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Direct Recording of High Resolution Mass Spectra of Gas Chromatographic Effluents

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A technique for the recording of complete high resolution mass spectra (resolving power: 1 part in 10 000 or better) of compounds emerging from a gas chromatograph is discussed. The pressure reduction system used for the connection of chromatographic column and mass spectrometer is short, simple in design, and made entirely of glass which make it suitable for the study of complex, polar, and high-boiling compounds. The quality and magnitude of mass spectral data obtainable by this technique demonstrate the value it will have in the identification or determination of the structure of compounds separated by gas chromatography. © 1965 American Chemical Society.

Both gas chromatography and—although less widely available—mass spectrometry have become valuable techniques in organic chemistry. While the former is, strictly speaking, a separation technique and the latter provides unique characterization of organic molecules, two of their common characteristics, namely high sensitivity and the requirement of a vaporized sample, make a combination of these two techniques a desirable method more powerful than either one considered individually. Some of the immediately obvious advantages are: an increase in the speed of analysis, elimination of the necessity for isolation of minute quantities of pure sample, elimination of sample alteration when coming in contact with the atmosphere (air oxidation, hydrolysis), and greater certainty in identification of the eluted component than that achieved by retention time alone.

A number of laboratories have successfully operated a gas chromatograph in combination with a conventional single focusing mass spectrometer. The earlier work ranges in implementation from the simple monitoring of a single mass[10,11] to the fast scanning of a preselected part[12] of the spectrum, or all of it, using oscillograph recorders[5,7,13,14,17] or oscilloscope photography.[5,10] In-line trapping of fractions in a manifold system located between gas chromatograph and mass spectrometer has also been used.[8,15]

Coupling of gas chromatography and mass spectrometry is extremely useful in the study of natural products where one may encounter complex mixtures containing trace components that are difficult to isolate and identify. In a study of neutral fecal steroids, impressive results have been obtained with a combination of a gas chromatograph and a fast scanning, conventional (low resolution) mass spectrometer.[18]

Data obtained with a mass spectrometer of much higher resolving power provide, however, unique information not derivable from conventional spectra and this becomes very important in the work on complex molecules of unknown structure.[2] Careful measurement of the molecular and fragment ions permits determination of their elemental composition[1] which enables one to make a less ambiguous interpretation of the spectrum than when examining a conventional low resolution mass spectrum in which case a number of different elemental compositions have to be considered for each nominal mass. A resolution of 1:10 000 is generally sufficient to permit mass determination with the accuracy required for work with compounds of molecular weight up to 500, although 1:20 000 or more is sometimes required. We have, therefore, explored the feasibility of obtaining complete high resolution mass spectra of compounds such as amino esters, alkaloids, carbohydrates, and steroids while they emerge from the gas chromatograph directly into a double focusing mass spectrometer.

We are using a CEC 21–110 mass spectrometer which is based on the Mattauch–Herzog design and thus focuses all ions in a focal plane. A photographic plate placed in this focal plane is used for the recording of the high resolution mass spectra. This technique eliminates many of the problems inherent in the direct combination of gas chromatograph and mass spectrometer. First, the disturbing effect of the change in sample concentration associated with the emergence of gas chromatographic fractions is eliminated due to the integrating effect of the photographic emulsion which is bombarded by all the ions all the time. Second, since the entire high resolution mass spectrum is simultaneously recorded, the necessity of rapid scanning during the time of emergence of the gas chromatographic fraction is eliminated. A point concerning the relative sensitivities of a photographic plate *vs.* electron multiplier should be made. When using magnetic or electric scanning at high resolving power the electron multiplier is exposed to each peak in the spectrum only for a very

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small fraction of the time taken for the total scan. When the lifetime of the sample in the ion source is so short that one must scan the spectrum in 1–10 s, the signal recorded for each peak in the spectrum becomes diminishingly small. On the other hand, the photographic plate is exposed to all the ions simultaneously and thus achieves greater over-all sensitivity. Based on the sensitivity of photographic emulsion (10^5 ions/mm^2 necessary to produce visible blackening[16]) one can conclude that a measurable line (1 mm long, 10 microns wide) will result if at least 1000 ions of a given *m/e* are produced during the residence time of the sample in the ion source.

In addition, the high resolving power of a double focusing mass spectrometer makes it possible to feed continuously a calibration compound into the ion source to provide mass standards for the mass determinations. The calibration compound is carefully chosen so that there is no overlap with compounds being analyzed—i.e., at any given nominal mass the calibration line is well resolved from the compound lines. This contribution of added calibration compound is later automatically removed during the computation process.[6]

PRESSURE REDUCTION SYSTEM

The most critical part in any such combination of gas chromatograph and mass spectrometer is the pressure reduction system connecting the two instruments. To minimize memory effects (tailing *after* the column) and catalytic or thermal decomposition, the connection has to be as short as possible (particularly in the region of high pressure), easily heatable, and free of metal.

The pressure reduction system we are using serves two purposes. First, it provides a pressure drop from 1 atm to 10^{-5} mm Hg and, second, it leads to concentration of the sample in the gas stream entering the ion source of the mass spectrometer.

Figure 1 shows a schematic of an improved modification of our earlier system.[19] It involves a fritted glass tube instead of the metal valve generally used[8,9,11,14,15] to pump off the excess carrier gas. The ultrafine porosity of the tube has the additional effect of permitting helium, the carrier gas, to be removed to a higher rate (due to preferential effusion) than the eluate (sample), the molecular weight of which is, of course, much higher than that of helium. This results in the relative enrichment of the sample (with respect to carrier gas) which passes through the second capillary constriction into the ion source of the spectrometer.

