

AUTOMATION IN THE ELUCIDATION OF THE STRUCTURE OF ORGANIC COMPOUNDS

W. SIMON

*Department of Organic Chemistry, Swiss Federal Institute of Technology,
Zürich, Switzerland*

There is much confusion of thought over the precise definition of the term automation. It is unfortunately often used to describe any procedure by means of which human effort is diminished. There is however the tendency to define automation as the regulated control and internal selfchecking of a process by feed-back of information. The process involved may be an analytical procedure or the processing of data. Automation in this sense is used more and more in connection with the application of physicochemical methods for the elucidation of structure of organic compounds.

One of the first steps in the elucidation of the structure of an unknown compound, especially of a natural product, is the determination of its elemental composition and its molecular formula. Before about 1955 this was done almost exclusively by conventional elemental analysis (Pregl, Dumas, Unterzaucher) in combination with a molecular weight determination (Rast). At present mass spectrometry in combination with other instrumental methods of analysis has partly eliminated the conventional approach. This trend is clearly shown in *Figure 1* which describes the situation in the Department of Organic Chemistry of the Swiss Federal Institute of Technology. Although the number of scientists increased from about 70 in 1950 to 147 in 1966 there has in fact been a decrease in the

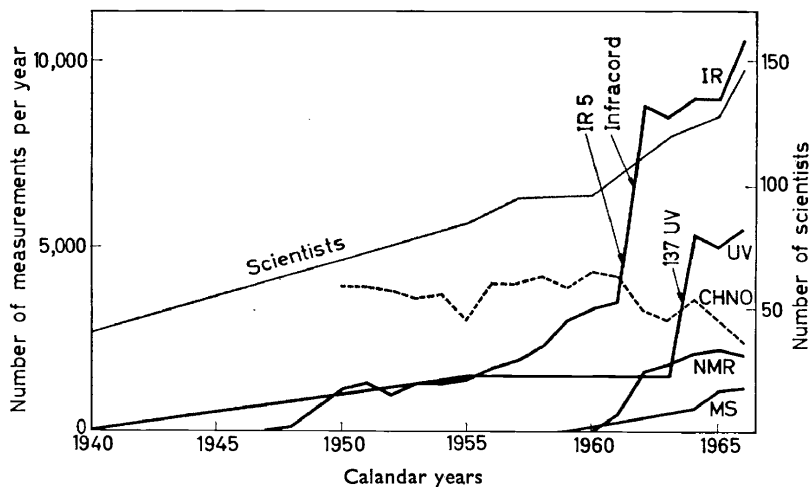


Figure 1. Number of measurements performed in the Department of Organic Chemistry of the Swiss Federal Institute of Technology

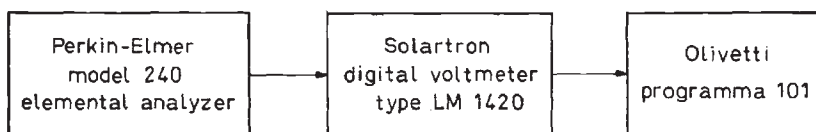
number of elemental analyses performed within the same period of time. This trend however is due partly to changes in the type of research activities. It is obvious that a group involved in mechanistic studies needs less carbon/hydrogen determinations than a group which is interested in the elucidation of the structure of natural products.

Although physicochemical methods of analysis are of increasing importance, Pregl/Dumas type determinations are still in widespread use. Some of the larger laboratories of the Swiss chemical industry are performing up to 25,000 such determinations a year¹ (up to 50,000 in 1967). The most serious drawback in the application of these classical procedures is the fact that (in order to obtain adequate results) they have to be used by well-trained people, having considerable experience and patience². Clearly there have been efforts directed at the automation of this analytical procedure. The first instrument capable of analyzing up to 25 weighed samples for carbon and hydrogen (0.6 to 1.3 mg) without any manual help having a digital print out of the results has been described in 1961².

This approach was however rather too slow and too expensive to be of general practical importance and has therefore been replaced by more efficient methods³. An instrument capable of analyzing 0.5 to 3.0 mg samples simultaneously for carbon, hydrogen and nitrogen* in 13 minutes with an accuracy equal or superior to Pregl/Dumas methods (Analyzer precision better than 0.3%) is shown in *Figure 2*³⁻⁷. In this instrument the weighed sample is made to undergo combustion in an oxygen atmosphere. Combustion is aided by chemicals such as silver tungstate and magnesium oxide located in the combustion tube. The gaseous combustion products are flushed by a helium stream through a reduction tube. Excess oxygen and unwanted combustion products are removed, and the oxides of nitrogen reduced to molecular nitrogen. The remaining mixture, consisting now of only water vapour, carbon dioxide, nitrogen and the helium diluent are gathered into a glass vessel of 300 ml capacity, which is reproducibly filled to a pressure of approximately 2 atm and held at a constant temperature. After equilibration, the sample mixture is expanded into an elongated sampling system, then swept into a series of thermal conductivity cells. Situated between the first pair of thermal conductivity cells is an adsorption trap containing a dehydrating material (magnesium perchlorate). As the gas passes through, water is removed from the stream. The thermal conductivity difference before and after the trap, as read on a microvolt-meter, reflects the water concentration and therefore the amount of hydrogen in the original sample. A similar measurement is made of the signal output of a second pair of conductivity cells between which is a trap which removes carbon dioxide. The remaining gas now consists only of helium and nitrogen. This gas passes through a conductivity cell, the output of which is compared to that of a reference cell through which flows pure helium from the gas cylinder to give the nitrogen concentration⁶.

