of this compound were consistent with the mass spectra. Further evidence of this structure was its similarity of characteristic to the previously determined 120 and 148.

M/e 131 also was an important field ionization peak. Analysis of the ^{13}C,O,N, and H possibilities from the library and the knowledge that the retention time was for a hydroxyl structure allowed postulation of the cyclic structure $\underbrace{^{13}CO^{13}CO^{13}C(OH)=}{}^{13}CH^{13}CO$. Its fragments of 44, 73, 87,

and 102 are all present in Figure 19. The aldo structure is also possible but is less consistent with the fragment pattern.

The two other odd molecular ions were 101 and 159. Neither ion amounts to a very large percentage of the total field ionization, and both are also likely fragments from the other ions. This low concentration made their identification inconclusive.

Two unexpected structures were obtained for the ions at m/e 146 and m/e 116. Each had two possible cyclic structures, one of which contained a five membered ring with an amine and the other was a six membered ring of C, O, and H only. The final selection was based on the LC retention times, nature of the other ions in this fraction, and preference to the resonance stabilized six membered ring of m/e 116. The ion at mass 116 was dihydroxybenzene (probably para), and the 146 mass was $^{13}CO^{13}C(OH)=^{13}CH^{13}CO^{13}C(OH)=^{13}CH$. Both structures appeared to be reduced relatives of hexahydroxybenzene and rhodozonic acid which earlier investigators suggested to be present.

The mos- intense intermediate mass fragment ion was m/e 73, which the exact mass data predicted to be $^{13}C_4H_5O$ and which had a structure similar to $-^{13}CH_2{}^{13}CH=^{13}CH^{13}COH$. The second most important fragment was m/e 89 with a molecular formula of either $^{13}C_3H_4NO_2$ or $^{13}C_4H_5O_2$. Mass 89 was also in the field ionization spectrum. The other fragment ion of interest was m/e 57, which has a formula of $^{13}C_3H_2O$.

The number of possible structures of m/e 189 was too great to obtain any meaningful information about. Unfortunately, this fact was true for most of the molecules above this mass also.

Theoretically, there should have been very little connection between the molecular weights, but an m/e 30 difference occurred in most

of the fractions. This difference in most cases was caused by the loss of two oxygens and the gain of a carbon and four hydrogens.

Fraction B. Fraction B produced the most complicated mass spectrum of all the fractions. The first important peaks in the high and low voltage mass spectra were the m/e 77 and 79, which were not prominent in any of the other fractions. These two masses corresponded to the two field ionization peaks which have previously been identified as amino-glycol and hydroxyacetamide.

The low voltage scan again yielded 146 and 147 mass ions, which were the same two compounds discussed in Fraction A. Their presence in both fractions was accounted for by the overlapping of LC peaks during fraction collection.

The most prominent low voltage ion was at m/e 132 and is a molecular ion. Considering C, H, O, and N as the only possible combination that could produce molecular ions, and requiring the presence of at least two oxygens in the molecule, the number of possible formulas was reduced to one, $^{13}C_6H_6O_3$. The most viable structure is trihydroxybenzene (probably in 1, 3, and 5 positions.)

The low voltage spectra also produced ions at four masses (117, 121, 124 and 172) but their contribution to the field ionization spectrum was very low.

Fraction B also contained an m/e 189 which was unidentifiable in fraction A, and remained so here. The occurrence of molecular ions at 232 was also noted, but insufficient information was obtainable for assigning a structure.

Fraction C. The field ionization spectrum and the mass spectrum of

fraction C concurred on seven molecular ions (272, 188, 175, 160, 159, 132, and 115). Three of the ion masses (188, 159, and 132) were detected and discussed in Fraction B, but actually occurred in both fractions because of nonbase separation of the early eluted peaks.

The ion formed at mass 115 was determined to be in Fraction C and in Fraction D. Only one likely formula appeared to exist in light of the percentage of oxygen in the lower weight fragments. The structure of $O^{13}C^{13}CH=^{13}CH^{13}CO^{13}CO$ was easy to predict from the molecular weights of 131 and 147.

Analysis of the data obtained from the ion at mass 160 allowed for two possible molecular formulas. The first formula yielded the cyclic structure $^{13}CO^{13}CO^{13}CH=^{13}C(OH)^{13}CO^{13}CO$ while the second showed a molecular formula of $C_7H_7NO_3$; and because the presence of amides was established earlier, it followed that the structure would be $H^{13}CO^{13}CH=^{13}CH^{13}CO^{13}CH=^{13}CH^{13}CONH_2$. Very little evidence could be presented to support either formula over the other. In fact, the first structure was closer in nature to the surrounding ions, but the second structure would contain nitrogen, which was consistent with some of the higher exact mass data of the fragments.

