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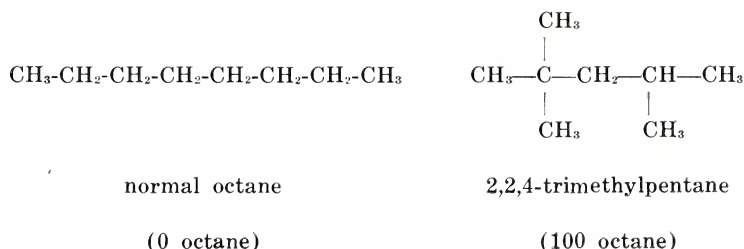
Chromatography and Spectroscopy—The New Team in Chemistry

FRANK A. GUTHRIE

Rose-Hulman Institute of Technology, Terre Haute, Indiana 47803

Chemistry has undergone a number of very significant changes since most of you last had formal course work in the subject. I would like to outline a few of these changes in an attempt to bring you somewhat more up to date in this rapidly evolving scientific discipline.

Surely you will recall the great concern which the chemist of old had for the composition of matter. When considering automotive fuels it becomes readily apparent that composition alone is not the key to efficiency, nor is it necessarily even a suitable means of differentiating between two chemical compounds. Two isomeric octanes, in spite of identical compositions, exhibit both structural differences and striking differences in performance.



The geometry of the molecule does make a difference. The arrangement of the atoms, with respect to one another, affects the shape of the molecule and in turn the geometric arrangement of the molecules in a solid influence the use to which it may profitably be put.

Perhaps the greatest challenge facing the research chemist during the past two decades has been to establish a systematic approach for the determination of structure of chemical compounds. Subsequently we will consider some of the fruits of his efforts. Normally it is essential that one has a reasonably pure compound before setting out to characterize it. Many of our older or more established separation techniques, such as recrystallization or distillation, are not adequate for the effective separation of compounds which exhibit only subtle differences in structure. Life itself often depends upon just such small structural variations which permit the body to function in a normal manner. Biochemists and pharmacologists can attest to the dramatic influence of a properly placed methoxy or hydroxy group in a wide variety of pharmaceutical products.

Fortunately, several new separation techniques have been developed in recent years which have changed our approach to chemical problems and often have resulted in solutions to problems which were not recognized to be problems a generation or two ago.

In 1941, Martin and Synge (3) published a paper on liquid-liquid partition chromatography for which they were later awarded the Nobel prize. In this paper the authors predicted the possibility of developing gas-liquid partition chromatography and emphatically expressed the potentialities of such a method. A decade later Martin and another graduate student (2) published their first paper on gas-liquid partition chromatography and thus established a powerful new method of separation. However, unlike most new developments in chemistry, gas-liquid chromatography received far greater developmental support from industry than from academic efforts. Within a decade the publication level of papers concerned with gas-liquid chromatography reached a rate of nearly 2,000 papers annually.

Gas-Liquid Chromatography

Let us now consider briefly the principle upon which the gas-liquid chromatograph is based. The column, which is the heart of the chromatograph, is usually a small diameter tube with a length of a few feet. It is packed with a finely divided solid on whose surface a thin layer of a non-volatile liquid has been deposited. A sample mixture, vaporized at a heated injection port, is carried into the column by a stream of inert carrier gas. Each component of the sample mixture tends to dissolve in the liquid layer according to its own distribution coefficient. Thus, a portion of each component enters the liquid layer and another portion remains in the vapor phase in the stream of carrier gas. However, true equilibrium is not really established because of the continual movement of the carrier gas which sweeps the sample vapors through the column to a fresh layer of liquid phase where partial dissolution again occurs. Eventually each sample component emerges from the column at its own time interval. This elution time is dependent upon the liquid phase, the temperature of the oven in which the column is housed and the flow rate of the carrier gas as well as the nature of the sample itself.

A typical gas chromatograph (Fig. 1) may cost as little as a few hundred dollars, or as much as a few thousand dollars. Relatively simple instruments usually afford the operator a variety of operating parameters which may be varied to effect a more satisfactory separation.

As little as a microliter (*i.e.*, 0.001 milliliter) of a 20 to 30 component mixture may be separated in a matter of minutes. The retention time, for a given set of operating parameters, is often useful in identification and the peak size is related to the amount of any given component. A typical gas chromatogram is shown in Figure 2.