In combination with a low cost desk computer (Olivetti Programma 101, approx. US \$ 4000) a direct print out of the percentage of C, H and N is obtained (*Table 1*). The only manual operations are the weighing of the

* Oxygen determinations may be performed on the same instrument⁶.



(a)

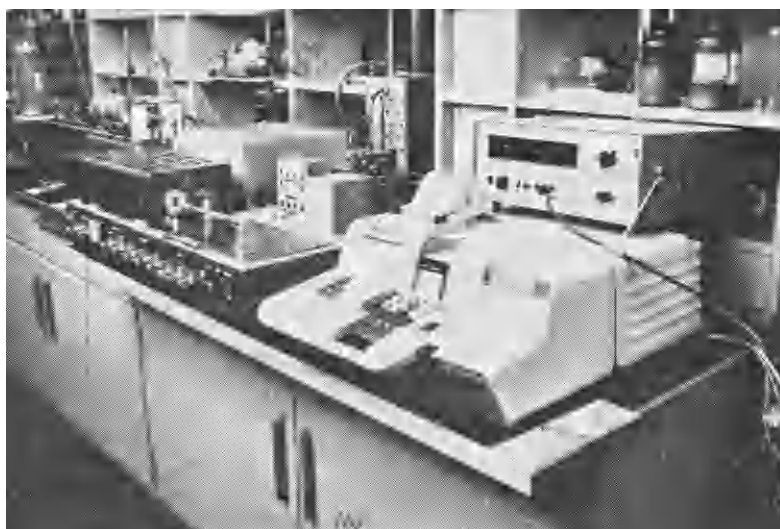


Figure 2. Automated C, H, N analyzer (a) diagrammatic sketch (b) Olivetti Programma 101 with Solartron Digital voltmeter on top and C, H, N analyzer model 240 on the left (uncovered)

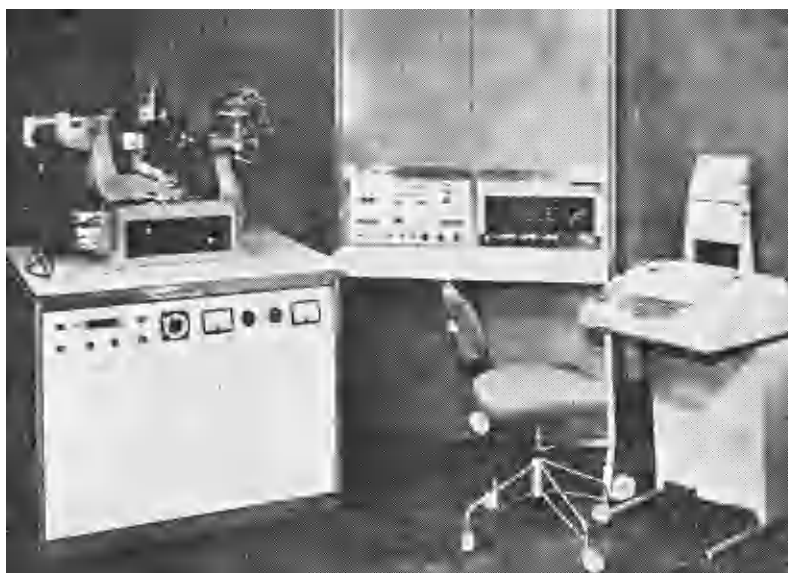


Figure 13. Photograph of an automatic diffractometer system⁴⁵ (Left: generator with diffractometer on top; middle: console with PDP-8 computer; right: teletype (input, output))

sample and its introduction into the combustion train after feeding its weight into the computer⁸ (see also ref. 9). The special feature of this instrument as compared to others⁹⁻¹¹ is its high accuracy and the fact that samples smaller than 1 mg can be used if necessary but need not be used. This is due to the self-integrating thermal conductivity approach incorporated^{3-5, 7}.

Table 1. Output of the C, H, N analyzer

Sample identification		1258	S
Sample weight (mg)		1.7155	S
Calibration factors	N	0.02872	D ◇
	C	0.10480	E ◇
	H	0.01340	F ◇
Blank	N	50.00000	d ◇
	C	1.00000	e ◇
	H	6.00000	f ◇
Electrical zero	N	46	S
	C	40	S
	H	11	S
Electrical signal	N	712	S
	C	1198	S
	H	872	S
Percentage found	C	70.68120	C ◇
	H	6.67851	A ◇
	N	10.31274	b ◇

For the transformation of the result of an elementary analysis into the molecular formula the help of computers^{8, 12} instead of tables is indicated especially for complex molecules. This brings the advantage that all possible formulae are presented immediately with the corresponding errors. A simple example for a compound which yielded 50.25 ± 0.3 per cent carbon and 8.40 ± 0.3 per cent hydrogen is given in Table 2 [output of an IBM 1620 (20K) computer]⁸.