The nature of the mass ion at 175 was difficult to determine. Any of four molecular formulas ($^{13}C_9H_{10}O_3$, $^{13}C_8H_9NO_3$, $^{13}C_7H_4O_5$ and $^{13}C_6H_3NO_5$) could produce molecular ions which would fit the fragment data. The $^{13}C_9H_{10}O_3$ and $^{13}C_8H_9NO_3$ formulas had a higher H/O ratio (a more reduced structure) than any of the previously produced ions, and, therefore, were less probable than the other two. The six carbon ions showed the cyclic structure $^{13}CO^{13}CO^{13}(OH)=^{13}C(NH_2)^{13}CO^{13}CO$, while the seven carbon structures were a straight chain pentacarbonyl, unsaturated at the second or

third bond.

Fraction D. A larger total ion current was observed for this fraction, which concured with the liquid chromatography detector response. Fraction D contained sufficient samples to obtain a clean low and high voltage mass spectrum, shown in Figures 22 and 25, respectively. Six molecular ions (233, 187, 175, 160, 132, and 115) were of interest to this study. The four lowest have already been described in Fraction C and the two highest ions were unidentifiable.

Fraction F. It is obvious from the liquid chromatogram, Figure 10, that there is a larger amount of sample in the first four fractions (A, B, C, D) than in any of the remaining fractions (assuming approximately equal absorption). This caused only Fraction F and J to contain enough samples for reliable mass spectrometric analysis.

Several molecular ions, 84, 115, and 143, were formed which should have allowed some characterization of the structures of this fraction. The standard compounds with retention time in this area were the neutral esters and the nitrogen compounds. However, the variety of possible structural formulas of a given molecular formula is high for the nitrogen or ester compound. Combining this with the large number of molecular, made assignment of any formula unfeasible.

Fraction J. The first noticeable characteristic of fraction J is the presence of a much larger 46 than the other samples. This is expected because it has the retention time of an acid, which suggest a $-^{13}COOH$ m/e fragment 46 peak. A second important peak is at m/e 62, which is also not present in the other fractions either. This m/e 62 is also in the field ionization, which corresponded to either acetic

acid or -hydroxyethanal. When considering the LC retention time corresponded to that of an acid, acetic acid is a much more likely choice.

One feature of the masses investigated in this section by observing the possible formulas is that they in general, either contain a higher percentage of oxygen per molecule than the other fractions or they are almost saturated. An example of this is the mass at ion 125 where the two most likely formulas are $^{13}C_5H_6O_5$ and $^{12}C_5H_{12}O_3$. The five carbon molecule would have a totally saturated trihydroxypentane structure while the three carbon formula would be a trihydroxyproponic acid. Again the LC retention time would predict the acid structure over the other possibility.

The same argument can be made for selecting the acid structure of $H_2{}^{13}C(OH)^{13}C(OH)={}^{13}C(OH)^{13}COOH$ (or double bonded at the third bond) instead of a $^{13}C_6H_{12}O_3$ formula for the ion at m/e 138. The ion at m/e 152 was much more difficult to determine. Two possible formulas of $^{13}C_4H_4O_6$ and $^{13}C_6H_{10}O_4$ were both able to form acids structures but the second structure would be more saturated. The four carbon formula would have a HOOCC(OH)=C(OH)COOH structure similar in character to other ion formed.

Important Considerations

The identification of polar organic compounds (especially those containing a high percentage of oxygen) was a very time consuming undertaking. Efforts were constantly made to avoid factors such as air oxidation or dehydration. Acid or base hydrolysis of the sample could also occur while in the buffer mobile phase media.

Mass spectral identification was also difficult because of possible

dehydration, dehydrogenation, and thermal decomposition rearrangements. Most organic compounds will form reliable molecular ions and fragment patterns at mass of 200 to 400 but these oxygenated compounds behaved just the opposite.

Carbon-13 FT-CMR could have been a useful tool in the identification of the individual fractions but even the largest fraction would have required 48 hours to even possibly obtain identifiable peaks and the instrument time was not available.

Further complications occurred while analyzing the isotopically enriched compounds. The isotope abundance spread the molecular ions over three or four masses instead of producing almost a single parent on, thus decreasing sensitivity. Nitrogen is generally detected in ^{12}C compounds by the presence of an odd mass molecular ion. However, even this was not true with ^{13}C present.

Finally, the inavailability of literature data and spectral information produce a completely new problem while working with these carbon-13 compounds.