When coupled with a computer, many gas chromatographs may be operated simultaneously to monitor the output of a petroleum re-

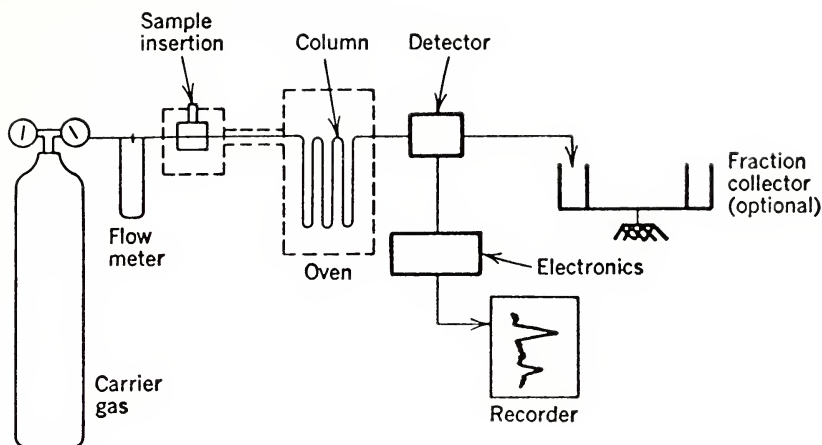


FIGURE 1. Block diagram of a simple gas chromatograph.

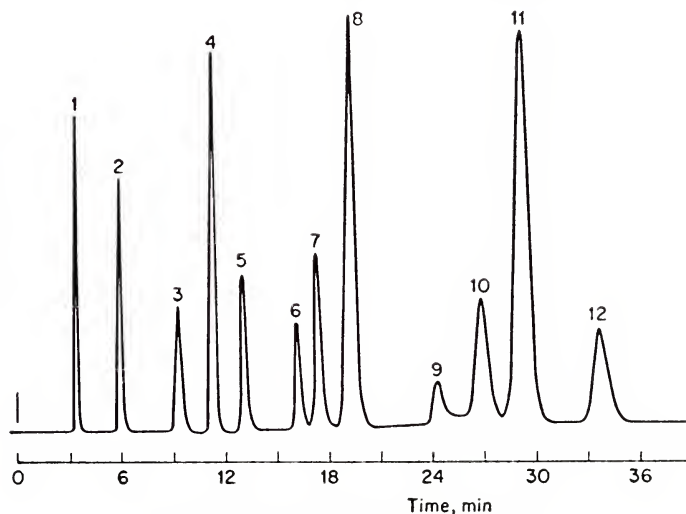


FIGURE 2. Typical gas-liquid chromatogram.

finery. Through the use of larger samples and columns it is practical to separate milliliter quantities of a desired compound with extremely high purity. In other cases, which we will consider later, it is common to pass the effluent from a gas chromatograph directly into a spectrometer for immediate structural examination.

In spite of the fact that gas chromatography is applicable to only about 15% of the organic compounds and an even smaller fraction of the inorganic compounds, because of the requirement of at least moderate volatility at temperatures up to about 500°C, this new method has quickly earned its place in the chemistry laboratory. It has been established as a powerful tool for the rapid separation

of complex mixtures and in many instances has displaced the far more cumbersome and costly mass spectrometer.

Gel Permeation Chromatography

The many variations of column chromatography, including ion exchange resins, collectively have greater overall versatility than gas chromatography. Because of relatively greater familiarity with column chromatography it seems inappropriate to discuss these methods here. However, one relatively new technique, known as gel permeation or gel filtration chromatography, does merit at least brief consideration.

Gel permeation chromatography is unique in its ability to separate giant molecules by size. Within a homologous series this effectively means separation by molecular weight. Commonly an open column is filled with beads of a carefully cross-linked polymer, or a modified dextran, which contains a microscopic grid or pore system with openings in the range of 10 to 1,000,000 Angstroms across. A solution of the sample mixture is poured into the column and subsequently eluted with a solvent. The smaller molecules find a greater number of pores into which they can permeate, whereas the larger molecules tend to pass by these openings and are eluted first. Thus, because of variation in permeation due to variation in size, a concentration gradient is established as a function of molecular size or weight and separation on this basis results.

