Description of the Oklahoma State University Combination Mass Spectrometer-Gas Chromatograph

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INTRODUCTION

Recent advances in mass spectrometry instrumentation have been so striking that when the new types of instruments are used in biochemical research, they can either singly or in combination with the gas chromatography column provide an exceptionally powerful tool for extending our knowledge. Various sample handling systems are needed; in cases where the samples are easily decomposed (e.g., steroids or peptides) it is necessary to insert the sample directly into the ion source so as to develop the maximum vapor pressure in the vicinity of the ionizing beam of electrons. A modern mass spectrometry laboratory for biochemical research should be capable of obtaining the mass spectra of compounds of low volatility and high molecular weight. A low-resolution combination mass spectrometer-gas chromatograph should be capable of detecting 0.1 to 10 nanograms of compound in a gas chromatography column effluent, of providing a useful mass spectrum (m/e = 12 - 600) on a few nanograms of sample within 2 to 10 seconds, of making accurate isotope ratio measurements on compounds with m/e ratios up to 300, and of obtaining useful spectra on submicrogram quantities of sample when a direct probe is used. It is essential that the spectrometer have a certain minimum performance in order to obtain the desired mass spectrum. In the large molecules frequently encountered in biochemical research a resolving power of 600 to 1200 is necessary in order to separate peaks of adjacent mass numbers using a single-focusing instrument. Finally, it is of major importance that the instrument be reliable and operable by a biochemist without special training in electronics.

While in Stockholm, Sweden, in 1963-64, the author worked out an arrangement with Dr. Ragnar Ryhage, Director of the Mass Spectrometry Laboratory at the Karolinska Institutet, Stockholm, Sweden, to build such an instrument for research use at Oklahoma State University. The National Science Foundation, Washington, D. C., graciously supplied the funds for purchase of this instrument.[1] The mass spectrometer-gas chromatograph[2] was installed in the biochemistry laboratories in January, 1966. Several additions have been made since that time to the original equipment. Additional accessories remaining to be installed are a mass marker and double collectors. The latter are for use in determining amounts of stable isotopes present in compounds with m/e ratios up to 300.

A partial description of an earlier version of this instrument has been given by Ryhage (1967).

GENERAL DESCRIPTION AND FUNCTION

The Oklahoma State University mass spectrometer-gas chromatograph instrument is a single-focusing mass spectrometer featuring sample introduction by three different inlet systems. It is especially designed for operation with the gas chromatography inlet, but the array of high capacity vacuum pumps and valves enables the operator to change quickly from one inlet system to another. The three systems available are: a) the

[1]Supported by Research Grant No. GB-3482 from the National Science Foundation, Washington, D. C.
[2]The instrument is commercially available as the LKB-9000 Gas Chromatograph-Mass Spectrometer from LKB Instruments, Inc., 12221 Parklawn Drive, Rockville, Maryland, 20852.

gas chromatography system, b) a heated glass inlet system and c) a direct probe. A block diagram showing the salient features of the mass spectrometer-gas chromatograph is shown in Figure 1.

The instrument consists of two main units; the analyzer unit, which is shown in Figure 2 (top view), Figure 3 (drawing of top view), and Figure 4 (front view), and a control unit, shown in Figure 5. The analyzer unit houses the gas chromatograph, the other inlet systems and spectrometer parts while the control unit houses most of the electronic subunits and recorders. The main features in each of these two units are pointed out in the number titles associated with Figures 2 through 5. Detailed electronic specifications will not be given except in a few instances; however, some technical specifications are summarized at the end of this article.

Ion Source—Ionization is achieved by electron impact. A schematic drawing of the ion source is shown in Figure 6. The filament consists of a piece of rhenium ribbon, heated to an electron-emitting temperature. The filament is entirely encased in an insulated shield except for a small central opening through which the electrons are emitted. This shield serves to direct the electron beam through the ionization chamber towards the trap which collects the electrons. Gaseous sample from either the dual inlet valve (No. 6) or from the direct probe enters the ionization chamber where it intersects the electron beam. The ions formed from the molecules of the sample are drawn out of the chamber by the electric field between chamber and extraction plates, and are accelerated by a still higher potential difference towards the focusing plates. After the ions have passed these plates the beam is collimated into its final shape by adjustment of the appropriate voltages on the deflection plates. This adjustment is made so that maximal ion transmission to the first dynode of the electron multiplier is obtained. An example of normal operating voltages would be as follows: Ionization chamber, 3500 volts; trap, 3650 volts; extraction plates, 3490 volts; focusing plates, 2500 volts.

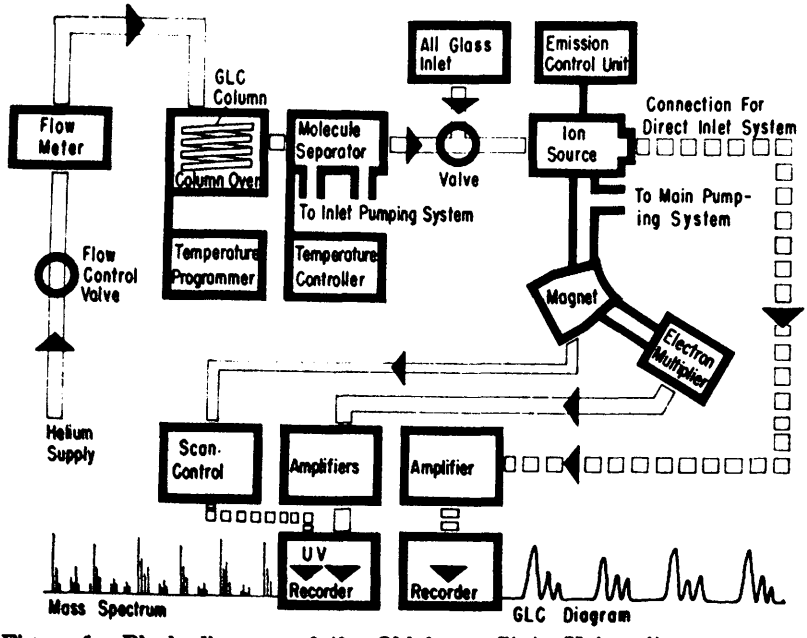

Figure 1. Block diagram of the Oklahoma State University mass spectrometer-gas chromatograph.

The ions then pass through a plate (electrode) pierced centrally by a rectangular hole. About 90% of the positively charged ions from the ion source reach the analyzer tube (No. 11) and the remainder, 10%, are collected by this electrode (attached to No. 9). Prior to reaching the analyzer tube, the ions are passed through a fixed focus and an adjustable focus control (No. 8). The ions that are collected on the total ion current electrode produce a current that is amplified (No. 9) and then recorded in the normal fashion with a strip chart recorder (No. 19). The record obtained is a graphic representation of the ion source output and is referred to as the total ionization current.

Analyzer — After traversing the 60° magnetic sector (No. 11), in which groups of ions are deflected according to their m/e ratios, a group of ions of equal m/e ratio is focused (Nos. 7 and 8) to impinge on a collector plate of the electron multiplier (No. 14) (i.e., the first dynode). The signal that is produced is amplified and its intensity recorded on the high-speed recording oscillograph (No. 22). By sweeping a range of magnet field strengths, it is possible to scan a wide range of masses and therefore obtain a recording of the m/e ratios of all fragment ions of a particular sample, and the abundance of each type.