AMC Reaction Efficiency

Continuous extraction with acetone removed a majority of the organic matter. The yellow-red extract comprised about 4% of the gray stabilized material from the reaction. About 58.5g of the solid original AMC product was available, plus probably 10 gm in water. Thus, 2.74 gm of organics were produced by the reaction 8.87 gm of C=O. If H, N, and Cl from NH_4Cl are not present in the products, then the reaction was approximately 31% effective in the use of C=O.

CHAPTER VI

SUMMARY

Analytical Techniques and Methods

The Separation Scheme

A major emphasis of this study was to develop and improve various methods and techniques so that they can be used to separate mixtures of polar organic compounds. This was accomplished by using high performance liquid chromatography with a strong anion exchange column. The resolution could be enhanced by first optimizing the pH, followed by ionic strength gradient.

This HPLC technique was effectively used to first separate a mixture of standard and then applied to a synthesis mixture containing polar carbon-13 compounds, and was able to separate the mixture into 25 peaks. The column performance was evaluated by measurement of the resolution and other separation parameters. The average HETP for the separated standards was excellent, especially considering ion exchange chromatography is generally lower than the other LC techniques.

The Identification Procedure

The retention time of the standards was used as a starting criteria for identifying the carbon-13 mixture. To this data was added mass spectrometry, IR, NMR and CMR information, using the same standard and

some more specific techniques.

The nature and complexity of the samples then required the implementation of high resolution and field ionization mass to allow identification of the mixture. Most of the data was taken in duplicate in an attempt to avoid erroneous information.

To these analyses were added the mass spectra data from the liquid chromatography. By combining all the information, the identity of compounds was determined at varying degrees of assurance. Some of these compounds agreed well with that proposed by earlier investigators, however, several new compounds were found. A summary of the compounds identified is presented in Table VI.

No single method, technique, or instrument is capable of separating or analyzing all samples. However, a combination of these when used in the correct manor will produce valuable information about the sample. This investigation has presented a scheme which was effective for separation and identification of polar organic compounds which hopefully will be of use in the future.

Future Areas of Study

Although this investigation has presented an effective method for separation of polar organic compounds, further research and development of techniques is still an important problem for researchers interested in the aquatic environment. The purification procedures presented could be useful in isolating synthetic samples as well as samples produced by nature. More complex mixtures are going to require development of equally sophisticated separation schemes if identification and quantification are to be performed.

TABLE VI

CARBON-13 COMPOUNDS IDENTIFIED

Mass	Structure or Formula	Mass	Structure or Formula
31	H_2CO	120	$C_4H_4O_4$
33	H_3COH	89	$C_3H_2NO_2$ &
45	CO_2		$C_4H_5O_2$
47	HCOOH	90	$C_3H_3O_3$
		115	OCCH=COCO
		116	dihydroxybenzene
59	$HCOCH=CH_2$	131	COCOC(OH)=CHCO
60	HCOCOH	132	trihydroxybenzene
62	CH_3COOH or	146	COC(OH)=CHCOC(OH)=CH
	$HCOCH_2OH$	147	COCOC(OH)=C(OH)CO
73	C_4H_5O	160	COCOCH=C(OH)COCO &
77	$H_2C(OH)CO(NH_2)$ or		$HCOCH=CHCOCH=CHCO(NH_2)$
	$H_2C(NH_2)COOH$	175	COCOC(OH)=C(NH_2)COCO &
79	$HC(NH_2)(OH)CH_2(OH)$		HCOCH=CHCOCOCOCOH
94	$C_3H_9NO_2$		
118	$HCOCH_2C(OH)=CHCOCOH$		

The instruments and methods used for identifying the organic matter are, and should continue to be, a constant area of advancement. Even during the short span of this investigation, identification techniques and instrumentation became available which were scarcely being used at its beginning. Areas for improvement still exist and will require constant updating.

A final area that will require further consideration is the production of isotopically (stable) labeled organic compounds for tracer studies. Their importance in biomedical investigation may prove as extensive as their laboratory or environmental use.

BIBLIOGRAPHY

(1) Schreiber, R. E., U.S. Atomic Energy Commission, LA 4759-MS, 1971.

(2) Matwiyoff, N. A. and D. G. Ott, Science, 181, 1125 (1973).

(3) Klein, Peter D., Chairman, VIII Annual AVA-ANL Biology Symposium, Argonne National Laboratories, Argonne, Illinois, 1973.

(4) Leyden, D. E. and R. H. Cox, "Analytical Applications of NMR," John Wiley and Sons, New York, New York, 1976.

(5) Schoeller, D. A. and J. M. Hayes, Anal. Chem., 47, 408 (1975).