A wide choice of gels is available, with molecular weight resolution ranges varying between very low values and values well in excess of several million molecular weight units. This permits applications such as the desalting and separation of sugars such as raffinose, maltose and glucose. The determination of molecular weight distribution in polymers and large scale production of lactose-free milk are illustrative of other applications. Molecular weight determination for proteins typically utilizes proteins of known molecular weight as "markers".

One unique feature of gel permeation chromatography is the molecular weight determination on crude samples which require no previous purification. Other methods of molecular weight determination require pure samples which often are difficult to obtain in ample quantities. In this application the molecular weight of each component is related to the volume of solvent required to elute that particular component.

Once separation has been accomplished, if the original sample requires it, the task of structure determination can begin. I would like to consider only three spectroscopic methods each in a brief introductory manner. These new methods have gained tremendous popularity during the past decade and are clearly a part of the chemistry of the future. First, however, it seems appropriate to review the basic requirements of a spectrometer.

Imagine the process of dipping a tea bag into a cup of hot water. As the color of the resulting solution attains the intensity

associated with a good flavor it is natural to remove the tea bag. This simple example of a colorimetric method of analysis contains all of the basic components of spectrometry. First, a source of energy is required. In this case it is room light or daylight, but it might have been monochromatic in the ultraviolet, infrared or another region of the electromagnetic spectrum. The sample is a second requirement. It might be a solution, such as the tea, through which light is passed. It might be a pure liquid or even a paint or fabric sample, from which light would be reflected. Transmission of X-rays through aluminum foil, used to measure and control the thickness of the foil during the rolling process, illustrates still a different type of source and sample. The third basic requirement is a detector. In the case of the tea it is the human eye. However, unlike the ear which has the ability to distinguish between various instruments in an orchestra or the tongue which is capable of tasting various flavors simultaneously, the eye is unable to disperse radiant energy into its component colors. For example, an object may appear to be green when in reality it is actually an appropriate mixture of blue and yellow. Because of the eye's inability to see many colors or energies of interest, and because of eye fatigue which all of us have experienced early on a Sunday morning, the scientist usually turns to other detectors. Commonly they include phototubes, photomultipliers, photographic film, thermocouples, Geiger-Müller detectors, etc.

Absorption of ultraviolet radiation is usually indicative of the presence of aromatic systems or conjugated olefins which are common in many organic compounds. A more informative spectral region is usually the infrared region.

Infrared Spectrometry

Because of limited time, I wish to consider only a narrow segment of infrared spectrometry for illustrative purposes. Absorptions of infrared radiation are due to vibrational and rotational interactions within the molecule. More specifically, infrared absorption is indicative of the presence of certain bond types or certain functional groups within the molecule and thus has come to be known as the "finger print" region of the spectrum. Absorption between 3100-cm^{-1} and 3000-cm^{-1} is typical of an aromatic compound whereas the region between 3000-cm^{-1} and 2800-cm^{-1} is indicative of the presence of aliphatic structure such as the methyl or methylene group. Both of these structural types absorb energy in other regions too, but by other modes. Here, for example, the bond between the carbon atom and the hydrogen atom may be thought of as a spring. It may stretch or shrink; in other regions it may bend or twist as it absorbs energy.

One can compare absorption of infrared radiation by a particular bond, at a particular wavelength or frequency, with human behavior. A given individual may absorb musical energy in the form of jazz by tapping his foot whereas his response to another type of music may result in hand clapping, or perhaps no response at all.

Alcohols and acids tend to form hydrogen bonds, which to some extent depend upon concentration, resulting in broad absorption bands often extending from 3600-^{cm} to 3200-^{cm}. Other unique features, such as the iso-propyl or tertiary butyl group, alter the normal carbon-hydrogen bending vibration with a resulting split in the absorption peak. The nature and extent of aromatic substitutions is usually evident in the 900-^{cm} to 600-^{cm} region. Many other structural and/or environmental features also leave their tell-tale marks.

Correlation tables are common and a number of excellent books on infrared interpretation have appeared on the market in recent years and are recommended for the reader anxious to extend this brief presentation. Scientific studies in many disciplines should utilize infrared spectrometry more extensively. With the advent of the \$3,000 instrument, infrared is rapidly becoming common place in many sophomore organic chemistry laboratories for checks of product purity and identification of unknown compounds. At the other end of the financial spectrum is a recently introduced infrared spectrometer with a built-in computer. It is capable of collecting infrared spectral data following only a half-second examination of the sample. Priced in excess of \$65,000, this instrument has the capability of examining each sample component as it emerges from a gas chromatographic column and reproducing its spectrum on request.