This enrichment was found to be, for example, 50-fold for diethyl ether, by introducing a mixture of helium and diethyl ether both through the pressure reduction system as well as the conventional reservoir into a Bendix Time-of-Flight mass spectrometer and measuring the abundance ratio of mass 31 *vs.* mass 4 in each case. The enrichment factor is, of course, related to the molecular weight of the sample—i.e., better for higher molecular weight compounds. For the purpose of this experiment the gas chromatographic column was removed. From this enrichment factor and an estimate of the total amount of gas entering the ion source one can roughly estimate that at least 10%, but probably not more than 50%, of the sample injected into the gas chromatographic column reaches the ion source, the rest being lost through the walls of the fritted tube. The fritted tube is held by means of two silicone rubber O-rings at either end of a small copper pipe which constitutes a vacuum chamber when connected to a mechanical pump.

The conditions of effusion[20] must be satisfied inside the fritted glass tube for the pressure reduction system to provide an enrichment of the sample with respect to the carrier gas. To maintain molecular flow (effusion) the mean free path of the gas must be 10 times the diameter of the pores through which it passes. Since the pores in the fritted tube are approximately 1 micron wide, the pressure inside the tube should be of the order of a few millimeters of mercury. Thus the diameter of the entrance constriction is important in effecting a pressure drop sufficient to satisfy conditions for effusion and in establishing the flow rate into the pressure reduction system. The exit constriction diameter controls the sensitivity of the apparatus limited only by the maximum pressure that can be tolerated in the ion source of the mass spectrometer. The constrictions are prepared by collapsing the capillary walls by a process

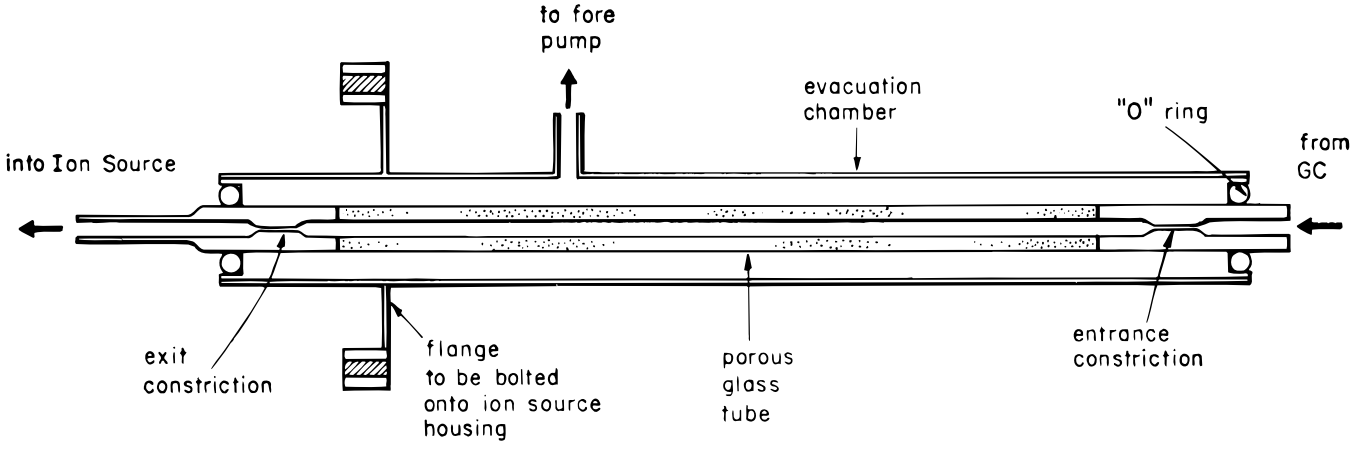

Figure 1. Pressure reduction system, the link between gas chromatograph and mass spectrometer.

of trial and error. A diameter of approximately 0.2 mm for the entrance orifice and 0.1 mm for the exit orifice were found to be best.

The apparatus is shown disassembled in Fig. 2. The gas chromatographic column is made of 3-mm i.d. borosilicate glass (Pyrex) tubing (6 ft effective length) wound into a helix (2-in. o.d., 8 in. long) The injection port consists of a serum cap of silicone rubber. Helium is supplied at a slight over pressure (0.5 psi) to the injection port via the borosilicate glass (Pyrex) tube intersecting the column just below the serum cap. The first 2 in. of the column below the injection point are filled with glass wool and wrapped with a heating tape to form the vaporizer. The column is connected directly to the capillary containing the entrance constriction to the pressure reduction system via a short piece of silicone rubber tubing. The porous glass tube (8-mm o.d., 2-mm wall thickness) is 8 in. long and of ultrafine porosity (average pore diameter 1 micron, obtainable from Corning Glassware, Corning, N.Y.). Beyond the exit constriction a short piece of glass tubing (4-mm o.d.) extends through the walls of the ion source just into the ionization chamber. Thus the effluent from the gas chromatograph contacts only glass and is channelled directly into the electron beam of the ion source, no more than 14 in. from the end of the column packing.

