are of particular significance. Polyols such as glycols or glycerol are reacted with natural oils containing carboxyl functions to obtain the partial ester of the polyol and the oil. Then a dibasic acid such as adipic acid or phthalic acid is added to complete the polyesterification. About 900 million lb. of alkyds were consumed in the paint industry in 1974.

Another ester, glycerol trinitrate, is used in explosives and as a heart stimulant. Rosin esters of glycerol are used in ester gums as varnish components. Food grade glycerol fatty esters may be produced either by alcoholysis or by direct esterification. Product monoglyceride contents vary according to manufacturing procedure and end use. For example, glycerol monostearate is marketed at several price levels depending on purity and end use, the three main products being food grade (e.g., Drewmulse), cosmetic grade, and technical grade. The food grade, of premium quality, is used for shortening and margarine, the cosmetic grade for creams and lotions.

Many special triglyceride products are required of different fatty acid homolog distribution than those of parent or hydrogenated fats and oils. These are prepared by splitting the fats or hydrogenated fats, fractionating the fatty acids, upgrading the glycerol, and reesterifying the desired fractionated acids with glycerol. One example is lauric triglyceride from coconut oil, suitable for use as a cocobutter substitute constituent in confectionery coating fats.

Glycerol ethers, i.e., polyglycerols, have many of the properties of glycerol and offer greater flexibility and functionability than glycerol itself. Polyglycerol esters' derivatives, (e.g., decaglycerol decaoleate, tetraglycerol cocoate) are useful in emulsifiers, plasticizers and especially in the bakery, food and industrial applications (e.g., the Caprols, the Drewpols).

The mono, di and triacetates of glycerol, called acetins are useful industrial chemicals. Triacetin is used as a cellulose plasticizer in cigarette filters, and as perfume solvent.

Glycerine is also used as one of the fundamental building blocks in polyethers for urethane polymers. It is the initiator, to which propylene oxide alone or with ethylene oxide is added to produce trifunctional polymers, which on reaction with diisocyanates produce flexible urethane foams.

Glycerol itself is widely used as a humectant in the soaps and cosmetics industry and is one of the feedstocks for producing emulsifiers and detergents in the surfactants industry. For example, sodium glycerol ether sulfonate is a patented component of one of Procter and Gamble's detergents. The sulfates and phosphates of monoglycerides are useful surfactants with good foaming and cleaning properties. Purified monoglycerides like the Myverols serve as emulsifiers in creams and lotions. The fatty acid moiety may be stearic, lauric, ricinoleic or oleic acid. Glycerol monocaprylate or caprate (Neobee oils) are emollient constituents in oils and creams.

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Recent Advances in the Analytical Chemistry of Fatty Acids and Derivatives

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ABSTRACT

Recent advances in the analysis of industrial fatty acids and their derivatives almost always involve complex instrumentation. One of the most important developments in the analysis of fatty acids and their derivatives was the application of gas chromatography (GC). The result has been so effective that the time-consuming fractional distillation and detailed analysis of fractions previously employed are rarely used. Even though the so-called GLC technique has now been applied for over twenty years, new advances continue to be made in this area. Perhaps the most potentially valuable new development is the coupling of GLC with mass spectrometry (GLC-MS). Two newer chromatographic methods which have great potential in the field of fatty material analysis are thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The former is applicable to difficult separations; e.g., separation of broad lipid classes, in microquantities. The latter has already been applied with some success to the separation of individual component triglycerides of fats and oils and fatty acids using reverse phase HPLC. Instrumentation techniques include supportive methods which are frequently used in conjunction with other methods. Among these, the techniques of nuclear magnetic resonance (NMR) and infrared absorption (IR) are the most prominent. ^{13}C NMR is useful in defining fine structure of fatty acids, particularly with respect to branching. X-ray diffraction is used to study polymorphism in fatty acids.

INTRODUCTION

Recent advances in the analysis of fatty acids and their derivatives almost always involve use of complex instrumentation. One of the most important instrumental developments was the application of gas chromatography to the analysis of fatty acids. Historically, fatty acids were the

first compounds to be separated by gas liquid chromatography (1). This occurred over a quarter of a century ago. However, important developments continue to be made in this field, particularly with the recent advent of the powerful gas chromatography-mass spectrometry (GC-MS) combination.

Two more recent developments in the field of chromatographic analysis are high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). This latter method is the one exception to the recent trends toward ever more increasing use of expensive instrumentation. It is possible to do quite remarkable TLC separations using only very simple inexpensive equipment that can be put together for a few dollars.

Many other instrumentation methods are used in the analysis of fatty acids and derivatives. These include infrared spectrophotometry (IR) Nuclear Magnetic Resonance (NMR) and others. In this paper, the various subjects are briefly reviewed. The most significant recent advances in the various instrumental techniques are treated at length.