Mass Spectrometers

Mass spectroscopy has had a long and interesting history of development. However, it has been only in the past decade that mass spectrometers have been used to any appreciable extent in solving structural problems. Relatively simple mass spectrometers are usually too expensive for use in undergraduate education. Units with higher resolution and greater flexibility often cost well in excess of \$100,000. Obviously educational applications are limited, at least at the present time. Nonetheless, mass spectrometry is a powerful tool which deserves consideration in solving modern chemical problems.

As with other forms of spectroscopy, the basic requirements of a source, a sample and a detector remain in the forefront. Unlike optical spectroscopy which uses a prism or diffraction grating to separate component colors or energies present, the mass spectrometer uses electrostatic or magnetic fields to separate particles of different energies, or mass to charge ratios.

The source consists of an electron beam which bombards the sample to generate a positive ion by dislodging an electron from the sample. These positive ions are accelerated by electrostatic fields or accelerator plates which propell them into a magnetic field. Here the lighter particles are deflected to a greater extent than the heavier ones. Variations in accelerating voltage or magnetic field strength permit scanning by focusing first one mass then another on the detector.

Of the several capabilities of the mass spectrometer, the scientific community probably first recognizes the possibility of identification of

the molecular weight of the sample. However, this information alone is often of relatively little value unless a high resolution instrument has been used. In such a case, where the molecular weight is known to three or four decimal places, it is possible to actually identify how many atoms of each kind are present and thus obtain the molecular formula of the compound. As we noted earlier, in the case of the octane isomers, composition alone is often not sufficient to solve a problem.

Isotope effects often make it easier to determine which of several elements is present in a molecule. For example, chlorine occurs in nature as approximately 25% in the form of the mass 37 isotope and about 75% as the mass 35 isotope, with an average atomic weight of nearly 35.5. However, the mass spectrometer does not report averages. Instead, it reports the two chlorine isotopes as peaks two mass units apart, with relative intensities of approximately 3 to 1. Such spacing and intensity ratios may appear at a number of points throughout the spectrum, depending upon how the molecule is fragmented at the source. Similar observations may be made for bromine, due to the naturally occurring isotopes, Br^{79} and Br^{81} , which have nearly equal populations. A number of other elements are readily identified by this type of elemental analysis.

Increased ionization voltage often results in cleavage or fragmentation of the molecule at several points, rather than merely the loss of an electron. Fragmentation patterns typically display certain common features because of easier rupture of certain geometric structures. For example, mass peaks of 15 are usually indicative of the methyl group whereas additional multiples of 14, such as 29, 43, 57, etc., represent one methyl and one or more methylene groups and suggest an aliphatic structure. A mass 77 peak represents the phenyl group and is indicative of benzene or one of its derivatives, whereas a mass of 91 commonly signifies the presence of a benzyl group.

Sometimes the difference between two mass values is equally instructive in establishing the initial structure since the difference represents atoms which were lost. This, of course, is indicative of structural weaknesses and often is helpful in placing certain functional groups in an appropriate position within the molecule. For example, branched chains tend to fragment more readily than do straight chains and cleavage occurs more readily at the carbon atom adjacent to a hetero-atom than elsewhere in the molecule. This latter observation is useful in dealing with alcohols and amines.

Nuclear Magnetic Resonance Spectrometry

Although NMR dates back to the mid-1940's, it has only been a really practical structural tool during the past decade. The recent advent of \$25,000 spectrometers and a number of grants by various federal agencies have been very largely responsible for the introduction of NMR into the undergraduate chemistry curriculum. Of the several atoms capable of examination by NMR, only the proton has really

widespread interest at the present time. This is understandable when one considers the tremendous number of chemical compounds which contain one or more hydrogen atoms. Briefly, a sample can absorb electromagnetic radiation in the radio frequency region when examined under appropriate conditions. Thus NMR is another form of spectroscopy not at all unlike infrared or ultraviolet spectroscopy.