The molecular formula can then be selected knowing the molecular weight of the compound in question, which is at present usually obtained either by mass spectrometry or vapour pressure osmometry¹³. It has been shown recently that number average molecular weights of up to at least 200,000 can be determined with vapour pressure osmometers¹⁴ (Figure 3).

The trend in modern laboratory instrumentation has been in the direction of making commercial instruments externally ever simpler to operate and more pleasing to the eye¹⁵. These instruments however have become internally more sophisticated and complex in their electronic and optical circuitry¹⁵. The organic chemist is often therefore no longer in a position to see clearly the limitations and idiosyncrasies of such instruments¹⁵. Since he is interested in using these devices as a tool for the determination of the structure of organic compounds and since he is not interested in the instrument as such, it is obvious that there is a tendency to use these analytical instruments as "black box" devices and to concentrate efforts on the interpretation of their read-out¹⁵⁻¹⁷ (see Figure 4). Some universities therefore have started to give systematic courses in the combined application of infrared (i.r.), nuclear magnetic resonance (n.m.r.), mass (m.s.) and electron spectroscopy (u.v./v.i.s.) in organic chemistry.

As has been shown clearly by Silverstein and Bassler¹⁸ the combined application of these four techniques (i.r., n.m.r., m.s., u.v./v.i.s.) is especially powerful because the areas of application are complementary and partly overlapping. Due to this overlap of the areas of application the successful

Table 2. Calculation of molecular formulae⁸

NR. R 1258 BLATT 1			
ANALYSE			
SYM	NR	P	E
C	1	50.27	.30
H	2	8.40	.30
N	3		
O	4		
BRUTTOFORMELN			
	NR	ANZ	P E
	1	9	50.44 .17
	2	18	8.46 .06
	3	4	26.14
	4	2	14.93
MG =		214.2750	F = 3
	1	10	49.97 — .29
	2	20	8.38 — .01
	3	6	34.97
	4	1	6.65
MG =		240.3180	F = 4
	1	11	50.36 .09
	2	22	8.45 .05
	3	2	10.68
	4	5	30.49
MG =		262.3130	F = 2
	1	12	50.50 .23
	2	23	8.12 — .27
	3	5	24.54
	4	3	16.82
MG =		285.3560	F = 4
	1	12	49.98 — .28
	2	24	8.38 — .01
	3	4	19.43
	4	4	22.19
MG =		288.3560	F = 3
	1	13	50.30 .03
	2	26	8.44 .04
	3	8	36.10
	4	1	5.15
MG =		310.4150	F = 5

Output IBM 1620 (20K). Input: Result of carbon and hydrogen analysis with errors. The assumption has been made that the compound contains nitrogen and has between 7 and 15 C atoms. From the total of 12 possibilities the first six are shown.

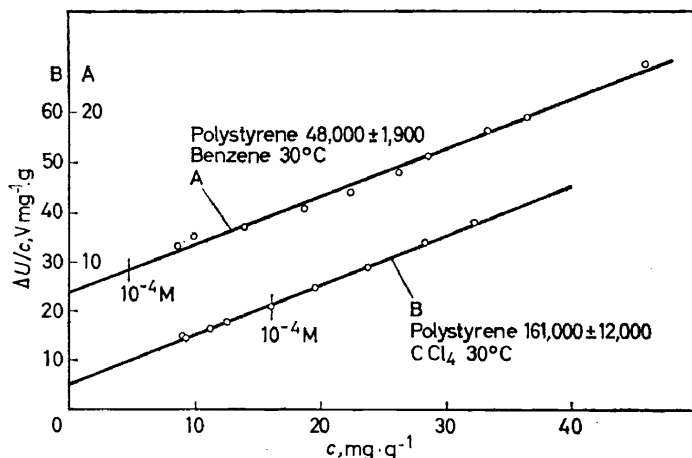


Figure 3. Determination of high number average molecular weights by vapour pressure osmometry (The molecular weights of the standards tested are 51,000 and 160,000 respectively Pressure Chemical Co., Pittsburgh))

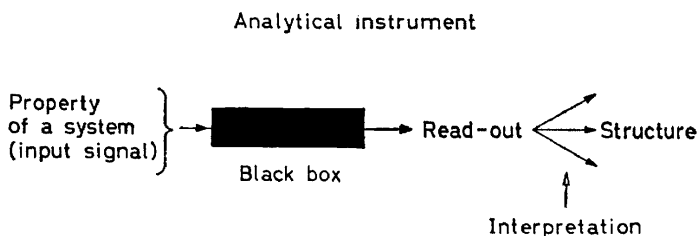


Figure 4. Trends in the application of analytical instrumentation by organic chemists

interpretation of the spectra is tremendously simplified so that the knowledge of experts is only necessary in exceptional cases. Furthermore the result of the interpretation obtained with one technique may be checked against another technique. With only a few exceptions¹⁷ it is usually possible to find the structure of an organic compound of molecular weight below about 250 without any further information if the infrared, nuclear magnetic resonance, mass and electron spectra are available^{17, 18}. One such example is given in *Figures 5 to 7* and a great number of additional exercises are available¹⁸⁻²².