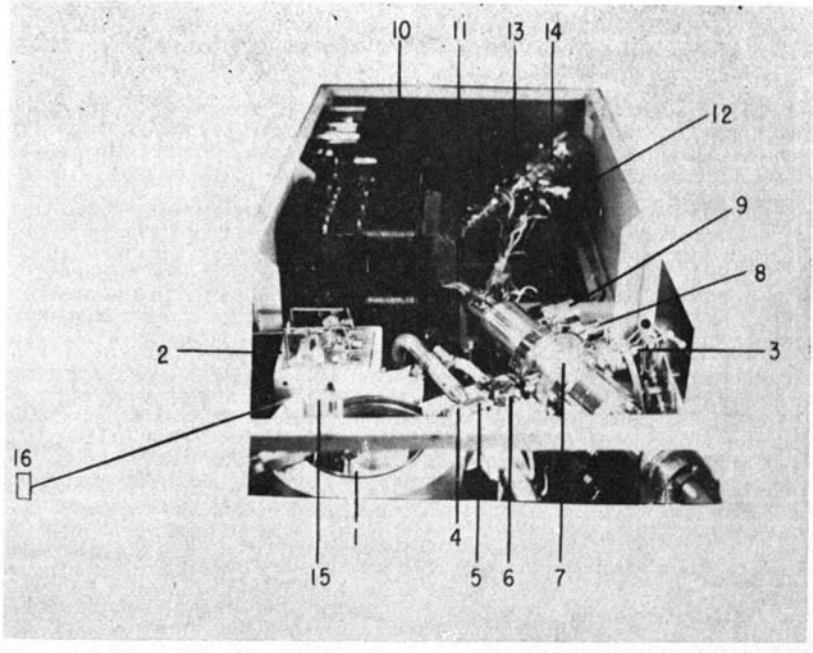

Figure 2. Top view of analyzer unit. 1. Gas chromatography oven with a ¼ in. × 10 ft. glass column. 2. Heated glass inlet. 3. Direct probe. 4. Separator No. 1. 5. Separator No. 2. 6. Diaphragm valve. 7. Ion source. 8. Externally adjustable focusing slits in source. 9. Pre-amplifier for total ionization current monitor. 10. Magnet. 11. Analyzer tube. 12. Externally adjustable collector focusing slits. 13. Externally adjustable control for varying the angle of the collector slits. 14. Electron multiplier. 15. Injection port. 16. Helium gas source.

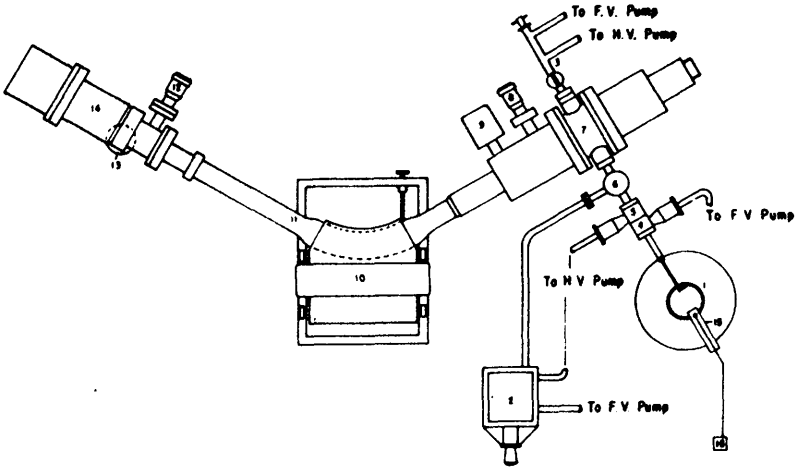

Figure 8. Drawing of analyzer unit showing vacuum connections. Numbers are the same as in Figure 1.

The exit slit (No. 8), collector slit (No. 12) and collector slit angle modifier (No. 13) are adjustable by micrometer controls outside the vacuum system. The width of the slits determine, in part, the degree of resolution and intensity.

Of considerable importance in design of this instrument was the type of vacuum pump used for the analyzer tube. In this instrument an oil diffusion pump with a capacity rate of 550 liters/second is mounted directly under the source. A picture of this pump is shown in Figure 7. It is important to have a high pumping capacity directly to the analyzer system so that one compound can be pumped out before another compound enters when the gas chromatography inlet system is being used.

Magnet — The function of the magnet (No. 10) is to effect a separation of the ions according to their masses. By continuous variation of the magnetic field the entire spectrum of ions is allowed to enter the collector slit in order. The magnet is capable of producing fields up to 10 kG during manual operation and 15 kG during automatic scanning. A selected portion of the mass range may be scanned. Different scan rates may be selected.

The instrument utilizes a truncated-sector type coiled electromagnet designed for a spectrometer tube with a radius of 20 cm.

Ion Collection and Recording — The function of the electron multiplier (No. 15), preamplifier (not shown), galvanometer, amplifier (No. 23) and recording oscillograph (No. 22) is to amplify and record the electrical signal produced when ions of a selected m/e impinge upon the collector (i.e., first dynode of the electron multiplier). Successive dynodes increase the number of secondary electrons. The gain of the multiplier may be varied by adjusting the voltage from 1700 to 3700 V (No. 25). This produces variations in gain from 10^3 to 10^7 times.

Each peak (group of ions of a particular m/e ratio) is recorded simultaneously by three galvanometers of different gains and the reading is made from the tracings which are produced on light-sensitive photographic paper.

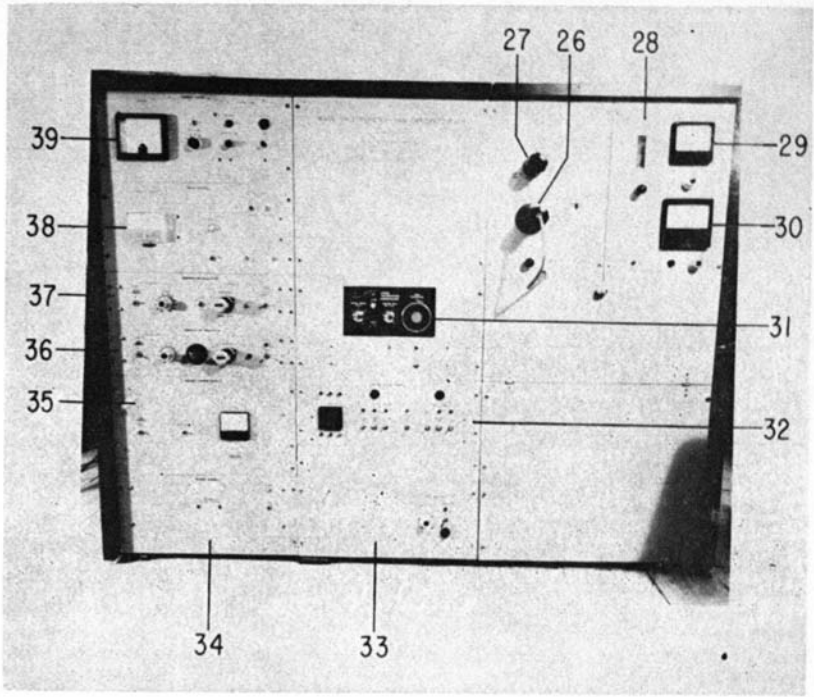

Figure 4. Front view of analyzer unit. 26. Sample holder for heated glass inlet system. 27. Vacuum control knob for heated glass inlet system. 28. Flow meter for carrier gas. 29. Temperature gauge (11 positions). 30. Pirani gauge for GLC and heated glass inlet systems. 31. Linear temperature programmer for gas chromatography oven. 32. Main power supply unit. 33. Controls for direct probe. 34. Electron multiplier focusing unit. 35. Magnet current supply unit. 36. Temperature regulator for heated glass inlet system and molecule separators. 37. Temperature regulator for GLC injection port. 38. Pirani gauges. 39. Penning gauge and automatic safety controls.

Vacuum Systems — To attain and maintain the high vacuum necessary for the operation of the mass spectrometer, two complete high vacuum systems are used. The larger system is used on the analyzer and consists of a mechanical forepump, a booster (mercury diffusion pump and an oil diffusion pump (Fig. 7) in series. The smaller system is used on the various inlet systems and consists of a mechanical forepump (see left foreground in Figure 7), a booster (mercury diffusion) pump and an oil diffusion pump in series.

Pirani-type vacuum gauges (Nos. 30 and 38) are used in the inlet systems and a Penning gauge (No. 39) is used in the analyzer.

The analyzer tube and inlet vacuum systems are equipped with magnetic valves in their forepump-mercury pump line. These serve to protect the diffusion pumps in case of power, fore vacuum or valve failure.

Safety Devices — Sensitive parts of the mass spectrometer (i.e., filament) are protected from possible damage due to: a) vacuum failure, b) water failure and c) power failure.