(6) Stothers, J. B., "Carbon-13 NMR Spectroscopy," Academic Press, New York, New York, 1972.

(7) Allen, H. E. and J. R. Kramer, "Nutrients in Natural Waters," John Wiley and Sons, New York, New York, 1972.

(8) Faust, Samuel J. and Joseph Hunter, "Organic Compounds in Aquatic Environments," Marcel Dekker, Inc., New York, New York, 1971.

(9) Eglinton, G., et al., "Environmental Chemistry," Burlington House, New York, New York, 1975.

(10) Ciaccio, L. L., ed., "Water and Water Pollution Handbood," Vol. VI Marcel Dekker, Inc., New York, New York, 197 .

(11) Junk, G. A., et al., J. of Chromat., 99, 745 (1974).

(12) Biemann, Klaus, "Mass Spectrometry, Organic Chemical Applications," McGraw-Hill Book Co., Inc., New York, New York, 1962.

(13) Hugentobler, E. and J. Loliger, J. Chem. Ed., 49, 610 (1972).

(14) Millard, Brian J., "Quantitative Mass Spectrometry," Heyden Press, London, England, 1978.

(15) Frigerio, Alberto and Neal Castagnoli, "Advances in Mass Spectrometry in Biochemistry and Medicine," Vol. I, Spectrum Publications, Inc., New York, New York, 1976.

(16) McLafferty, F. S., "Mass Spectrometry of Organic Ions," Academic Press, New York, New York, 1963.

(17) McDowell, Charles A., "Mass Spectrometry," Mc-Graw Hill Book Co., Inc., New York, New York, 1963.

(18) Beckey, H. D., "Field Ionization Mass Spectrometry," Pergamon Press, Oxford, England, 1971.

(19) Scheppele, S. E., Anal. Chem., 48, 2105 (1976).

(20) Wiberg, Kenneth B. and B. J. Nist, "The Interpretation of NMR Spectra," W. A. Benjamin, Inc., New York, New York, 1962.

(21) Axenrod, T. and G. D. Webb, "Nuclear Magnetic Resonance of Nuclei Other Than Protons," John Wiley and Sons, New York, New York, 1973.

(22) Levy, George C., "Topics in Carbon-13 NMR Spectroscopy," John Wiley and Sons, New York, New York, 1977.

(23) London, Robert E., V. H. Kollman, and N. A. Matwiyoff, J. Amer. Chem. Soc., 97, 3565 (1975).

(24) Mareci, Thomas H. and Katherine N. Scott, Anal. Chem., 49, 2130 (1977).

(25) Shoolery, James N., Varian Instrument Applications (VIA), 10, 10 (1976).

(26) Scaublin, S., A. Hohener and R. R. Ernest, J. Magn. Reson., 13, 196 (1974).

(27) Ammon, Herman L., Paul H. Mazzochi, and Elen J. Colicelli, J. Org. Magn. Reson. 11, 1 (1978).

(28) Giddings, J. Calvin and R. A. Keller, "Advances in Chromatography," Marcel Dekker, New York, New York, 1965.

(29) Hadden, Nina, et al., "Basic Liquid Chromatography," Varian Aerograph, Walnut Creek, California, 1971.

(30) Williams, R. C., et al., Am. Lab., 5, 45 (1973).

(31) Kirkland, J. J., "Modern Practices of Liquid Chromatography," Wiley-Interscience, New York, New York, 1971.

(32) Scott, Charles D., et al., J. Chromat. Sci., 11, 96 (1973).

(33) Rajcsanyi, Peter M. and Elisabid Rajcsonyi, "High Speed Liquid Chromatography," Marcel Dekker, Inc., New York, New York, 1975.

(34) Snyder, L. R., J. Chromat. Sci., 7, 352 (1969).

(35) Kirkland, J. J., J. Chromat. Sci., 9, 206 (1971).

(36) Schill, G., Acta Pharm. Suecica, 2, 119 (1965).

(37) Wittmer, D. P., N. O. Nuessle and W. G. Haney, Anal. Chem., 47, 1422 (1975).

(38) Rieman, William III and Harold F. Walton, "Ion Exchange in Analytical Chemistry," Pergamon Press, New York, New York, 1970.

(39) Scott, Charles D., Dennis D. Chilcote, and Norman E. Lee, Anal. Chem., 44, 85 (1972).

(40) Ertingshausen, G. and H. J. Alder, J. Chrom., 44, 620 (1969).

(41) Kirkland, J. J., J. Chromat. Sci., 8, 72 (1970).

(42) Jones, J. K. N. and R. A. Wall, Can. J. Chem., 38, 2290 (1960).