The mass spectrum in *Figure 5* gives the molecular weight of 185 for the unknown compound. Since it is odd numbered the molecule contains an odd number of nitrogen atoms²³. The signals at $M-19$ and m/e 69 are an indication for a $-\text{CF}_3$ group in the molecule. From the singlet at 3.8 ppm in the n.m.r. spectrum (*Figure 6*) the group $\text{CH}_3-\text{O}-\text{CO}-$ is evident. Furthermore, the signals at 7.3 and 4.1 ppm have to be correlated with group of the type $-\text{CH}_2-\text{NH}-$. The infrared spectrum in *Figure 7* is a confirmation for the ester ($> 1700 \text{ cm}^{-1} \sim 1200 \text{ cm}^{-1}$) and $-\text{NH}-$

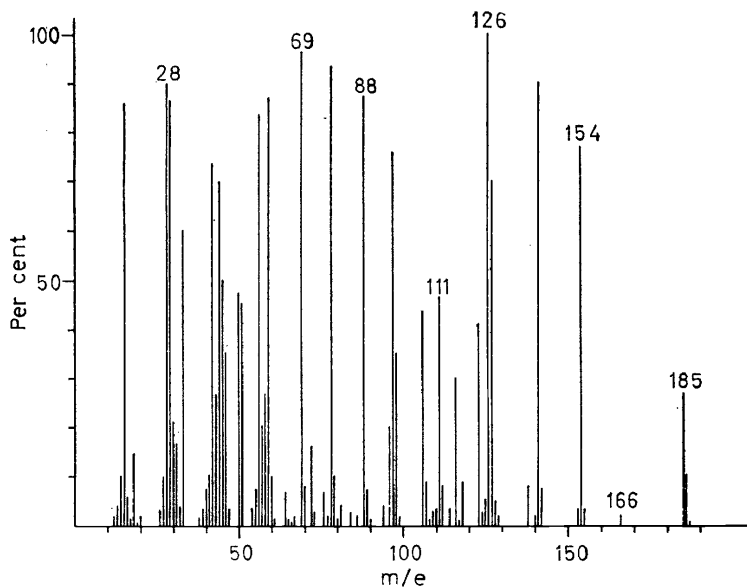


Figure 5. Mass spectrum of an unknown compound (the signal at m/e 126 is attenuated by a factor of 4)

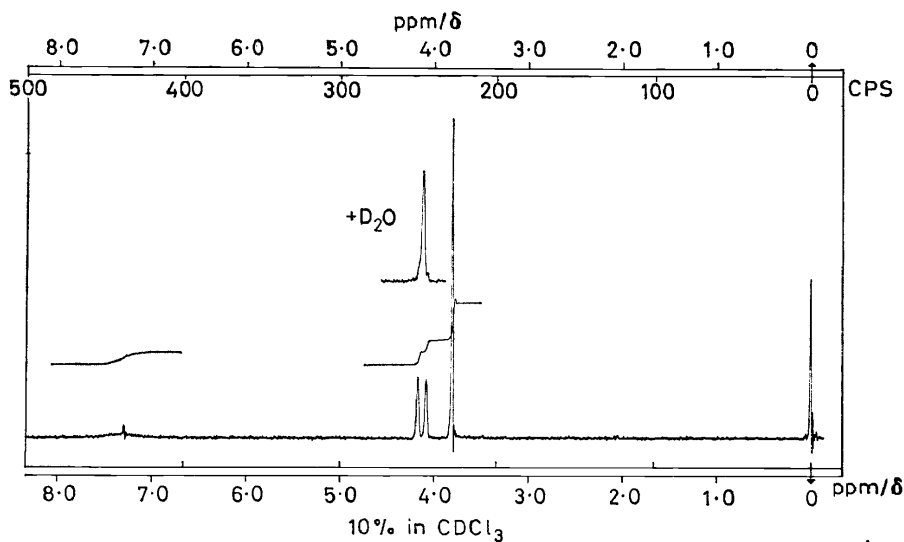
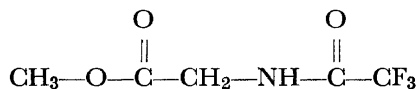


Figure 6. N.m.r. spectrum of an unknown compound

grouping ($\sim 3300 \text{ cm}^{-1}$) and indicates the presence of second carbonyl group ($> 1700 \text{ cm}^{-1}$). The different groupings give the final structure