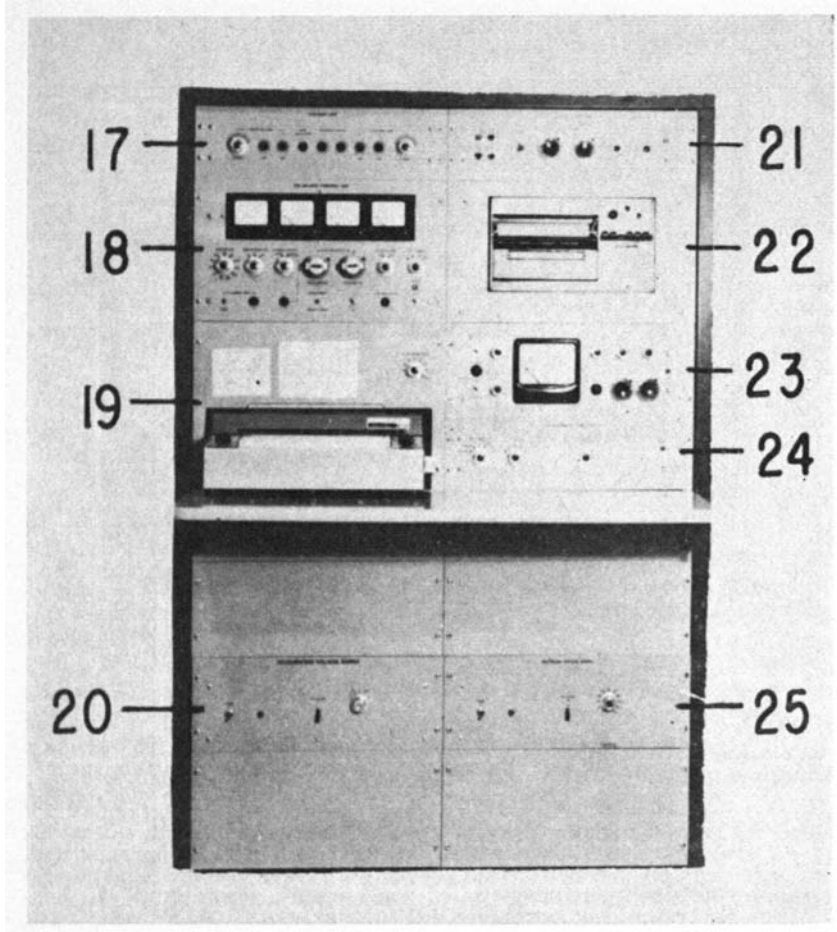

Figure 5. Front view of control unit. 17. Ion beam focusing controls. 18. Ion source controls. 19. Pen and ink recorder for monitoring total ionization current. 20. Acceleration voltage supply. 21. Gas chromatography peak splitting unit. 22. Oscillographic recorder. 23. Magnet power control unit. 24. Galvanometer amplifier. 25. Electron multiplier voltage supply.

Gas Chromatography Inlet System — Samples separated by gas- liquid chromatography (GLC) require subsequent analysis in order to determine the structure of their constituents. In this instrument, the mass spectrometer is used as a specific detector and the GLC effluent is fed directly through a series of helium separators into the ion chamber of the mass spectrometer. The system used is similar to that described originally by Ryhage (1964) and Ryhage, Wikstrom and Waller (1965). This type of combination instrument has been in use in the Mass Spectrometry Laboratory of the Karolinska Institutet for several years.

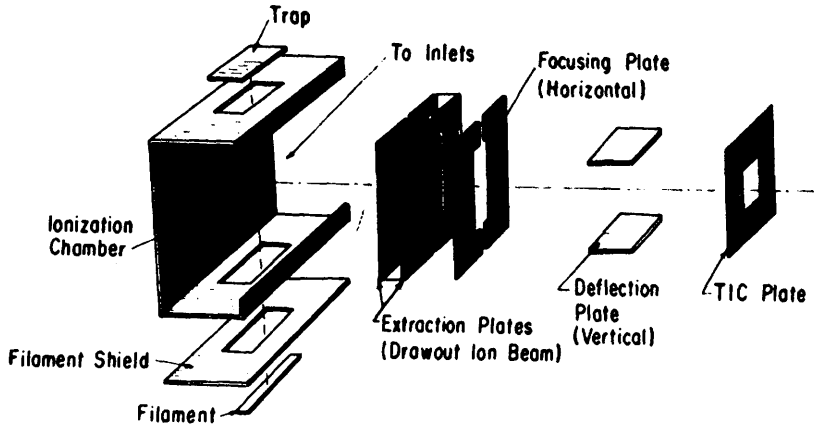

Figure 6. Schematic of ion source.

One of the main problems in coupling a gas chromatograph to a mass spectrometer is in obtaining an efficient method of removal of the carrier gas, or, stated conversely, of enriching the sample. This should be done so that the carrier is removed effectively without removal of the compounds of interest also. A unique system was developed by Ryhage using the molecule separator principle first employed by Becker in separating uranium isotopes. A schematic drawing of the molecule separators[2] used in this instrument is shown in Figure 8. This type of molecular separator (Nos. 4 and 5) uses a pressure drop in two jet openings. Both jets must be so dimensioned that the ratio of the mean free path to the diameter of the orifice is small. In our instrument the first separator (No. 4) is evacuated by a forepump with a pumping speed of 160 liters/minute which gives a pressure of 0.1 to 0.2 mm Hg for a flow rate of about 40 ml/min measured in the pumping line. The variation in the pressure of the first vacuum chamber of the separator (labeled F.V. pump in Figure 3) depends upon the conditions of operation (i.e., the type of column used). The second separator (No. 5) is connected to an oil-diffusion pump (labeled H.V. pump in Figure 3) with a pumping speed 150 liters/second (for air pressure). The pressure in this separator is measured about 20 cm from the jet and is usually less than 10^{-3} mm Hg. The orifice diameters of the jets currently being used are 0.1 mm and correspond to the diameters d_1, d_2, d_3 and d_4 in Figure 8. The distances between the jets (L_1 and L_2) can be varied but they are usually about 1 mm. Since the size of each of the jets and the distances between the jets may be varied, a large number of combinations can be obtained. From evidence obtained by Ryhage (1964), the diameter of the first jet is the most important one to control. This is necessary so that the correct pressure drop across the first separator system can be obtained. This pressure drop plays an important role in determining the optimum flow rate of carrier gas in the chromatography column and of the forepumping speed needed in the first separator. Experimental results obtained by using different diameters of the orifices in the jets and distances between jets have been reported by Ryhage (1967). A pictorial view of separators 1 and 2 is shown in Figure 2 as Nos. 4 and 5.

[2]The principles of the molecule separator are protected by patent in the USA, West Germany, France, Great Britain, Italy, Canada, Japan, and Sweden.