If one considers the proton to be a small bar magnet it is reasonable to assume that it might be aligned in either of two directions as is suggested by the two equal but opposite spin quantum numbers. Therefore, as a sample is placed in a uniform magnetic field, the protons have the option of being aligned either with or against the field. The energy necessary to align all of the protons in the same direction is dependent upon the strength of the magnetic field and the environment in which the protons reside.

Our friends in physics noted that protons did not show consistent behavior, which they had anticipated, but instead absorbed energy at a wide variety of frequencies. This disturbing effect was given the presumably disrespectful title of the "chemical shift". However, the chemist is aware of the influence of the environment and recognizes that it is responsible for these variations. In turn, this leads to the assignment of structural features when properly interpreted.

Upon examining a low resolution spectrum of ethanol,



one observes three absorption peaks, with sizes in the ratio of 3 to 2 to 1 which is in direct proportion to the number of methyl protons, methylene protons and hydroxyl protons. Each type of proton is located in a somewhat different chemical environment which gives rise to somewhat different chemical shifts and thereby accounts for the three separate peaks rather than a single peak.

When examined with a high resolution instrument we discover that adjacent groups exert an influence on each other which commonly results in the splitting of a peak into several smaller peaks. The accumulative area under these small peaks remains the same and is still indicative of the total number of similar protons. This splitting is due to the fact that each proton, acting as a small bar magnet, may either add to or subtract from the total magnetic field depending upon the alignment of the proton. The adjacent protons then find themselves in several slightly different magnetic fields and react to each independently. This is illustrated in Figure 3, in which interaction between the methyl and methylene group is depicted.

The two hydrogen atoms in the methylene group may be aligned so that they oppose the magnetic field or so that they add to the magnetic field in three different ways. Likewise, the three hydrogen atoms in the methyl group may be aligned in four different ways. Statistically, the intermediate alignments are most frequent and give rise to larger central peaks and smaller extreme peaks. The various

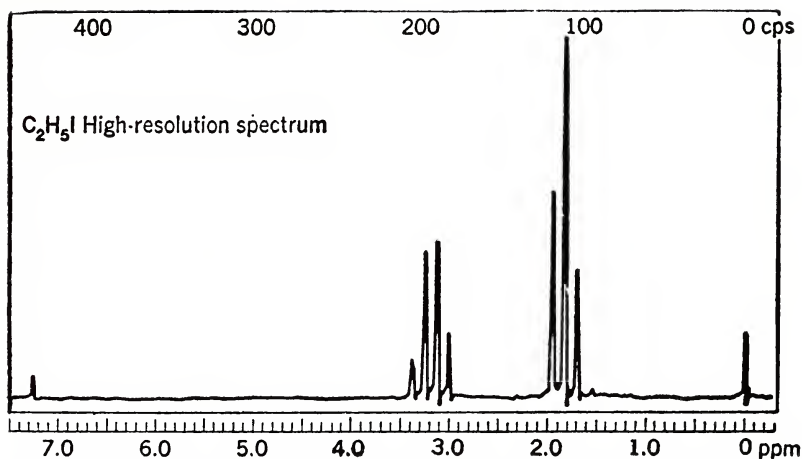


FIGURE 3. NMR spectrum of ethyl iodide dissolved in deuteriochloroform (CDCl_3).

alignments are depicted below, where the letters A, B and C represent the protons in a particular group.

Methylene Protons, $-\text{CH}_2-$

$2 \uparrow 0 \downarrow \{A \uparrow B \uparrow\} [1]$

$1 \uparrow 1 \downarrow \left\{ \begin{array}{cc} A \uparrow & B \downarrow \\ A \downarrow & B \uparrow \end{array} \right\} [2]$

$0 \uparrow 2 \downarrow \{A \downarrow B \downarrow\} [1]$

Methyl Protons, $-\text{CH}_3$

$3 \uparrow 0 \downarrow \{A \uparrow B \uparrow C \uparrow\} [1]$

$2 \uparrow 1 \downarrow \left\{ \begin{array}{ccc} A \uparrow & B \uparrow & C \downarrow \\ A \uparrow & B \downarrow & C \uparrow \\ A \downarrow & B \uparrow & C \uparrow \end{array} \right\} [3]$