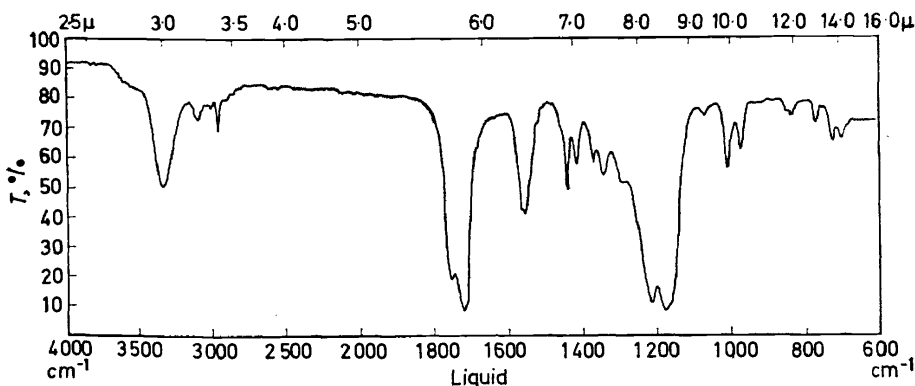
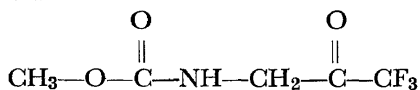


Figure 7. Infrared spectrum of an unknown compound

The alternative structure



is not in agreement with the position of the carbonyl bands in the infrared spectrum. Some information concerning the present routine application of the four spectroscopic methods mentioned is given in *Table 3*.

Table 3. Routine application of infrared, nuclear magnetic resonance, mass and electron spectroscopy in organic chemistry

<i>Method</i>	<i>Sample size (mg)</i>	<i>Application</i>	<i>Cost of instrumentation 1967 (U.S. \$)</i>
Nuclear magnetic resonance	1-20	Bond type, relative position of atoms, stereochemistry	17,000-60,000
Mass spectroscopy	0.05	Skeleton of molecule, molecular weight, elemental formula	35,000-100,000
Infrared Spectroscopy	1-5	Functional groups (skeleton of molecule)	4,000-20,000
Electron spectroscopy	0.1	Unsaturated systems	4,000-17,000

If the combined application of infrared, electron, nuclear magnetic resonance and mass spectroscopy fails to give a clear answer usually x-ray analysis or chemical methods have to be applied. Occasionally special methods may give structural information which cannot be obtained with comparable efforts by the spectroscopic methods mentioned. It has been shown for instance, that the determination of the acidity can be of help especially in the elucidation of the structure of cyclohexane carboxylic acids^{1, 17, 24}. Using about 0.7 mg of each of the two isomers of 4,4-dimethyl-decaline-9-carboxylic acid it has been possible to make a clear decision about the configuration of the rings in the two isomers²⁵.

Spectroscopic methods are usually applied after a separation procedure and it is therefore obvious to combine the two techniques. Gas chromatography and mass spectrometry have both become powerful techniques in organic chemistry. Since gas chromatography is essentially a separation technique and mass spectrometry an extremely useful method for the identification and elucidation of the structure of organic compounds, and since both techniques have high sensitivity and the requirement of a vaporized sample, a combination of the methods is indicated. The coupling of gas chromatographs with single as well as double focusing mass spectrometers has been described on different occasions (for a review see ref. 26) after the demonstration of the principle in 1957^{27, 28}. The potentialities of this instrumental combination are striking. During the recording of three gas chromatograms of a complex mixture (cigarette smoke), each giving about 150 signals, a total of 300 mass spectra were taken. These mass spectra led to the determination of the structure of 68 compounds, to the probable structure of an additional 11 compounds, to the elementary composition of additional 32 compounds and to the molecular weight of an additional 39 compounds, using literature data only²⁸. Components in quantities $\leq 0.01 \mu\text{g}$ give adequate mass spectra²⁶. While it took about half a day to record the three gas chromatograms (50 min each²⁸) with the 300 mass spectra, the reduction of the data and their interpretation took about 14 days. Part of the time was spent in reducing the data from the presentation on the recorder (see *Figure 8*) to a form suitable for interpretation by the

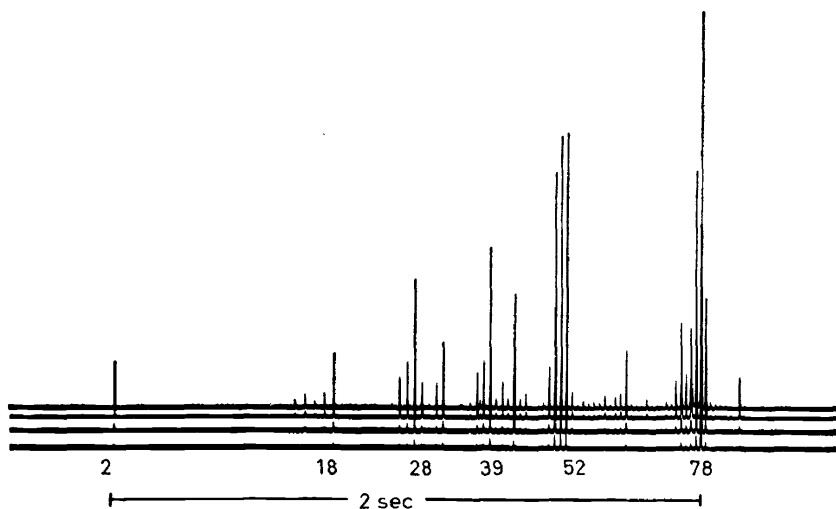


Figure 8. Mass spectrum of component mixture of cigarette smoke showing one signal in the gas-chromatogram²⁸ (Benzene, 3-methylbutanone 2)

chemist (*Figure 5*). The most obvious solution to this problem is the use of computers.