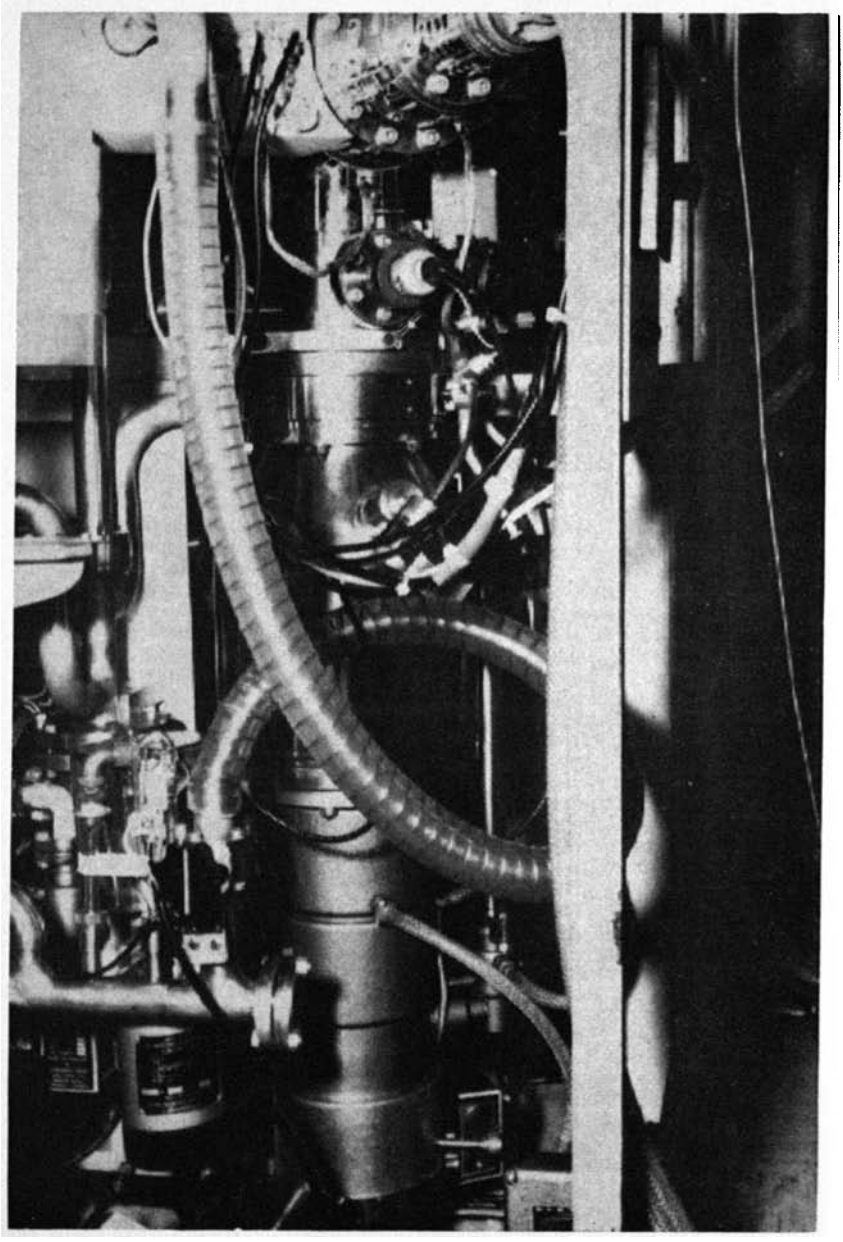

Figure 7. View of oil diffusion pump.

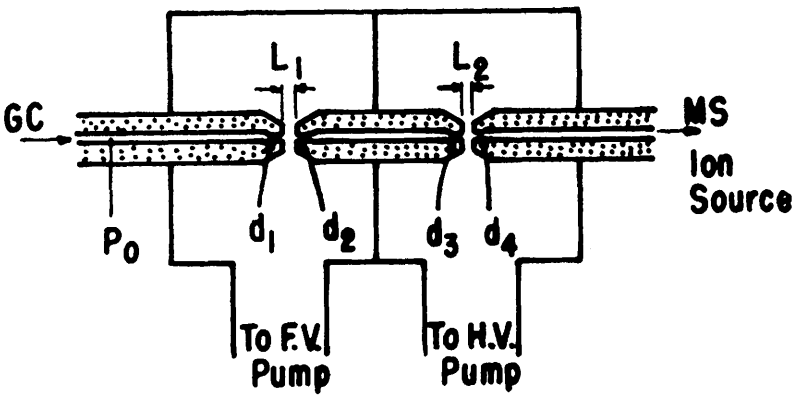

Figure 8. Schematic of molecule separators.

To obtain maximum sensitivity, it is important to obtain a high separating factor since under these conditions one gets as much sample as possible into the ionization chamber with a correspondingly low helium flow rate. The separating factor may be calculated as follows:

$$\frac{\%\ \text{sample/helium (ml) before entering the separator}}{\%\ \text{sample/helium (ml) after emerging from the separator}}$$

Ryhage (1967) has tested samples with molecular weights as high as 300 and found that from 50 to 75% of the sample in the gas chromatograph effluent also passes the separators.

Helium is used as the carrier gas. The helium passes through a flow meter (No. 29) and a flow control to the injection port. The injection port (No. 15) with its surrounding heater is mounted on top of the oven on a track so that its position can be adjusted to accommodate chromatographic columns of varying dimensons. The column is housed in a stainless steel cylinder 6 inches in diameter and 12 inches in depth. A linear temperature programmer (No. 31) operates the oven by proportional control. Rates of temperature change for the oven are from 1 to 15 C per minute. The temperature range is from ambient to 350 C. The oven injection port and separators have independent temperature controls (Nos. 36 and 37). Thermocouples are placed in all units and temperature is read on the same meter (No. 30).

Heated Glass Inlet — Liquid or solid samples of pure material are inserted in a glass tube of 4 mm × 12 mm which is pushed into the sample holder (No. 26). By turning knob (No. 27) the sample is evacuated, stepwise, until the line is finally opened directly to the source (No. 8). The sample is heated slowly and spectra are taken at appropriate intervals. This inlet has a 1-liter glass reservoir which can serve as storage for a mass marker such as perfluorokerosene (needed for m/e ratios above 1000).

Direct Probe Inlet—Recently we have installed the direct probe (No. 3) on our instrument. This enables one to obtain a mass spectrum of submicrogram quantities of a pure compound of very low volatility. The probe is constructed so that the sample is placed next to the ionization chamber and is heated at whatever rate is desirable. As the sample is volatilized into the chamber, it is ionized and the ions accelerated, collected and measured as previously described.

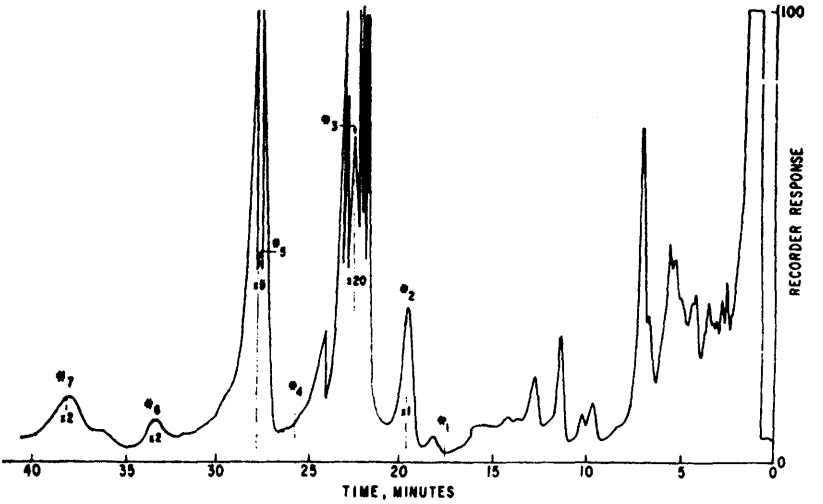

Figure 9. Gas liquid chromatograph tracing of the hydrocarbons obtained from *Nepeta cataria* L. steam distillate. A capillary column was used. The dimensions were 100 ft. × 0.02 in. inside and the coating used was Apiezon L. The injection port, column, separators and ion source temperatures were 140, 80, 225 and 290 respectively. The helium flow was 8 cc/min. Slash (/) marks indicate the places where mass spectra were taken. Three μl of an ether solution was injected.

Control Unit—The accelerating voltage power supply (No. 20) provides positive high voltage in three adjustable steps of 2.5, 3.0, and 3.5 kV to the units related to the operation of the ion source. The electron multiplier voltage power supply (No. 25) provides negative high voltage to the electron multiplier collector in 10 adjustable steps, ranging from 1.7 to 3.7 kV. The electron multiplier focusing unit (No. 34) is housed in the analyzer unit. The ion source control unit (No. 18) contains the controls for the operation of the ion source. The ion-focusing unit (No. 17) houses the controls that focus the ion beam (i.e. extraction, focusing and deflection plates, shown in Figure 6). Control No. 23 is an assembly for automatic scanning and manual focus of the mass spectrometer and No. 24 is the galvanometer amplifier. The signal from unit No. 24 is then sent to the recording oscillograph. The scan speed can be controlled by a 9-step selector switch and the photographic chart paper speed is controlled by 6 pushbuttons. Unit No. 21 is the gas chromatography "Peak Splitter" described by Sweeley, et al. (1966) which permits the separation of unresolved gas chromatography peaks by rapidly varying the accelerating voltage between two peaks that have an m/e ratio within 10% of each other.

Results and Discussion

To test the effectiveness of the oil diffusion pump on the analyzer, a test run using the hydrocarbon fraction[1] from *Nepeta cataria* L. oil was performed. In Figure 9 a gas-liquid chromatograph tracing is shown. Spectra were taken at places numbered on the tracing. In Figure 10, the

[1]Prepared by preparative thin-layer chromatography using the natural oil of catnip from Nepeta cataria L. plants grown in the greenhouse.