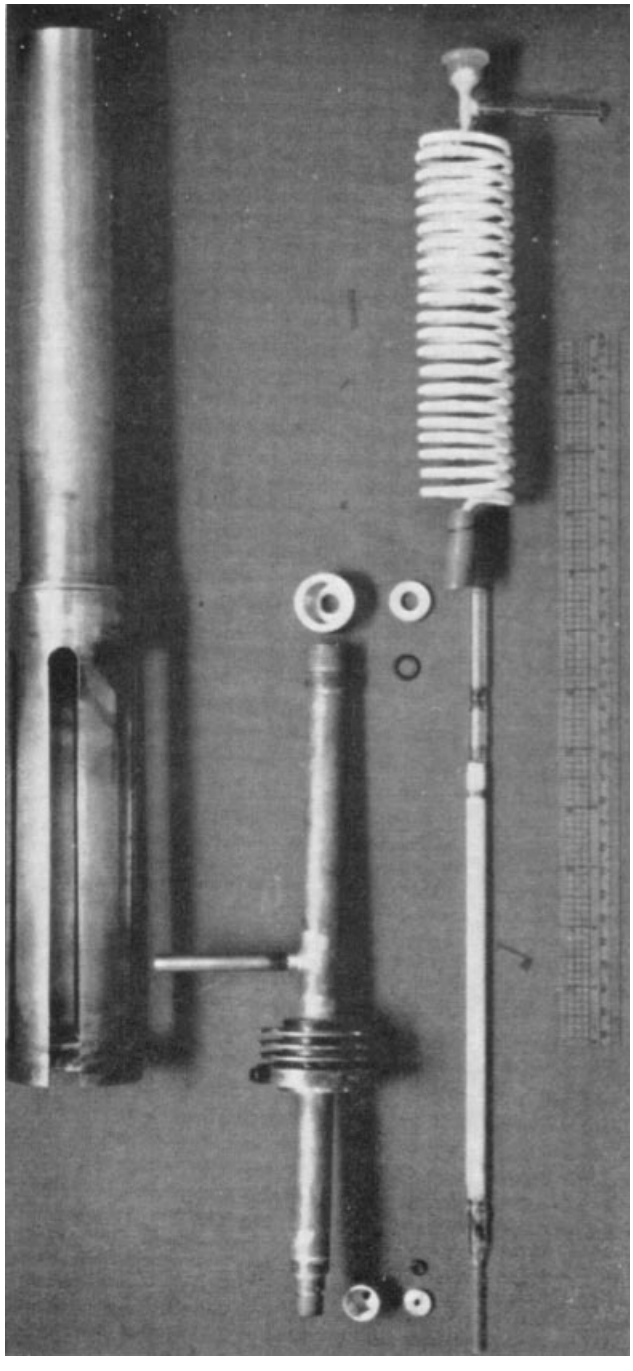

Figure 2. Pressure reduction system, disassembled.

The evacuation chamber consists of a $\frac{5}{8}$-in. copper pipe fitted on both ends with 'Quick-Connect' fittings which provide the means for vacuum-tight O-ring seals (silicone rubber) around the glass capillary. The small pipe at right angles to the evacuation chamber leads to a mechanical pump. The evacuation chamber is connected to the mass spectrometer ion source housing by means of a stainless steel flange and is wrapped with heating wire above the flange; the entire chamber is kept at a reasonably uniform temperature because of the high thermal conductivity of copper. Because of its low thermal conductivity, stainless steel was used for the flange to avoid heat loss to the ion source housing. The large cylinder at the top of Fig. 2 provides both a support as well as an oven for the gas chromatographic column when bolted to the circumference of the flange. The lower slotted portion is of stainless steel and the solid walled upper portion is of brass which provides even distribution of heat when wrapped with heating wire.

All of the components have separate heaters and their temperatures are monitored by appropriately placed thermocouples. Practical limits of the temperature of the components are as follows: injector, 350 °C; column oven, 300 °C; pressure reduction system, 275–300 °C; and ion source 225–300 °C.

Since we use the high resolution mass spectrometer as a highly sophisticated fraction detector and are—at least at present—more interested in qualitative work rather than quantitative aspects, it was possible to greatly simplify the gas chromatographic section of the system. It has been reduced to the only essential part, a heated column. Thus it represents more a modified inlet system for the mass spectrometer rather than a separate unit. The pressure reduction system remains permanently attached to the ion source housing and, with the helium flow clamped off and the mechanical pump running, does not interfere with the normal operation of the instrument using individual samples. The column is attached to the pressure reduction system by a simple silicone rubber connection which facilitates interchange of various columns without completely dismantling the apparatus.

For a display of the chromatogram in the conventional sense the variation in the intensity of total (unresolved) ion beam is recorded using the beam monitor of the mass spectrometer. As shown in Fig. 3, it intercepts and measures part of the ion beam after leaving the electric sector and before entering the magnetic field. In the absence of a chromatographic fraction entering the ion source, the beam is mainly due to the calibration compound (perfluorokerosene). Helium seems to contribute little since all the parameters are set

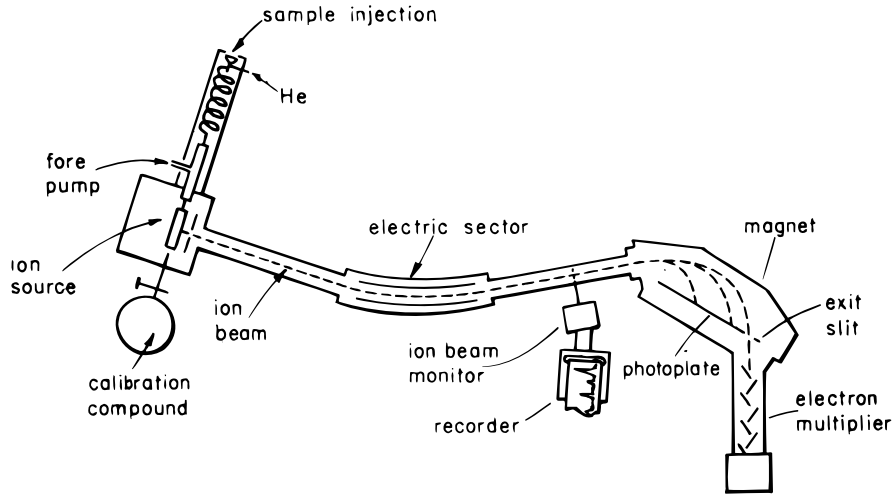

Figure 3. Schematic of spectrometer with gas chromatograph attached to modified ion source housing.

for maximum intensity at high masses (300–500). Mass discrimination is thus greater for very low masses preventing most of the helium ions from passing through the first slit of the electric sector.