(43) Marinsky, Jacob A. and Marcus Yizhak, "Ion Exchange I and II." Marcel Dekker, Inc., New York, New York, 1966.

(44) Schmit, J. A. and R. A. Henry, Chromatographia, 3, 497 (1970).

(45) Katz, Sidney, W. W. Pitt, and Charles D. Scott, Water Research, 6, 1029 (1972).

(46) Khym, Joseph X., "Analytical Ion Exchange Procedures in Chemistry and Biology," Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 1974.

(47) Armstrong, Dale E., et al., U.S. Atomic Energy Commission, LASL 4391, 1970.

(48) Hammond, Allen, L., Science, 176, 1315 (1972).

(49) Waller, G. R., S. D. Sasty, and K. Kinneberg, J. Chromat. Sci., 7, 577 (1969).

(50) Gunter, B. D. and J. D. Gleason, J. Chromat. Sci., 9, 191 (1971).

(51) Davidson, Charles N., Charles K. Mann, and Raymond K. Sheline, J. Phys. Chem., 67, 1519 (1963).

(52) Murry, Arthur III and D. L. Williams, "Organic Isotopes (I and II)," Interscience Publ., Inc., New York, New York, 1958.

(53) Neir, A. O. C., D. W. Wilson and S. P. Reimann, "Preparation and Measurement of Isotopic Tracers," Edwards Brothers, Ann Arbor, Michigan, 1946.

(54) Calvin, M., et al., "Isotopic Carbon, Techniques in its Measurement and Chemical Manipulation," John Wiley and Sons, New York, New York, 1949.

(55) Famenhaft, D., et al., Biochem. Biophys., 215 421 (1970).

(56) Ladenburg, A., "Hanworterbuch der Chemie," Verlag Von Eduard Trewendt, Breslav, Germany, 1888.

(57) Wohler, L. and A. Berzelius, POGG. Ann., 4, 31 (1825).

(58) Heller, G., J. f. pr. Chem., 12, 193 (1927).

(59) Heller, G., Ann. d. Chem. U. Pharm., 24, 1 (1927).

(60) Liebig, J., Ann. d. Chem. U. Pharm., 11, 182 (1834).

(61) Liebig, J., POGG. Ann., 33, 90 (1834)

(62) Brodie, B. C., Ann. d. Chem. U. Pharm., 113, 358 (1860).

(63) Nietski, R. and T. Benckisser, Ber., 18, 1834 (1885).

(64) Joannis, A., Compt. Rend., 116, 1518 (1803).

(65) Joannis, A., Compt. Rend., 158, 874 (1914).

(66) Guntz, M. M. and B. Mentrel, Bull. Soc. Chim. France, 29, 585 (1903).

(67) Pearson, T. G., Nature, 131, 166 (1933).

(68) Roederer, G., Bull. Soc. Chim. France, 35, 715 (1906).

(69) Sager, W. F., et al., J. Inorg. Nucl. Chem., 25, 187 (1963).

(70) Scott, Arthur F., Science, 115, 118 (1952).

(71) Karger, Barry L., Lloyd R. Snyder, and Csaba Horvath, "Introduction to Separation Science," John Wiley and Sons, New York, New York, 1973.

(72) Dean, John A., "Chemical Separation Methods," D. Van Nostrand Co., New York, New York, 1969.

(73) Miller, James M., "Separation Methods in Chemical Analysis," John Wiley and Sons, New York, New York, 1975.

(74) Berry, Laverne and Barry Karger, Anal. Chem., 45, 819A (1973).

(75) Conlon, Ralph D., Anal. Chem., 41, 107 (1969).

(76) Kirkland, J. J., Anal. Chem., 40, 391 (1968).

(77) Katz, Sidney, W. W. Pitt, and G. J. James, Jr., Clin. Chem., 19, 817 (1973).

(78) Underfriend, Sidney, "Fluorescence Analysis in Biology and Medicine II," Academic Press, New York, New York, 1969.

(79) Katz, S. and W. W. Pitt, Jr., Anal. Let., 5, 177 (1972).

(80) Bengtsson, L. and O. Samuelson, J. Chromatog., 60, 101 (1971).

(81) Kirkbright, G. F., T. S. West, and C. Woodward, Anal. Chim. Acta., 36, 298 (1966).

(82) Beynon, J. H., R. A. Saunders and A. E. Williams, "The Mass Spectra of Organic Molecules," Elsevier Pub. Co., New York, New York, 1968.

(83) Budzikiewiz, Herbert, Carl Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1963.

(84) Rozett, R. W., Anal. Chem., 46, 2085 (1974).