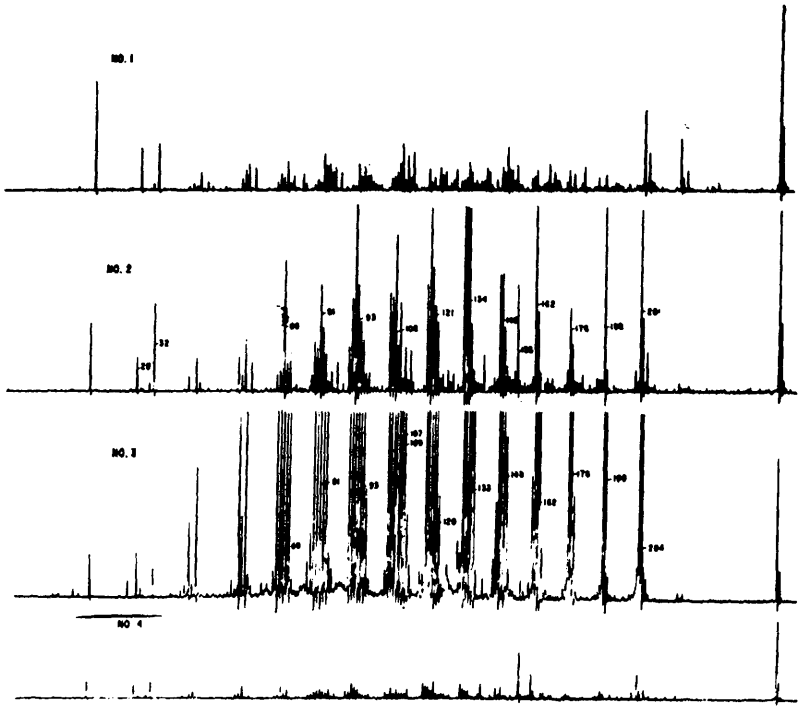

Figure 10. Drawings of original mass spectrum numbers 1-4 as shown in Figure 9. Only the highest galvanometer sensitivity (× 100) is shown.

original spectra (as marked in Figure 9) are reproduced. It can be seen that spectrum No. 1 represents primarily background, spectrum No. 2 represents a hydrocarbon with mass 204 (this is an unknown hydrocarbon), spectrum No. 3, which is rather intense, represents another hydrocarbon with mass 204 (identified as caryophyllene), and spectrum No. 4 indicates that the background is again low. This fast removal of a compound from the analyzer is very important so that background contribution to the following spectrum (No. 5 — not shown) is minimized. Another measure of the efficiency of removal of the carrier gas in the mass spectrometer is shown in Figure 11. In obtaining the results shown in Figure 11 a splitter with a flow ratio of 160 to 1 was used instead of the molecular separators.

It is desirable to perform preliminary gas chromatographic analyses using a conventional instrument (i.e., equipped with a thermal or hydrogen flame ionization detector)[5]. This prevents using the mass spectrometer merely as a gas chromatograph detector. Sometimes it may be desirable

[5]We have modified two Barber-Colman Model 5000 gas chromatographs equipped with hydrogen flame detectors and a Perkin Elmer F-11 gas chromatograph equipped with a thermal ionization detector so that the columns used in these instruments and the mass spectrometer-gas chromatograph are interchangeable. Suitable conditions for separating compounds of interest are worked out on these instruments prior to using the mass spectrometer-gas chromatograph.

[6]Also flow rates higher than 100 ml/min are to be avoided with separators of 0.1 mm diameter since the pressure increase in the source is higher than desirable.

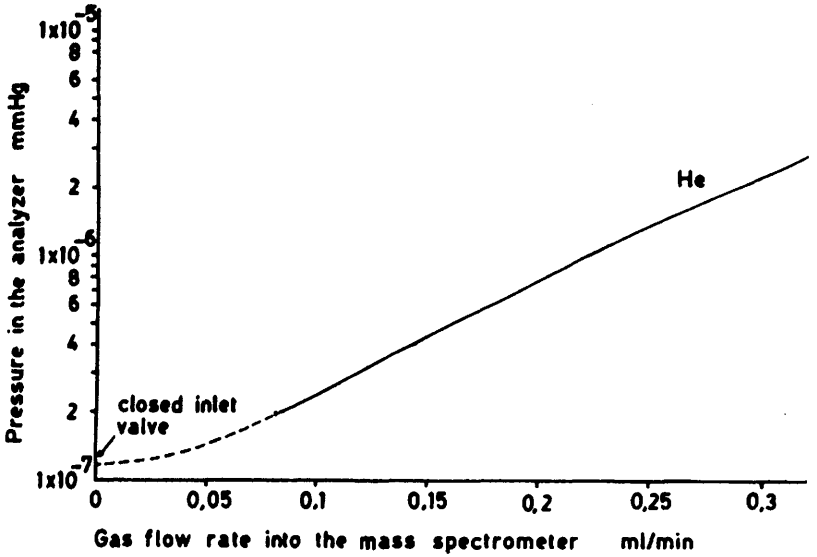

Figure 11. Pressure in the analyzer as a function of the gas flow into the mass spectrometer for helium (from Ryhage, 1967).

to use low flow rates; however, such rates are to be avoided if possible since when the column is connected to the molecular separators in the combination mass spectrometer-gas chromatograph, reduced pressure can occur at the column outlet. In Figure 12 a diagram is shown that compares the difference in the helium gas flow rate when a packed column is used at the normal air pressure outlet and when it is connected to the molecular separators. The difference in gas flow depends on the pressure change from vacuum to air pressure. It may be seen that from a helium container pressure of 0 the flow rate is about 12 ml/min. Figure 12 also shows that to have atmospheric pressure at the column outlet, a container pressure of about 43 psi is needed. In Figure 13 a plot of the helium inlet pressure is shown when the column is connected to the separators. For normal operation, which is at about 30 ml/min, the outlet pressure is about 7 psi or about 380 mm Hg; however, the helium pressure must be about 43 psi to obtain atmospheric pressure at the end of the column when connected to the separator.

Since the pressure in the analyzer system varies with flow rate, it was desirable to know just how the variation in pressure affected the resolution of the instrument. In Figure 14, the molecule ion mass range of methyl palmitate ($M^+ = 270$) taken at different pressures in the analyzer is shown. The resolution decreases from about 900 to 600 for an increase in the helium pressure in the analyzer from 5×10^{-7} to 7×10^{-6} mm Hg.

The results in Figure 15 show variations in the total ion current peak areas and the peak heights of mass spectra for different pressures in the analyzer for methyl palmitate ($M^+ = 270$). The maximum peak heights were obtained for a pressure of about 1×10^{-6} mm of Hg. This might be considered the optimum operating condition for use of the gas-liquid chromatography inlet system.

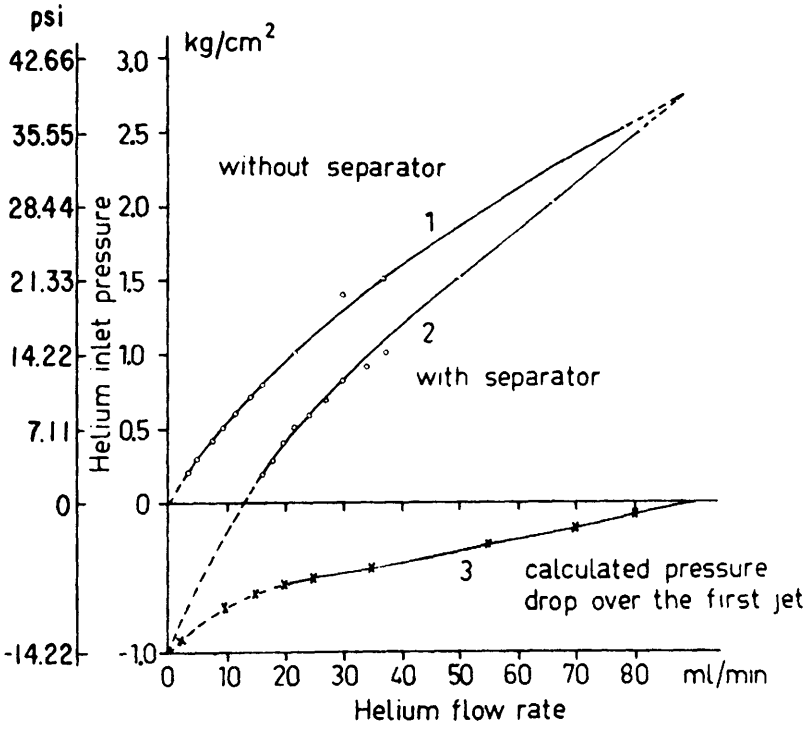

Figure 12. Helium inlet pressure as a function of the helium flow rate at the column outlet (from Ryhage, 1967). The GLC column used was 3 mm × 2 m and packed with 2% SE-30 on silanized Gas Chrom P, 30-100 mesh. The separator dimensions in mm are: $d_1 = 0.1$, $d_2 = 0.4$, $d_3 = 0.25$, $d_4 = 0.4$, $l_1 = 0.1$, and $l_2 = 0.2$ (Fig. 8). Column temperature 200 C.