When a fraction emerges from the chromatograph, the total ion intensity increases and this change is displayed on the pen-and-ink recorder connected to the beam monitor. Only if a very large amount of organic material—e.g., the solvent—emerges do the potentials in the source get sufficiently disturbed as to lead to a temporary loss of intensity by 'defocusing' the source and thus to a dip in the signal or even to a negative peak.

EXPERIMENTAL

In operation the pressure in the ion source is about 1–2 × 10^{-5} mm Hg when a good mechanical pump—i.e., Duo-Seal 1400B—is connected to the pressure reduction system. The entrance of the gas chromatographic column is kept at a slight overpressure (about 0.5 psi) of helium. Under these conditions a flow rate of 20 ml/min (into the column) is established.

Although the beam monitor was not specifically designed to function as a detector for a gas chromatograph, it behaves quite well as shown in Fig. 4. Since this trace represents the ion beam—i.e., sample concentration in the ion source *vs.* time—it also demonstrates that there are no prohibitive memory effects due to adsorption or condensation in the pressure reduction system and ion source.

Figure 4 is a chromatogram of 1 μl of a solution containing 2–4 μg each of 11 fatty esters (3% JXR on Gaschrom P, injector: 260°/column temp.: programmed, start at 160°/vacuum jacket: 240°/glass extension into ion source: 185°). The vertical slots in the chromato-

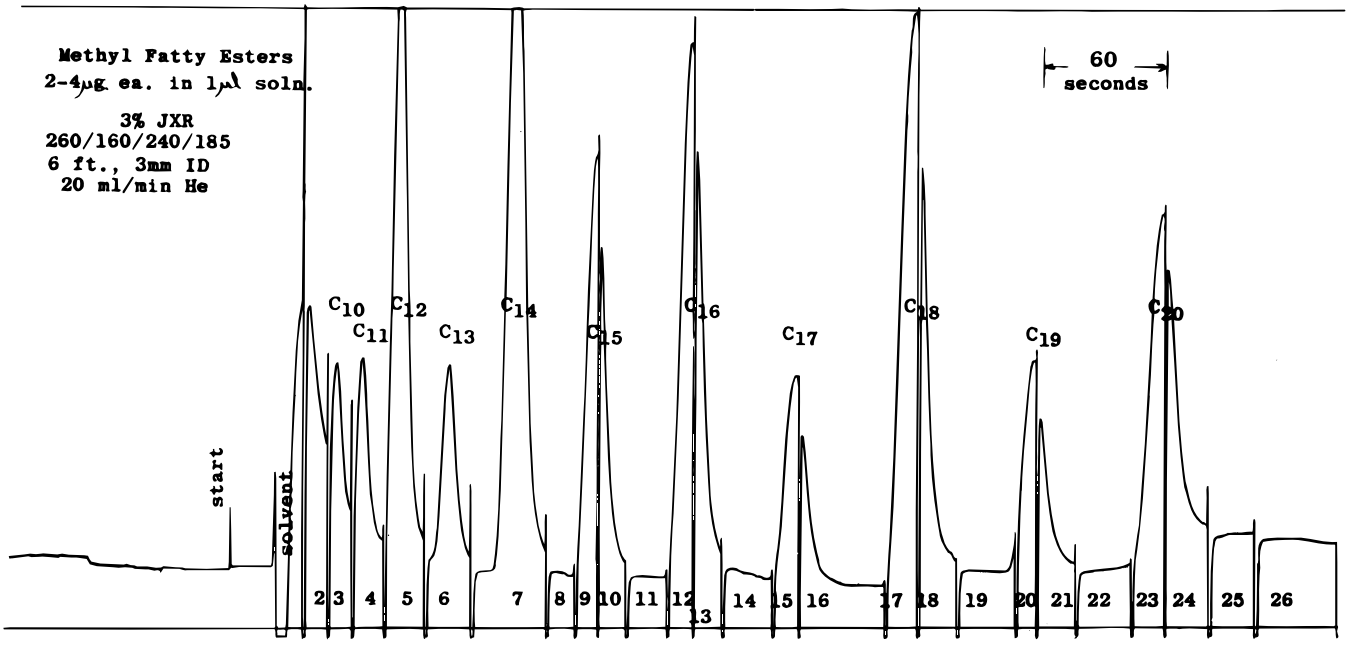

Figure 4. 'Beam monitor chromatogram' of a series of esters from methyl caprate to methyl arachidate.