In general, computers may be used for the purpose of: (1) Reducing the data fast enough (as compared with the potentialities of the analytical instrument) to make them adequate for interpretation by the chemist.

(2) Increasing the quality of the analytical information (e.g. signal to noise ratio). (3) Information storing and retrieval, and aid in the interpretation of data.

It has been demonstrated that direct analog recording even of high resolution mass spectra on magnetic tape is possible in 10 seconds to a few minutes depending on the resolution and mass range²⁹⁻³¹. Automatic conversion of this data to digital form makes possible computer calculation of exact masses and therefore normally the determination of molecular formulae, and preparation of element maps³⁰⁻³⁴. An element map as obtained using a computer technique which automatically plots data in three dimensions³⁵ is shown in Figure 9³⁶. The mass spectrum has first been recorded on

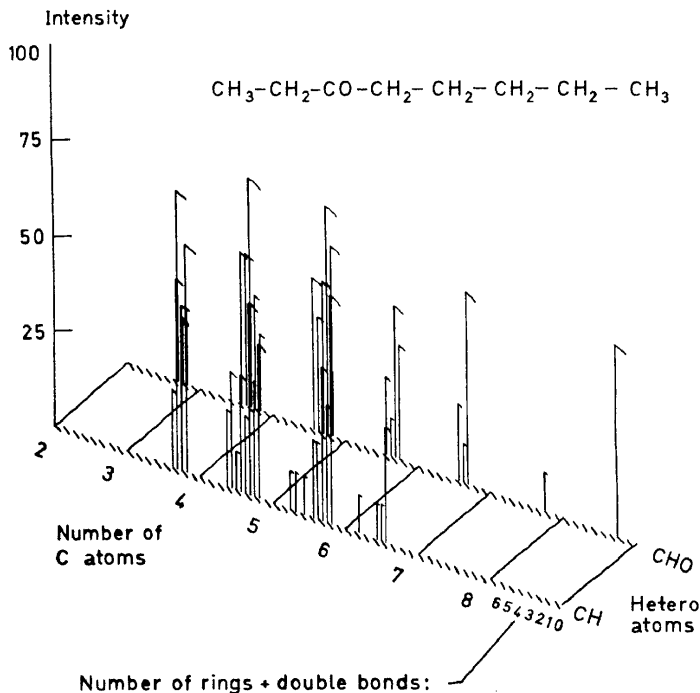


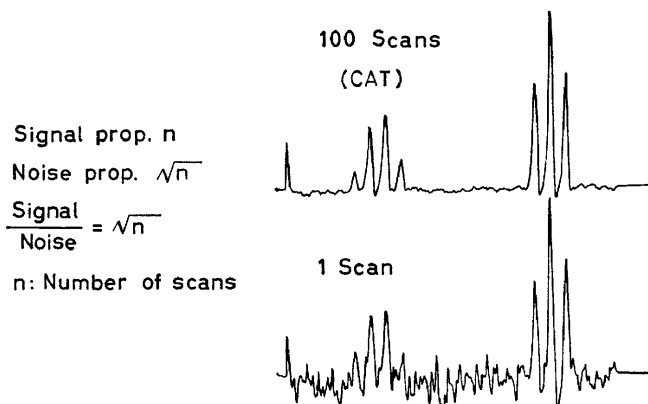
Figure 9. Element map (F. W. McLafferty and Rengachari Venkataraghavan, private communication³⁶)

a photoplate³⁶. Since the high resolution spectrum of a submicrogram sample of a high molecular weight compound may contain several hundred kinds of ions a presentation in tabular form for interpretation would have made it difficult to locate key spectral features³⁶. In the near future the most economic data reduction will probably be obtained by using a computer on line with the mass spectrometer^{37, 38} even for low resolution work.

In n.m.r. spectroscopy small on line computers are used to improve the signal to noise ratio by averaging the signals of repetitive scans as shown in Figure 10.

With such an arrangement useful n.m.r. spectra (protons) have been

taken on samples as small as $50\text{ }\mu\text{g}^{39}$ using conventional instrumentation. Standard n.m.r. spectrometers in combination with time averaging computers can be used to record n.m.r. spectra of carbon using the natural abundance (1.1%) of ^{13}C .