(85) Sano, M. Y. Yotsui, H. Abe, and S. Sasaki, Mass. Spect., 3, 1 (1976).

(86) Ogata, Koreichi and Teruo Hyakawa, "Recent Developments in Mass Spectrometry," Univ. of Tokyo Press, Tokyo, Japan, 1970.

(87) Pickup, John F. and Klim McPherson, Anal. Chem., 48, 1885 (1976).

(88) Kirkland, J. J., J. Chromat. Sci., 7, (1969).

(89) Snyder, L. R., Anal. Chem., 46, 1384 (1974).

(90) Scott, Charles D., Sci., 185, 186 (1974).

(91) Pitt, W. W., Jr., Robert L. Jolly, and Charles D. Scott, Environ. Sci., & Tech., 12, 1068 (1975).

(92) Hercules, David M., "Fluorescence and Phosphorescence Analysis," Interscience Pub., New York, New York, 1966.

(93) Godbille, E. and P. Devaux, J. of Chromat. Sci., 12, 564 (1974).

(94) Stothers, J. B. and P. C. Lauterbur, Can. J. Chem., 42, 1563 (1964).

(95) Gray, G. A., et al., J. Mag. Res., 1, 41 (1969).

(96) Johnson, Leroy F. and William C. Jankowski, "Carbon-13 NMR Spectra," Wiley Inter. Pub., New York, New York, 1972.

(97) Silverstein, R. M. and G. C. Bassler, "Spectrometric Identification of Organic Compounds," John Wiley and Sons, Inc., New York, New York, 1967.

(98) McDowell, Robin S., Anal. Chem., 43, 1192 (1971).