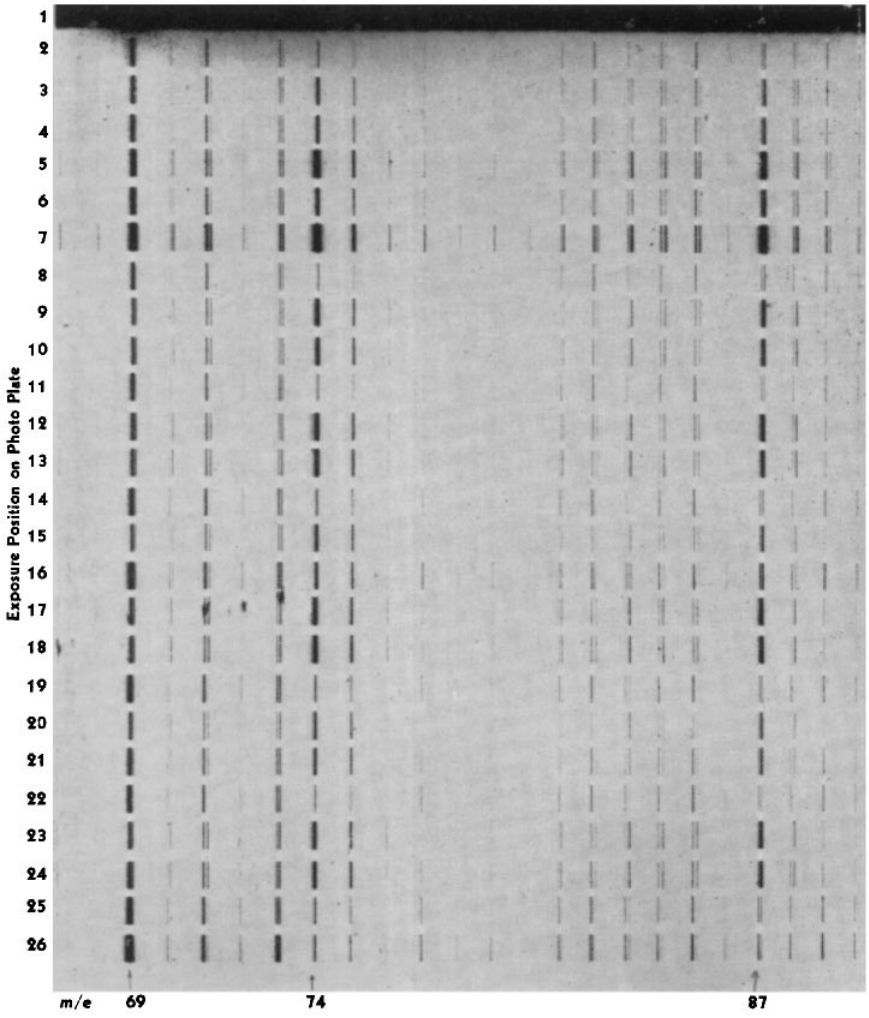

Figure 5. Section of photo plate containing spectra of the esters shown in Fig. 4.

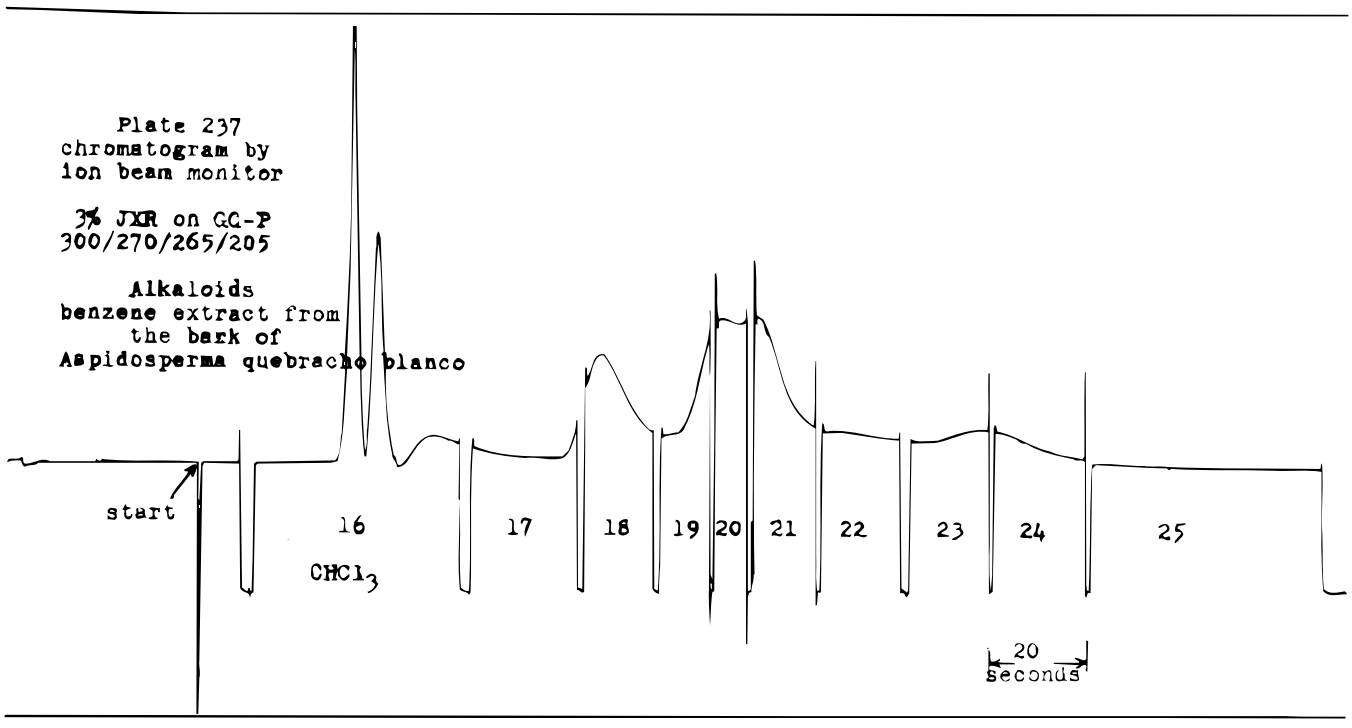

Figure 6. Trace obtained by recording the ion current after injection of a portion of *Aspidosperma quebracho blanco* bark extract.

gram trace indicate the short periods of time when the ion beam is deflected off the plate (and thus off the beam monitor) which is necessary when changing the position of the photographic plate, a process that takes about 2–3 s with our present arrangement. This pen deflection provides a useful indication of the exact portions of the chromatogram being recorded with the mass spectrometer and aids in the correlation of the spectra on the plate with the chromatogram.