0.2% Ethylbenzene in CCl_4

Bottom: single 4-min. trace (varian A-60)

Figure 10. Improvement in signal to noise ratio using multiple scan [L. C. Allen, LeRoy and F. Johnson. *Analyt. Chem.* **85**, 2668 (1963)]

One such spectrum obtained in about 3.5 hours (256 scans) is shown in Figure 11. Since the probability for two adjacent ^{13}C is very small, only the coupling between ^{13}C and the protons is visible. It is obvious that ^{13}C magnetic resonance spectroscopy has a potentiality in the elucidation of the structure of organic compounds comparable to that of proton magnetic resonance spectroscopy⁴⁰.

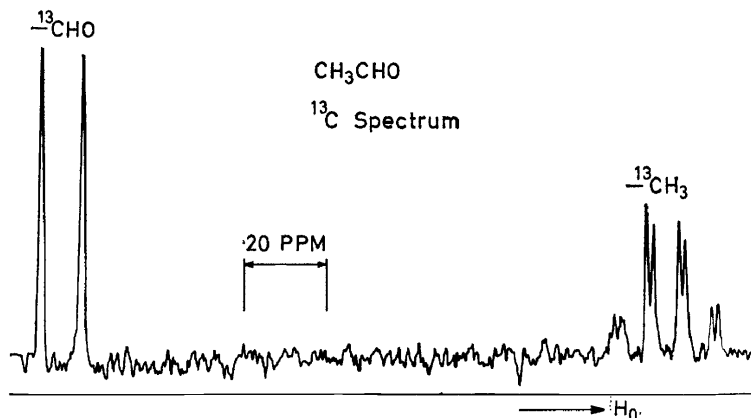


Figure 11. ^{13}C spectrum of acetaldehyde (256 scans in 3.5h, 14092 gauss) using n.m.r. spectrometer R10 (Perkin-Elmer Ltd., Beaconsfield)

Other computer methods for increasing the quality of the analytical information such as smoothing and differentiation of data by simplified least squares procedures have been described⁴¹.

There is unfortunately a certain limitation in the application of i.r., n.m.r., m.s. and u.v./v.i.s. spectroscopy in the elucidation of the structure of organic compounds since the correlation between the structure and the spectroscopic data is at present essentially empirical. It is for instance at present not possible to calculate *a priori* the chemical shift of organic compounds with an adequate precision by considering the structure (conformation and configuration) of the compound only. Estimations of the chemical shift which are based on a statistical treatment of a great number of compounds and the corresponding chemical shift data are however possible. Similar situations hold for the other spectroscopic methods mentioned. It is obvious that much data has to be stored and this must be easily retrievable. An attractive possibility is the use of a time-shared computer as has been shown in the storing retrieval and interpretation of mass spectroscopic data⁴².

Since the computer processors are extremely fast, and the input and output of data are relatively slow, a central computer can be shared among many input-output stations⁴³. The simplest such station is the Teletype or TWX machine, which can sit in the laboratory and can at any time be dialled directly into the computer⁴³. At his remote console, the user appears to "own" the largescale computer⁴³. Data can be entered as soon as it is ready, and answers return in a few minutes⁴³.

Although it is at present not economical there is no reason why the complete interpretation of the output of the different instruments could not be done exclusively by the computer. Since a 12-hour course in FORTRAN gives one the fundamentals of computer programming such a training would seem to be very desirable in the education of analytical chemists.

At present, x-ray analysis seems to be one of the most powerful methods for studying the spatial arrangement of atoms and is being used to investigate problems of crystal and molecular structure that arise in a number of different scientific disciplines (crystallography and solid state physics, metallurgy, inorganic and organic chemistry, molecular biology⁴⁴). It is more and more used to elucidate the structure of organic compounds whenever other physicochemical methods fail. Very recently a high degree of automation has been achieved. In earlier experiments, the collection of a complete set of three-dimensional data required thousands or even tens of thousands of separate measurements to be registered photographically⁴⁵. Commercial, four-circle single crystal x-ray diffractometers⁴⁵ provide a fully automatic method for collecting and recording the data associated with crystal diffraction (see *Figures 12 and 13**).

In connection with the use of antibiotics as tools for metabolic studies an ion specific behaviour of compounds such as nonactin homologues^{46, 47}, gramicidins^{48, 49} and valinomycin⁵⁰ has been observed in rat liver mitochondria. It was proposed that these compounds act via a mechanism related to cation movements, with the specific cation requirements depending