In using the combination instrument, we always find that a normal gas chromatograph tracing is not directly reproduced by the total ion current tracing obtained with the combination instrument. Figure 16 shows the results of such comparative tracings of the same oil; shorter retention times occur when the combination instrument is used. This may occur because there could be a slight pressure reduction at the chromatography column exit, but we consistently observe this phenomenon even when inlet pressures are increased (see Fig. 12). It can be seen from the graphs in Figure 16 that chromatographic resolution by the combination instrument is slightly poorer, but still it is quite adequate for the identification of unknown compounds.

It may also be worth pointing out that spectra may be taken repeatedly every few seconds so one can get quite a number of spectra during the period of time it takes for most compounds to emerge from the GLC column. In this manner, a mixture of compounds in one peak can be detected and sometimes identified.

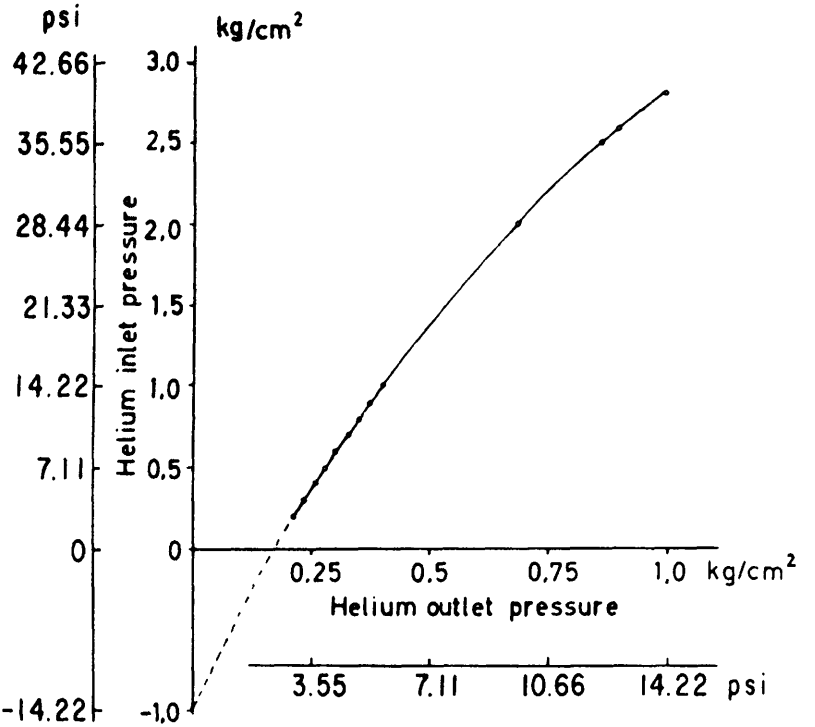

Figure 13. Helium pressure at the outlet as a function of the helium inlet pressure of the GLC column when connected to the separator (from Ryhage, 1967). The same GLC column was used as in Figure 12.

In Figures 16 and 9 the use of glass-packed and metal capillary columns has been demonstrated. In Figure 17 results of using a coiled glass capillary column are shown. It can be seen that very good separation of the isomeric 7-carbon hydrocarbons is obtained. The glass capillary column is slightly more delicate to handle than the metal capillary column but does offer considerable promise for obtaining high resolution and short retention times with short columns.

The usefulness of the gas chromatography inlet of the combination instrument can be extended by using the technique of Sweeley, et al. (1966) for the determination of unresolved compounds in gas chromatographic effluents. When no separation of individual components occurs during gas-liquid chromatography it is possible to separate these by monitoring two different peaks as the effluent emerges from the chromatography column. The technique employs an accelerating voltage alternator (No. 21, Figure 4) such that a continuous recording of two values of m/e separated by not more than 10% of the mass range can be obtained. Results from performing such an experiment on our instrument are shown in Figure 18. Deuterated cyclohexanone was mixed with nonlabeled cyclohexanone and put through the gas chromatography column. No separation was noted. The peak emerged over a 2-min time period (the retention time was about 32 min). By setting the high voltage switching unit so

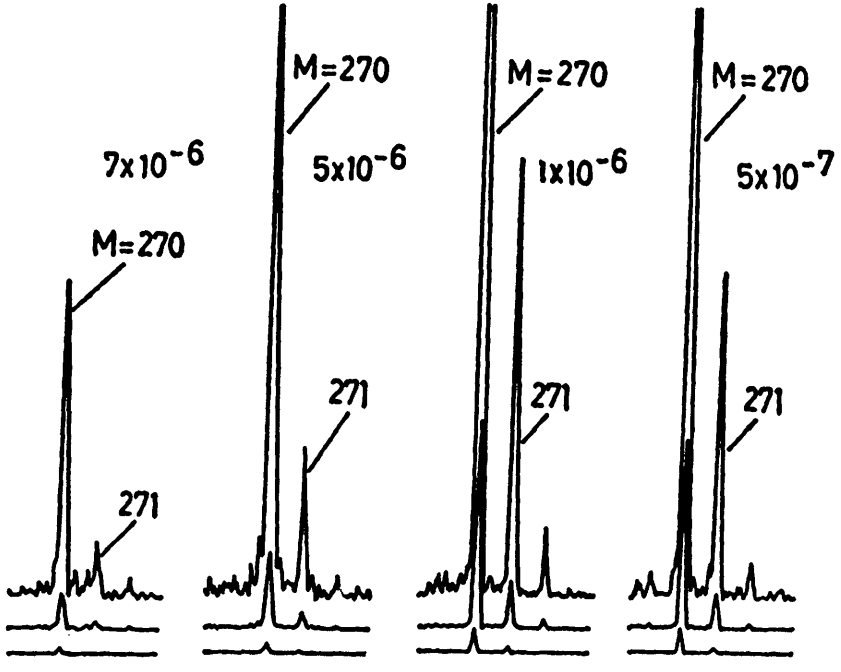

Figure 14. Molecule ion mass range of the mass spectra of methyl palmitate at different helium pressures in the analyzer (from Ryhage, 1967). The same GLC column was used as in Figure 12. 0.1μg was injected on the column.

that ions with m/e = 98 and m/e = 102 (corresponding to cyclohexanone and cyclohexanone-d_4, respectively) were monitored it was possible to distinguish between these two compounds as they emerged from the column as a single peak. It can be seen that the ratio of deuterated cyclohexanone changes continuously across the gas-liquid chromatography peak as would be expected when slight separation (i.e. 20 seconds) between the compounds has occurred. By measuring the relative areas underneath the peaks, the percent of labeled compound can be estimated.

Figure 19 shows the result of a total ionization current tracing of 0.2 μg of ricinine using the direct probe. Figure 20 shows the mass spectrum of 0.2 μg of ricinine. The mass spectrum is clearly discernible on this small amount of material and it is in full agreement with the published spectra (Waller, et al., 1966).

From the brief description given and the data presented it can be ascertained that the Oklahoma State University combination mass spectrometer-gas chromatograph meets the specifications outlined in the introductory paragraph of this article. During the first year of operation we have had about 25% down time. This is expected to improve in subsequent years.