The photographic plate provides space for up to 29 usable spectra and it is thus possible to analyze reasonably complex mixtures by this technique. The numbers along the baseline between the vertical slots correspond to the position of the photographic plate exposed for that length of time. Figure 5 shows the full width and approximately one tenth the length of the plate where many of the masses of the fatty esters are discernible (the magnetic field was set to focus the region from mass 12–360 on the plate). The column of numbers at the left in Fig. 5 refers to the exposure positions on the photographic plate. Those lines which are of similar density in all exposures are due to the calibration compound—e.g., *m/e* 69. Others, as for example, portions of the multiplets at *m/e* 74 and 87 appear and disappear; these are fragments which are characteristic of methyl esters of fatty acids. Multiplets are discernible at several nominal masses even under this quite low magnification, not only rather widely spaced ones, due to ions of the perhalogenated mass standard and the ester, but also narrow doublets of the C, H *vs.* C, H, O type from the ester—e.g., at *m/e* 83–85. Exposures 5 and 7 are overexposed as far as masses 74 and 87 are concerned, which could still be measured on exposure 8 as it contains the tail of the fraction represented by exposure 7. A very short exposure (1 s) during the emergence of such an intense peak would still be better.

Figure 6 shows a chromatogram of a fraction of alkaloids extracted from the bark of *Aspidosperma quebracho blanco*. In an attempt to improve the information obtainable from the poorly-resolved gas-chromatographic fraction, the doublet was recorded in three separate parts (19, 20 and 21 in Fig. 6). A closeup view of the photographic plate (Fig. 7) shows a definite trend in the appearance and disappearance of two different compounds in consecutive exposures during the emergence of the incompletely resolved fraction.

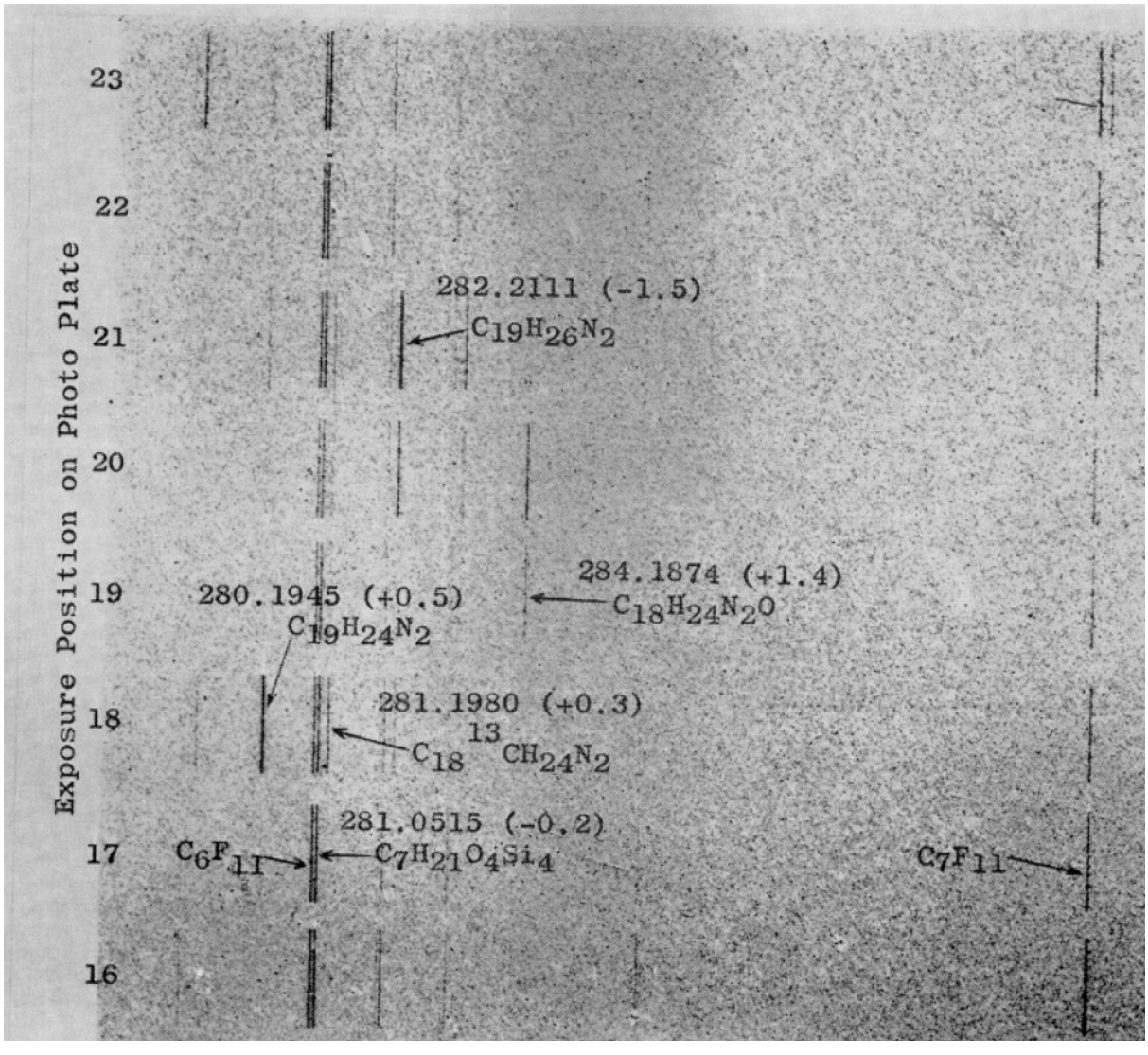

Figure 7. Magnification of a portion of photo plate containing spectra of alkaloids recorded during chromatogram represented by Fig. 6.

Figure 7 is of that region of the plate which corresponds to *m/e* 280 through *m/e* 293. The numbers at the left correlate the plate positions exposed to the various segments of the chromatogram (Fig. 6)—e.g., positions 17 and 22 are background. Nominal mass 281 is always a doublet; one peak, C_6F_{11}, is from perfluorokerosene, the other, C_7-$H_{21}O_4^{28}Si_4$, from the bleeding of silicone compounds from the gas chromatograph. The spectrum at position 18 was obtained during the emergence of the first chromatographic peak which contained a compound having a molecular ion at mass 280. Positions 19, 20 and 21 show the trend in the appearance first of a compound that has a fragment at mass 284. A mixture appears in position 20 and finally in position 21 mass 284 virtually disappeared and the compound with molecular ion at mass 282 clearly dominates the spectrum. The lines at 279 and 294 in exposure 23 illustrate that even one half of a very weak chromatographic peak gives well-discernible spectrographic lines.