* *Figure 13* is given on the page facing p. 99

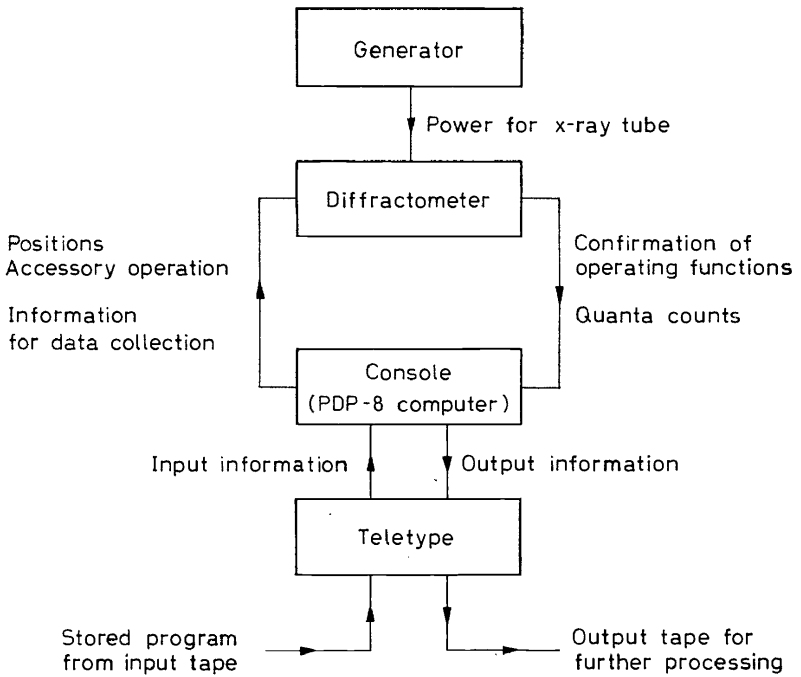


Figure 12. Schematic diagram of an automatic diffractometer system⁴⁵

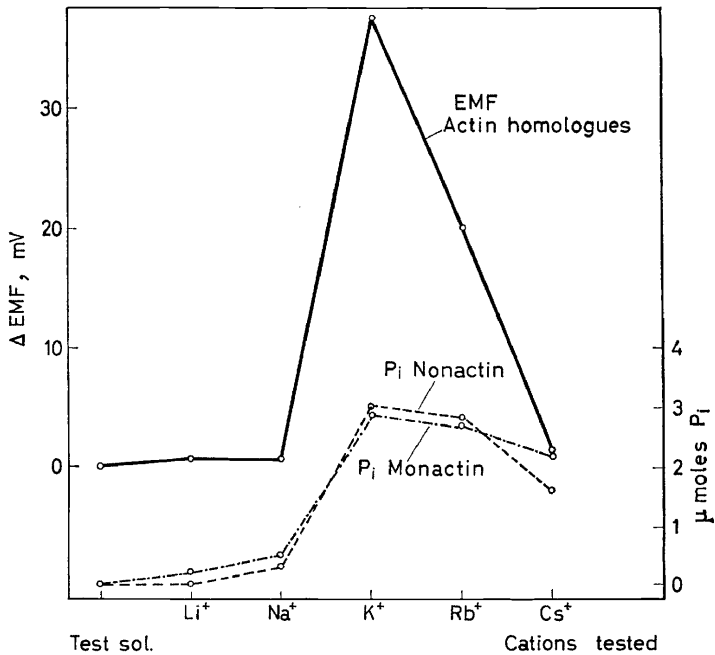


Figure 14. Comparison of the ion specific effects of nonactin homologues on the ATPase induction^{46, 47} with the e.m.f. response of electrochemical cells for monovalent cations.

in some manner on the molecular configuration of the antibiotic^{46, 47, 50}. E.m.f. measurements using membranes consisting of such antibiotics on inert supports (sintered glass) have shown an ion specificity comparable to the behaviour in liver mitochondria (*Figure 14*)^{51, 52}. It therefore seemed likely that this ion specificity was simply due to a specific complex formation between the antibiotics and the different cations⁵¹⁻⁵³. Similar thoughts have been published in the mean-time⁵⁴. Very recently crystalline 1:1 complexes between nonactin and monactin and sodium, potassium, and ammonium thiocyanate have been isolated⁵³. According to the constitution of the actin homologues, which has been determined earlier using a combination of chemical and spectroscopic methods^{55, 56}, these complexes are as shown in *Figure 15**. Using an instrument as shown in *Figures 12 and 13* the

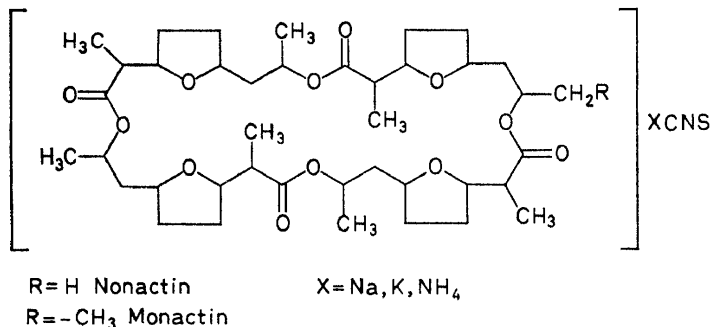


Figure 15. 1:1 complex of macroretrolides with monovalent cations

complete crystal structure of the nonactin-KCNS complex was determined in about one month. Half of that time was used for the collection of the diffraction data and the other half for the computation of the structural parameters. This means that in the relatively short space of one month the spatial arrangement of all 120 atoms in the molecule including the total geometry has been unambiguously established (see ref. 58).

One sees that these physico-chemical methods of analysis not only give information which is often impossible to obtain by purely chemical methods but does so faster by orders of magnitude.

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