$1 \uparrow 2 \downarrow \left\{ \begin{array}{ccc} A \uparrow & B \downarrow & C \downarrow \\ A \downarrow & B \uparrow & C \downarrow \\ A \downarrow & B \downarrow & C \uparrow \end{array} \right\} [3]$

$0 \uparrow 3 \downarrow \{A \downarrow B \downarrow C \downarrow\} [1]$

The methyl protons, when placed in the three slightly different magnetic fields created by the adjacent methylene protons, appear as three peaks. Likewise, the methylene protons, when placed in the four slightly different magnetic fields created by the adjacent methyl protons, appear as four peaks. This combination clearly signifies the presence of the ethyl group, $\text{CH}_3\text{-CH}_2-$, in the molecule. Coupling of this sort is also observed in a number of other groupings of adjacent proton-containing carbon atoms.

Most NMR instruments now have an integrator incorporated into their design. It may be used to draw a stepwise curve across the spectrum in such a manner as to indicate, by the size of each step, the relative number of protons associated with each absorption peak. This feature greatly simplifies the interpretation of many NMR

spectra, but sometimes requires additional evidence so that the relative number of protons of each type might be converted into the absolute number contained by the particular molecule. This is a particularly important point when dealing with molecules which are symmetrical.

Spectroscopic Identification of Insect Sex Attractants

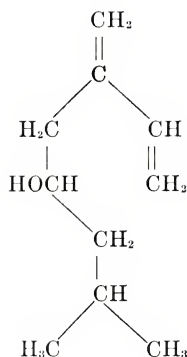
A recent report (1) indicated that the United States Department of Agriculture is attempting to banish the fire ant from our southeastern states. Its program calls for discharging 450 million pounds of material containing over 1.3 million pounds of Mirex, a powerful chlorinated hydrocarbon, on 150 million acres of land in nine states. Conservationists are seeking to enjoin this ambitious 12-year project since little is known about the impact of Mirex. DDT which once was considered to be the ultimate pesticide has been deemed an insidious killer and recently had severe restrictions placed on its use. Mirex has been shown to have undesirable characteristics which may also result in its ban.

The work of Silverstein (4) and other chemists has now opened the door to a potential new solution to pesticide problems. Their efforts combine the capabilities of chromatography, spectroscopy and entomology, to bring practicality to the new tools of chemistry which I have just described.

Silverstein notes that each year this country loses the equivalent of 5 billion board feet of timber to the bark beetle. This is six times the mortality due to fire, and thus far there has been no really effective method of control. Bark beetles apparently attack in two phases. Initially a few beetles construct nuptial chambers in a tree, and in the process expel frass—a mixture of fecal pellets and wood fragments. The fecal pellets contain an attractant that triggers the secondary invasion. In the case of *Ips confusus* the attractant released by the male soon results in three females mating with each male.

The collection of about 4.5 kg of frass, which represents the output of about 20,000 unmated male beetles, produced three terpene alcohols, which in various combinations served as the sex attractant for the female beetle. Following solvent extraction to remove these chemicals from the wood, fractionation was accomplished by the use of gas-liquid chromatography. Subsequent tests on each compound, and on various mixtures of compounds contained in the frass, resulted in the identification of substances which attract the female.

One of the active compounds was determined to have a molecular weight of 154, and a composition of $C_{10}H_{18}O$, by mass spectroscopy. The infrared spectrum showed a broad peak at 3380-cm^{-1} , indicative of an alcohol. A vinyl group and an iso-propyl group were also indicated by the infrared spectrum. The mass spectrum and NMR spectrum confirmed the iso-propyl group. Additional spectroscopic evidence and measurement of optical rotation were used to establish the structure as (—)-2-methyl-6-methylene-7-octen-4-ol, represented graphically as:



The other two components of the *Ips confusus* attractant were *cis*-verbenol and a compound similar to the one just described, but with a double bond in the 2-position.

Other species studied by Silverstein include the western pine beetle and the black carpet beetle.

It should be apparent to the imaginative reader that isolation, evaluation, identification and subsequent synthesis of a sex attractant has outstanding prospects for the elimination of many destructive insect species. Simultaneously, the objections to past and present practices in the use of pesticides can be largely overcome.

Although other fascinating and practical applications of chromatography and spectroscopy could be cited, it is my hope that this brief review will suffice to convince the reader that a new team is at work in the ever evolving field of modern chemistry.

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