APPENDIX A

PROGRAM FOR THE GENERATION OF A CARBON-13 EXACT MASS LIBRARY AND SAMPLE COMPARISON

```
C
C
C     PROGRAM FOR CALCULATION THE EXACT MASS OF COMPOUNDS
C     CONTAINING CARBON-13, NITROGEN-14, OXYGEN-16 AND
C     HYDROGEN-1
C
C
C     THE PROGRAM CONSIST OF FOUR BRACKETED DO LOOPS FOR
C     GENERATING ALL POSSIBLE COMBINATIONS OF C, O, N, AND H
C     WITHIN LIMITATIONS AND PRINTING THE ONES WITH THE SAME
C     MASS TOGETHER
C
C     JERRY CAPLINGER
C
C
C     TMASS = THE TOTAL MASS OF INTEREST
C     EMH = EXACT MASS HYDROGEN
C     EMC = EXACT MASS CARBON
C     EMN = EXACT MASS NITROGEN
C     EMO = EXACT MASS OXYGEN
C
      TMASS=13.0
C
C
C     SUM = SUM OF INDIVIDUAL MASSES OF ELEMENTS PRESENT
C     II = NUMBER OF OXYGEN PRESENT
C     JJ = NUMBER OF NITROGEN PRESENT
C     KK = NUMBER OF CARBON PRESENT
C     LL = NUMBER OF HYDROGEN PRESENT
C
      DIMENSION IMASS(307)
C
      EMH=1.0078246
      EMC=13.0033554
      EMN=14.0030738
      EMO=15.9949141
      EMCL=34.9688
C
      WRITE (6,997)
      IN=1
      ICOUNT=1
      READ (5,1001) (IMASS(IM),IM=1,307)
   50 SUM=0.0
      TMAX=TMASS+0.5
      TMIN=TMASS-0.5
      IN=ICOUNT
      DO 10 IM=IN,307
      REMASS=IMASS(IM)
      EMASS=REMASS/10000.
      IF(EMASS.LT.TMIN) GO TO 15
      IF(EMASS.GT.TMAX) GO TO 950
      WRITE (6,1000) TMASS,EMASS
      MM=0
      DO 500 M=1,10
      II=0
      DO 100 I=1,20
      JJ=0
      DO 200 J=1,20
```

```
      KK=0
      DO 300 K=1,20
      LL=0
      DO 400 L=1,42
      LL=L
      KX=KK*2
      KY=KX+2
      JX=JJ*1
      LX=KX+JX+2
      KT=II+JJ+LL
      IF(LL.GT.LX)GO TO 550
      SUM=SUM+EMH
      IF(SUM.GT.TMAX)GO TO 550
      IF(SUM.LT.TMIN.OR.SUM.GT.TMAX)GO TO 400
      IF(II.GT.KY.AND.II.GT.2)GO TO 400
      IF(JJ.GT.KY.AND.JJ.GT.2)GO TO 400
      IF(KK.GT.KT.AND.KK.GT.1)GO TO 400
      IF(SUM.LT.(EMASS-0.0050).OR.SUM.GT.(EMASS+0.0050)) GO TO 400
      WRITE (6,999) LL,KK,JJ,II,MM,SUM
  400 CONTINUE
  550 SUM=0.0
      LL=0
      KK=K
      KX=KK*2
      KY=KX+2
      JX=JJ*1
      LX=KX+JX+2
      KT=II+JJ+LL
      SUM=SUM+EMN*JJ+EMO*II+EMCL*MM
      SUM=SUM+EMC*K
      IF(SUM.GT.TMAX)GO TO 650
      IF(SUM.LT.TMIN.OR.SUM.GT.TMAX)GO TO 300
      IF(II.GT.KY.AND.II.GT.2)GO TO 300
      IF(JJ.GT.KY.AND.JJ.GT.2)GO TO 300
      IF(KK.GT.KT.AND.KK.GT.1)GO TO 300
      IF(SUM.LT.(EMASS-0.0050).OR.SUM.GT.(EMASS+0.0050)) GO TO 300
      WRITE (6,999) LL,KK,JJ,II,MM,SUM
  300 CONTINUE
  650 SUM=0.0
      LL=0
      KK=0
      JJ=J
      KX=KK*2
      KY=KX+2
      JX=JJ*1
      LX=KX+JX+2
      KT=II+JJ+LL
      SUM=SUM+EMO*II+EMCL*MM
      SUM=SUM+EMN*J
      IF(SUM.GT.TMAX)GO TO 750
      IF(SUM.LT.TMIN.OR.SUM.GT.TMAX)GO TO 200
      IF(JJ.GT.KY.AND.JJ.GT.2)GO TO 200
      IF(II.GT.KY.AND.II.GT.2)GO TO 200
      IF(KK.GT.KT.AND.KK.GT.1)GO TO 200
      IF(SUM.LT.(EMASS-0.0050).OR.SUM.GT.(EMASS+0.0050)) GO TO 200
      WRITE (6,999) LL,KK,JJ,II,MM,SUM
  200 CONTINUE
  750 SUM=0.0
```

```
      LL=0
      JJ=0
      KK=0
      II=I
      KX=KK*2
      KY=KX+2
      JX=JJ*1
      LX=KX+JX+2
      KT=II+JJ+LL
      SUM=SUM+EMCL*MM
      SUM=SUM+EMO*I
      IF(SUM.GT.TMAX)GO TO 850
      IF(SUM.LT.TMIN.OR.SUM.GT.TMAX)GO TO 100
      IF(II.GT.KY.AND.II.GT.2)GO TO 100
      IF(JJ.GT.KY.AND.JJ.GT.2)GO TO 100
      IF(KK.GT.KT.AND.KK.GT.1)GO TO 100
      IF(SUM.LT.(EMASS-0.0050).OR.SUM.GT.(EMASS+0.0050)) GO TO 100
      WRITE (6,999) LL,KK,JJ,II,MM,SUM
  100 CONTINUE
  850 SUM=0.0
      II=0
      JJ=0
      KK=0
      LL=0
      MM=M
      KX=KK*2
      KY=KX+2
      JX=JJ*1
      LX=KX+JX+2
      KT=II+JJ+LL
      SUM=SUM+EMCL*M
      IF(SUM.GT.TMAX)GO TO 15
      IF(SUM.LT.TMIN.OR.SUM.GT.TMAX)GO TO 500
      IF(II.GT.KY.AND.II.GT.2)GO TO 500
      IF(JJ.GT.KY.AND.JJ.GT.2)GO TO 500
      IF(KK.GT.KT.AND.KK.GT.1)GO TO 500
      IF(SUM.LT.(EMASS-0.0050).OR.SUM.GT.(EMASS+0.0050)) GO TO 500
      WRITE (6,999) LL,KK,JJ,II,MM,SUM
  500 CONTINUE
   15 ICOUNT=ICOUNT+1
   10 CONTINUE
  950 TMASS=TMASS+1.0
      IF(TMASS.GT.40.0)STOP
      GO TO 50
  997 FORMAT (1H0,15X,'H',13X,'C',13X,'N',13X,'O',13X,'CL',13X,'MASS')
  999 FORMAT (1H ,15X,I3,10X,I3,10X,I3,10X,I3,10X,I3,10X,F11.7)
 1000 FORMAT (1H0,F4.0,76X,F8.4)
 1001 FORMAT (6(I7,5X))
      END
```