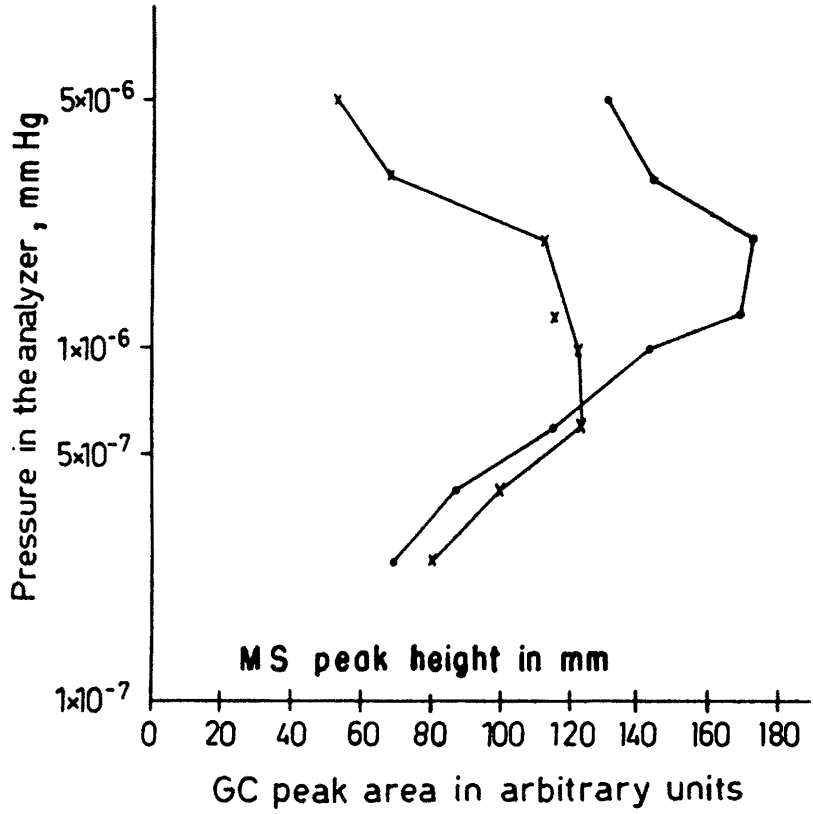

Figure 15. Pressure in the analyzer as a function of GLC peak areas and the mass spectrum peak height (from Ryhage, 1967).

x——x Peak height in mm of the molecule ions of methyl palmitate ($M^+ = 270$).

.——. Peak area in arbitrary units of the total ion current.

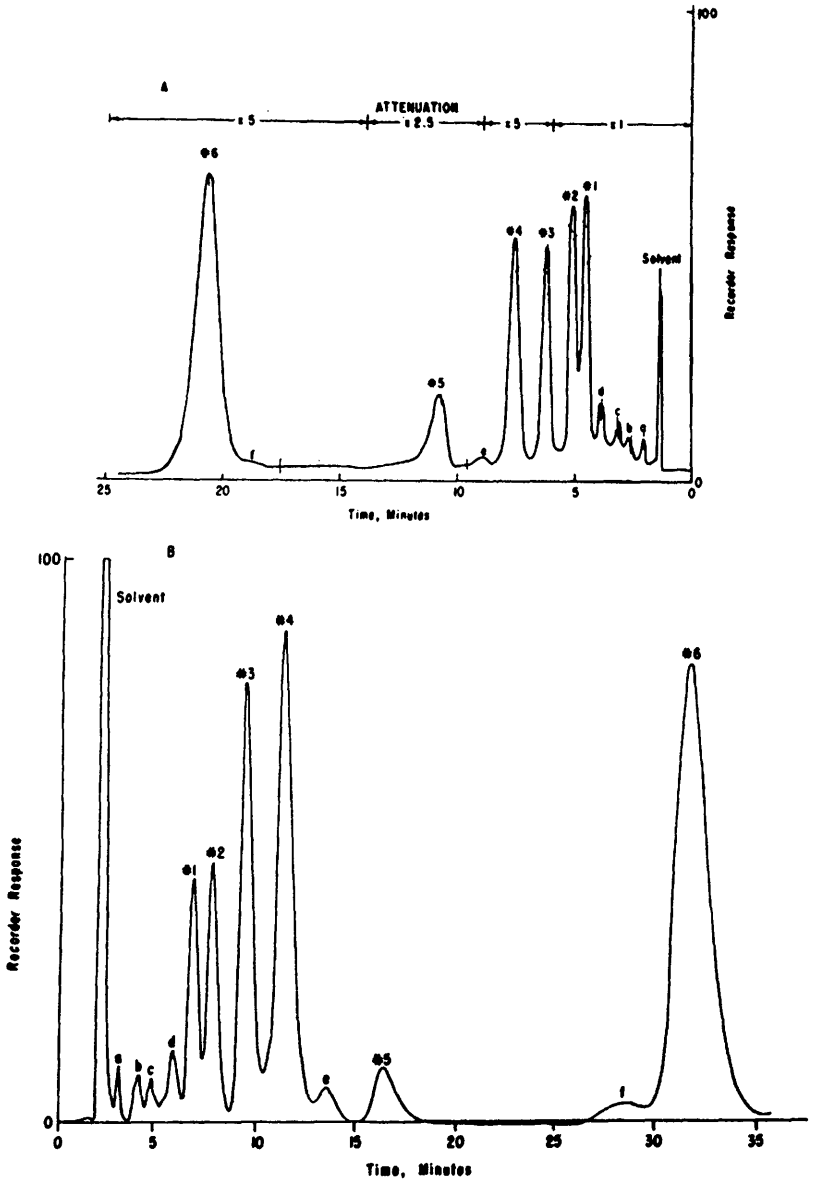

Figure 16. Mass spectrometric-gas liquid chromatographic analysis of *Santolina chamaecyparissus* L. oil. A. (top). Mass spectrometer-gas chromatograph tracing. The glass column was 10 ft. × ¼ in. and was packed with 6% LAC 728 on acid-

washed Chromosorb W. The column temperature was kept at 70, injection port at 150, separators at 250 and ion source at 310 C. The He flow rate was 50 ml/min. One μl. of oil was injected. Slash (/) marks indicate the places where mass spectra were taken. B. (bottom). Hydrogen flame gas chromatograph tracing. Column used and amount of oil injected were the same as in A above. The column was kept at 70, injection port at 150, and detector at 225 C; the He flow rate was 50 ml/min., the He pressure was 12 psi and the air pressure applied was 30 psi. The attenuation on the Barber-Colman Gas Chromatograph was 300. The peaks labeled c, 1, 3 and 5 were identified as due to α-pinene, β-pinene, myrcene and artemisia ketone, respectively.

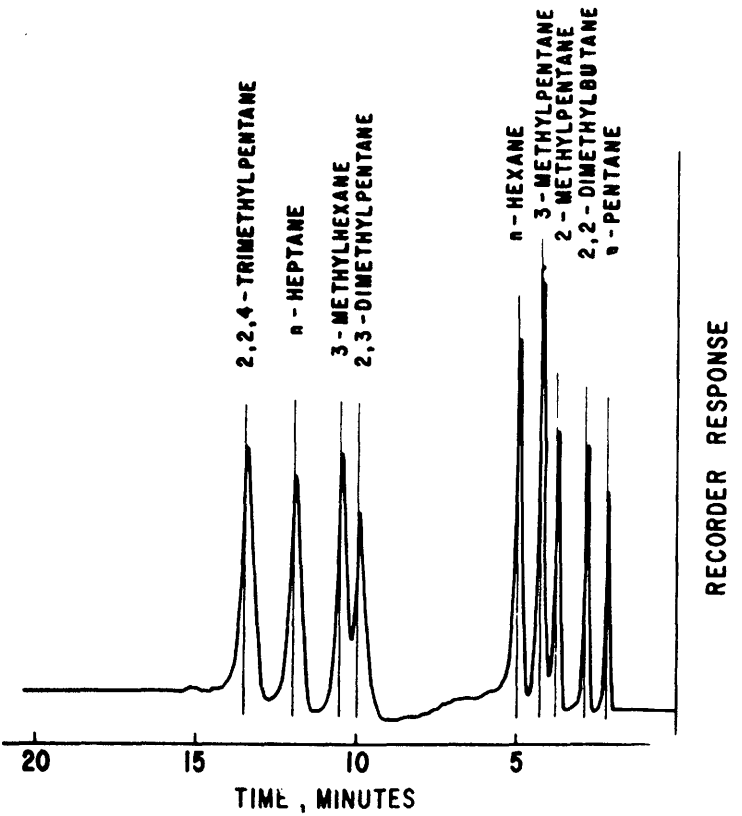

Figure 17. GLC tracing of hydrocarbons on a coiled glass capillary column. The column used was packed with squalane on Gas-Chrom R. The column dimensions were 20 ft $\times$ 0.2 mm. It was prepared by drawing out ¼-inch Pyrex tubing packed with Gas-Chrom R (one ft of ¼-inch tubing gives about 50 ft of capillary tubing). The injection port, column, separators and ion source temperatures were 50, 25, 200 and 310 C respectively. The He flow rate was 2 ml/min. 0.5 μl of liquid was injected. Slash (/) marks indicate the places where mass spectra were taken.