The two major fragments of the calibration compound (perfluorokerosene) in this region are C_6F_{11} and C_7F_{11} (see Fig. 7). The exact masses of these fragments are used to calculate the masses of all the other lines in the region by carefully measuring their distances relative to the positions of the two calibration fragments. This formidable task is accomplished using a semi-automatic comparator–densitomer[4] for the distance measurements, followed by feeding the resulting data into a digital computer (IBM 7094) for further conversion to masses and elemental composition[6] or further treatment.[2,3] The masses determined for a few lines are shown in Fig. 7; the difference (in millimass units) between the mass calculated from the lines on the plate and the theoretical mass of the combination of the elements indicated is given in parentheses. It can be seen that the mass measurement is sufficiently accurate to assign unambiguously a definite elemental composition to each of the ions observed.

While it may be difficult for the untrained eye to see the subtle differences in intensity on the plate, they are readily distinguished in densitometer scans of the same area of the plate as shown in Fig. 8. The bottom trace shows only the calibration compound with nominal masses at 269, 281 and 293. The third trace shows the

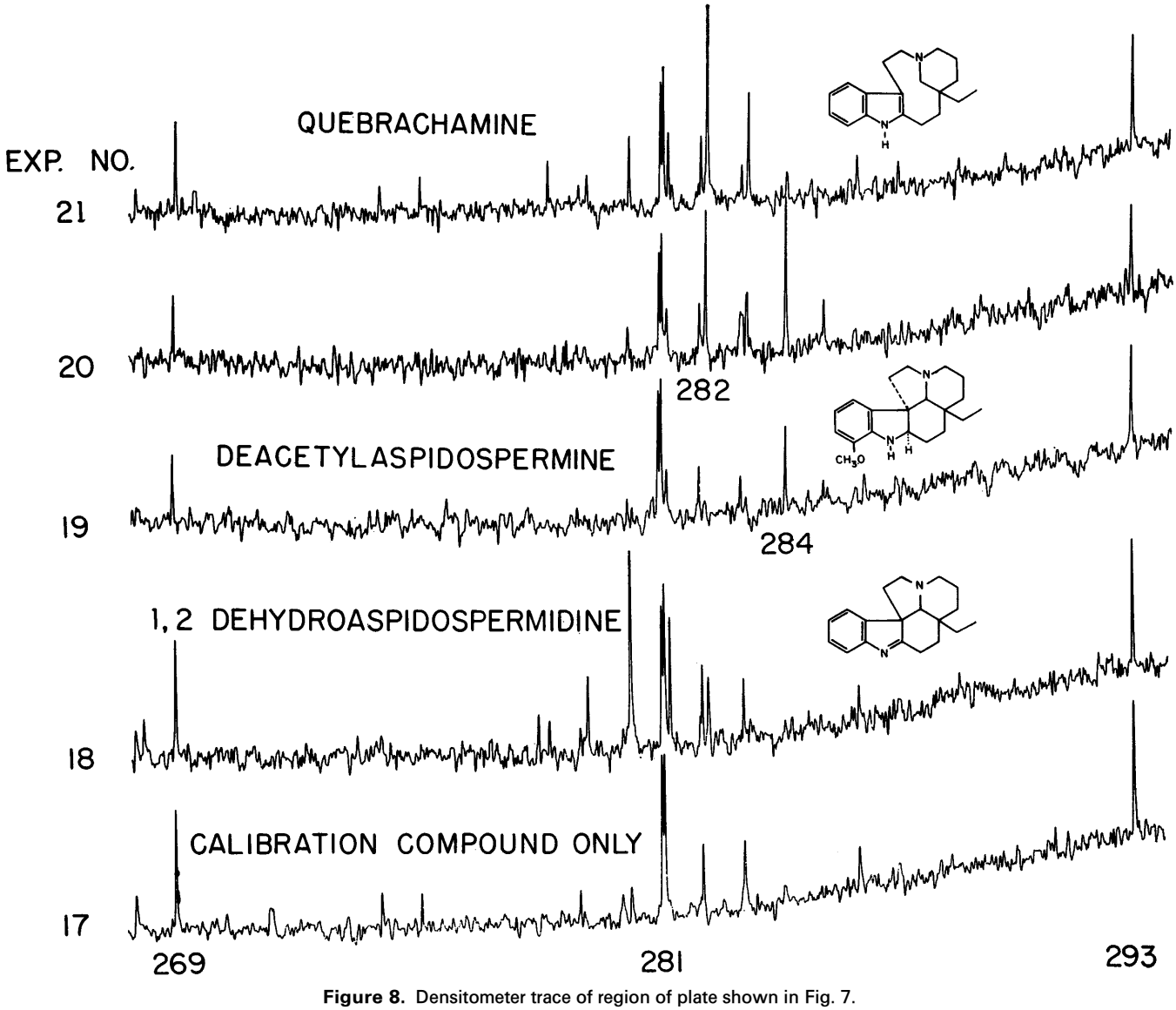

Figure 8. Densitometer trace of region of plate shown in Fig. 7.

appearance of mass 284 from deacetylaspidospermine (molecular weight 312) while the fifth trace shows the virtual disappearance of mass 284 as quebrachamine clearly dominates the spectrum with its molecular ion at mass 282.