APPENDIX B

PROGRAM TO CONVERT PHOTOGRAPHIC PLATE DATA TO EXACT MASSES

```
C
C     EXACT MASS CALCULATIONS ON ACETONE EXTRACT
C     TAKEN FROM SHEET NUMBER 4
C
C     PROGRAM PROVIDED COMPLIMENTS OF THE MASS SPECTROMETRY
C     GROUP AT OKLA. STATE UNIV. AND MODIFIED BY THIS INVESTIGATOR
C
C     THIS PROGRAM WILL CALCULATE EXACT MASSES FROM PHOTOGRAPHIC PLATE
C     DATA AND WILL PUNCH THOSE EXACT MASSES ONTO CARDS. THOSE CARDS CAN
C     THEN BE USED WITH THE MASSPEC PROGRAM TO PREDICT EMPIRICAL FORMULA
C
      IMPLICIT REAL*8 (A-H,O-Z)
      DIMENSION JPCHD(6)
      SQRT(X)=DSQRT(X)
      NPCHD=0
      HALF=0.5
      RMULT=10000.
      LPUN=7
C
C     IF THE NEXT CARD IS IFLAG=1 CARDS WILL BE PUNCHED, IF IFLAG=0
C     CARDS WILL NOT BE PUNCHED
C
      IFLAG=0
      WRITE(6,5)
    5 FORMAT(1H1,'PHOTOPLATE READER')
      WRITE(6,6)
    6 FORMAT(1H0,10X,'NOMINAL MASS',5X,'EXACT MASS',5X,'EXACT MASS H1',
     120X,'POSSIBLE FORMULAS',16X,'D1',7X,'D2',7X,'D3',/)
C
C     IF MORE THAN ONE DATA SET IS TO BE PUNCHED, A 1 SHOULD BE PLACED
C     IN COLUMN 2 OF THE FINAL CARD OF EACH DATA SET.
C     TO TERMINATE THE PROGRAM A 1 SHOULD BE PLACED IN COLUMN 1 OF THE
C     LAST CARD OF THE LAST DATA SET
C     AM1 AND AM2 ARE THE EXACT LOW AND HIGH MASS PFK PEAKS RESPECTIVILY
C     D1=LOW MASS,D2=HIGH MASS, D3=UNK. MASS MICROMETER READING
C
   10 READ(5,1)N,L,AM1,AM2,D1,D2,D3
    1 FORMAT(2I1,8X,2F10.6,4X,F8.4,3X,F8.4,3X,F8.4)
      IF(L)9,9,8
    9 CONTINUE
      IF(N)3,3,2
    3 SM1=SQRT(AM1)
      SM2=SQRT(AM2)
      R=D1-D2
      RX=D1-D3
      AMX=((RX/R)*(SM2-SM1)+SM1)**2
C
C     IF NEXT PEAK IN H1 + AMX, AMXH WILL PREDICT THIS EXACT MASS
C
      AMXH=AMX+1.00782
      WRITE(6,4)AMX,AMX,AMXH,D1,D2,D3
    4 FORMAT(14X,F4.0,10X,F8.4,9X,F8.4,53X,F8.4,1X,F8.4,1X,F8.4)
      IF(IFLAG)10,10,90
   90 NPCHD=NPCHD+1
      JPCHD(NPCHD)=AMX*RMULT+HALF
      IF(NPCHD-6) 10,100,100
  100 WRITE(LPUN,1000) JPCHD
      NPCHD=0
```

```
      GO TO 10
    8 IF(NPCHD)998,998,111
  111 WRITE(LPUN,1000) (JPCHD(I),I=1,NPCHD)
      NPCHD=0
  998 CONTINUE
      GO TO 10
    2 IF(NPCHD) 999,999,110
  110 WRITE(LPUN,1000) (JPCHD(I),I=1,NPCHD)
  999 WRITE(6,7)
    7 FORMAT(1H0)
 1000 FORMAT( 6(I7,5X) )
      STOP
      END
```

	H	C	N	O	MASS
13.					
	0	1	0	0	13.0033550
14.					
	1	1	0	0	14.0111799
	0	0	1	0	14.0030737
15.					
	2	1	0	0	15.0190048
	1	0	1	0	15.0108986
16.					
	3	1	0	0	16.0268250
	2	0	1	0	16.0187225
	0	0	0	1	15.9949141
17.					
	4	1	0	0	17.0346375
	3	0	1	0	17.0265350
	1	0	0	1	17.0027313
18.					
	2	0	0	1	18.0105438
19.					
20.					

VITA

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Candidate for the Degree of

Doctor of Philosophy

Thesis: ION EXCHANGE HPLC SEPARATION OF POLAR ORGANICS AND THE MASS SPECTROMETRIC AND NUCLEAR MAGNETIC RESONANCE IDENTIFICATION OF A ^{13}C MIXTURE

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