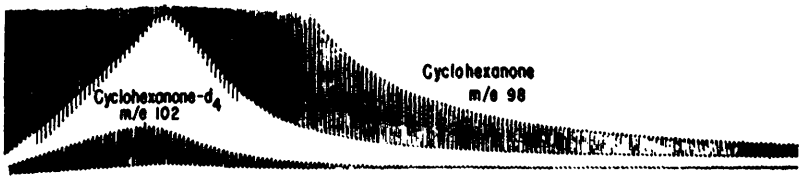

Figure 18. Continuous recording for m/e = 98 (cyclohexanone) and m/e = 102 (cyclohexanone-d_4) during elution from the GLC column. Percent relative ion intensity is shown on the abscissa and time in minutes is shown on the ordinate. A 10-ft. × ¼-inch coiled glass column packed with 25% Apiezon L on Gas Chrom Q was used. The injection port, column, separator and ion source temperatures were 165, 145, 225 and 310 C respectively. One μl of sample was injected.

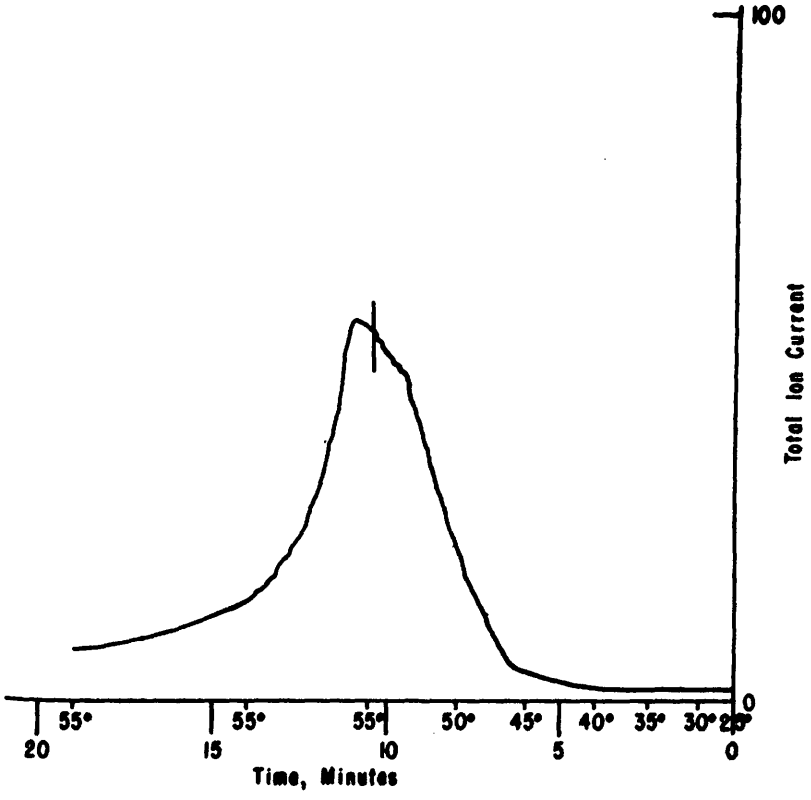

Figure 19. Pen and ink tracing of total ion current produced by 0.2 μg of ricinine using the direct probe.

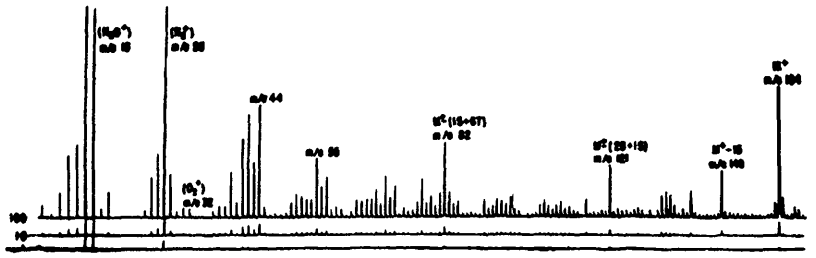

Figure 20. Tracing of mass spectrum of 0.2 μg of ricinine using the direct probe.

The use of mass spectrometry in solving biochemical problems (other than the determination of stable isotopes in gases) is a recent development and only a few problems have been attacked in this manner. As more biochemists become familiar with its capabilities and limitations, it is anticipated that this technique will be increasingly in demand. Already notable progress has been made in identifying and elucidating the structure of compounds of biological importance such as steroids, alkaloids, hormones, amino acids, peptides, fatty acids, and vitamins.

SUMMARY

The technical specifications of the Oklahoma State University mass spectrometer-gas chromatograph are summarized below:

Mass Range

Automatic scanning (continuously variable):

m/e 2-1250 at 3.5 kV accelerating voltage
m/e 2-1450 at 3.0 kV " "
m/e 2-1700 at 2.5 kV accelerating voltage

Manual adjustment (continuously variable):

m/e 2- 600 at 3.5 kV accelerating voltage
m/e 2- 700 at 3.0 kV " "
m/e 2- 850 at 2.5 kV " "

Resolution (Normal)

$M/\Delta M = 1000$ (10% valley definition).

Resolution (Maximal)

$M/\Delta M = 2000$

Sensitivity (at optimal performance)

The required sample amount is less than 0.01 μg (capillary column) and 0.1 μg (packed column).

Scanning

Magnetic scan, the range of which can be preset to any suitable value by means of limit controls. Scanning speeds 1-960 seconds in 9 steps for full scan.

Examples: $M/\Delta M = 1000$

m/e 12-100 in 2.5 sec.
m/e 12-400 in 4.2 sec.
m/e 24-800 in 4.2 sec.
m/e 48-1000 in 3.6 sec.

$M/\Delta M = 400$

m/e 12-100 in 1.0 sec.

m/e 12-400 in 1.7 sec.

	m/e 24-800 in 1.7 sec. m/e 48-1000 in 1.5 sec.
Accelerating Voltage	3-step selector for 2.5, 3.0 and 3.5 kV. Stability better than 0.01% per 10 min.
Magnetic Field	0-15 kilogauss, continuously variable. Stability better than 0.01% per min.
Electron Energy	10-100 eV. Automatic switching to pre-selected value during scan, e.g. from 20 to 70 eV.
Ion Source Exit Slit	0-0.4 mm. Continuously adjustable from outside the vacuum system.
Collector Slit	0-0.8 mm and rotatable ± 5°. Continuously adjustable from outside the vacuum system.
Electron Multiplier	14 dynodes, variable gain 10^3 - 10^7. The multiplier is fed by a high-voltage D.C. power supply (stability better than 0.01% per 10 min.), 1.7 - 3.7 kV in 10 steps.
Recording	A collector electrode, located just after the exit slit of the ion source, is connected through an electrometer amplifier to a strip-chart recorder. With GLC effluents typical chromatograms are produced by continuous recording of total ion current in the spectrometer. With other inlet systems the recorder serves to indicate optimal ion intensity for mass spectral analysis.
Inlet Systems	Gas chromatograph, heated all-glass system and direct probe.
Gas Chromatograph	The cylindrical oven with a circulating air bath accepts coiled columns of variable length but maximum dimensions of 140 mm (5½") coil diameter and 100 mm (4") height. Column temperatures are regulated by proportional control (25-350 C) for isothermal operations, and can be programmed linearly at rates up to 15°/min.
Molecule Separator[3]	2-step jet separator of the Becker-Ryhage type, column temperature regulated by proportional control. Most (50% to 75%) of the sample injected into the column passes through the separators and reaches the ion source of the mass spectrometer but only 0.5% to 1% of the carrier gas does so. The total enrichment factor is between 50 and 150.
Vacuum System	Two separate pumping assemblies are provided: one for the analyzer tube and one for the inlet systems, with two separate high-capacity pumps.
Analyzer tube:	Forepump 2.5 m³/h Oil diff. pump 550 l/sec. Mercury booster pump 12 l/sec. Cold trap for liquid N_2 or CO_2 Ultimate vacuum 10^{-7} mm Hg (closed dual valve).

	Inlet System:	Forepump	9.5 m³/h
		Oil diff. pump	150 l/sec.
		Mercury booster pump	2 l/sec.
	Gauges	Pirani and Penning vacuum gauges.	
Bake-Out	Analyzer tube and other high vacuum parts can be outgassed at 300-400 C by indirect heating.		
Mains Connection	220/380 V (220 V to ground, 380 V between phases) 3-phase A.C.		
Power Consumption	Approx. 8 kVA, unsymmetrical load max. 15 A/phase (at 220/380 V).		
Cooling Water Consumption	Approx. 3 l/min., temperature max. 25 C.		

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