It should be noted that the recording densitometer used for this scan (which is shown only as a pictorial representation of the relative densities but is not used for any actual measurements) was not designed to measure lines as sharp as those of a high resolution mass spectrum. The slit on this densitometer is much wider than the line width, hence the resolution in the doublet at *m/e* 281 appears to be quite poor in this trace in contrast to the appearance of the lines on the plate where they are completely separated (see Fig. 7). The baseline appears to be quite noisy; this is due to the slight grain in the photographic emulsion, an effect exaggerated in the densitometer scan because of the logarithmic representation of the output.

In a similar experiment a mixture containing androstane, androstane-17-one, androstane-3,17-dione, and Δ^4-androstene-3,17-dione, and Δ^4-androstene-3,17-dione was injected into the column (3% JXR on Gas-Chrom P, injector: 280 °C/column: 260 °C/pressure reduction system: 230 °C) and the chromatogram indicated incomplete resolution of the last two components under these conditions. To obtain spectra of each pure component, three exposures[15–17] were taken during the emergence of this chromatographic doublet. Figure 9 is that section (in the region of *m/e* 281–293) of the photoplate which contains the molecular ions of the saturated and unsaturated diketones. A definite trend can be seen starting with exposure 15 which contains the molecular ion of androstane-3,17-dione at mass 288. The next spectrum is that of a mixture because Δ^4-androstene-3,17-dione (mass 286) begins to emerge, which finally dominates in the third spectrum (exposure 17) while the line at *m/e* 288 virtually disappears.

Some of the determined masses and corresponding elemental compositions derived from measurement of line positions are incorporated in Figs 7 and 9. These values demonstrate that the molecular composition of even a rather complex organic molecule such as a difunctional steroid or an indole alkaloid, can be determined regardless whether emerging as a single fraction or as an incompletely resolved mixture. These values shown are but a few of the many mass measurements (350–600) performed on each spectrum using the techniques discussed elsewhere.[4] The completeness and accuracy of these measurements, even though obtained on a minute, transient sample such as a gas chromatographic fraction, are sufficient for data processing and conversion to complete high resolution mass spectra in the form of 'element maps,' the principle and interpretation of which has been discussed elsewhere.[2,3] Since the computer can automatically subtract the lines produced by the simultaneously recorded calibration compound as well as those of the column bleeding, spectra free of this background and representing only the emerging fraction are thus produced automatically.

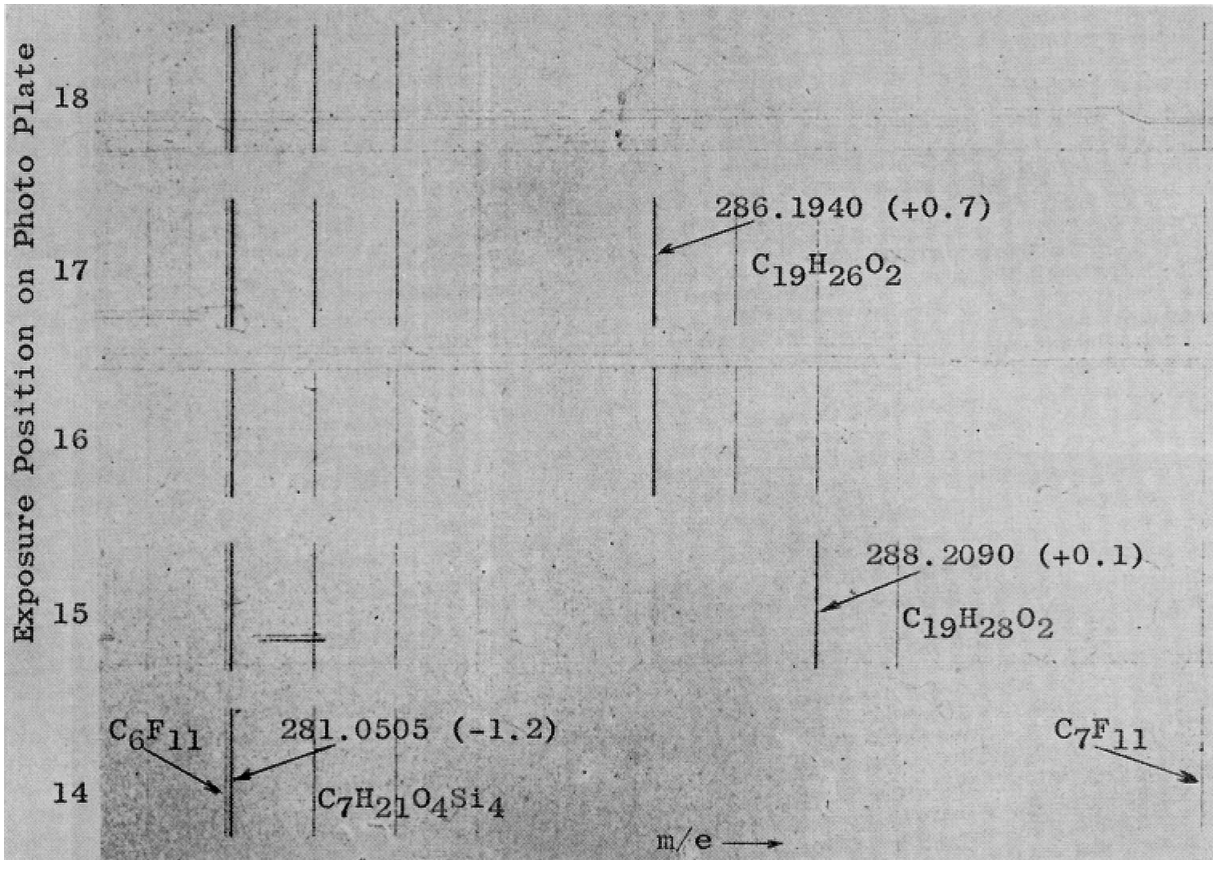

Figure 9. Magnification of photo plate in region of molecular ions of androstane-3,17-dione and Δ^4-androstene-3,17-dione.

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