GAS CHROMATOGRAPHY

Gas liquid chromatography has certainly been one of the important advances in the analysis of fatty acids and their derivatives. James and Martin separated volatile fatty acids in their first gas liquid chromatography experiments. A short time later they made a similar separation on volatile amines up to heptylamine (2). One can easily see that the concepts involved are not new. However, there are perhaps thousands of papers describing subsequent gas chromatography of fatty acids and their derivatives.

It was quickly found that the fatty acids were best separated as their methyl esters (3). There has been some gas chromatographic work on separating unesterified acids (4), but this work will not be discussed in this lecture. One of the most difficult and important problems in the early gas chromatography research was the separation of the saturated acids from the unsaturated ones. This problem was eventually resolved by using very polar columns (4). Examples of these were the polyester columns. Probably the best known liquid phase capable of separating the saturated and unsaturated esters and still one very popular is the diethylene glycol succinate polyester. With this type of column, not only is chain length separation achieved, but also the acids are separated on the basis of the number of double bonds present. The order of separation in a given chain length is saturate, monoene, diene, triene etc., using the polyester columns. Gas chromatography quickly replaced fractional distillation followed by detailed analysis of the fractions as an analytical tool for fatty acids. GC became an enormously powerful analytical tool in many industrial applications. Probably even more important was its application to the solution of biomedical problems as to the role of lipids in the body functions and disease. For the first time it was possible to perform complex fatty acid analyses of minute biological samples. Though all this is not new, many useful chromatographic developments continue to be made. A number of the most important recent developments will be discussed in the following section.

CIS AND TRANS SEPARATIONS

The rapid separation of the cis and trans isomers of fatty acids using packed columns has long been an intriguing problem. It can be done with capillary columns, but this is a time-consuming analysis. The introduction of the cyanopropyl silicone liquid phases was a great advance in the separation of the geometrical isomers of fatty acids (5,6) and also of some of the derivatives (7). These highly polar liquid phases are variously known as Silar 5C, Silar 10C, Silar 9CP, SP 2340 and OV-275. Sometimes the name Apolar instead of Silar is used. In general, columns 3-6 meters long packed with 12-15% liquid phase on Chromosorb are used for cis- and trans-separations. The trans-isomer will emerge before the cis. For example, elaidic acid emerges before oleic acid. It can easily be seen that when the samples contain polyunsaturated compounds, the chromatograms can get quite complex. These cyanosilicone liquids are more heat stable than polyesters, and columns can be run at temperatures over 250 C for short periods. This cannot be done with polyester columns since the liquid phase would quickly bleed off.

The cis- and trans-isomers of unsaturated amines and diamines can be separated in a manner similar to the fatty acids (7). However, the amines must first be converted to their trifluoroacetamides. If the free amine is placed on the cyano column, their basicity destroys the column's ability to do the geometric isomer separation. Some type of reaction occurs that must break down the cyanopropyl groups.

GLASS CAPILLARY COLUMNS

The highest number of theoretical plates is obtained with capillary or open tubular columns. Glass capillary columns, though inert, are difficult to coat. Recently developed techniques have made it possible to coat open tubular columns with polar liquid phase such as Silar 10C or OV 275 (8). These treatments usually involve etching, silanizing, or otherwise rendering the glass surface inert. Columns 50 meters long can have 150,000 theoretical plates. It is possible to separate positional and geometrical isomers and branched acids. This high resolution makes possible separations and gives fatty acid information on natural lipids never possible before (9) except with extremely laborious and time-consuming laboratory operations.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

The increased resolution obtained with better gas chromatographic methods also causes a dilemma for the lipid chemist. The advanced chromatographer begins to see small peaks that were not seen before. The identity of these new peaks becomes a tremendously difficult problem that is not readily solved by old approaches, such as collecting samples for characterization. One solution was the coupling of gas chromatography with mass spectrometry (GC-MS) (10). This combination provides a sensitive, convenient tool for the analysis of fatty acids and their derivatives. Good mass spectra can be obtained on nanogram amounts of sample. As peaks emerge from the chromatograph, complete mass spectra can be obtained in less than a second. This means that hundreds of mass spectra can be made in a GC-MS analysis of a complex mixture. You not only get the usual GC data, but also a tremendous amount of structural information. You can use electron impact (EI) for structural detail and chemical ionization (CI) for molecular weight data. Obviously technical details cannot be covered here concerning interfacing, columns and other GC-MS parameters. Let it suffice to say the whole bit is quite complicated and very expensive. However, you must remember that to get the same information by the old conventional classical methods could possibly take years of laboratory work for several people.

GAS CHROMATOGRAPHY-COMPUTER DATA PROCESSING

When you couple a gas chromatograph to a mass spectrometer, an enormous amount of data suddenly appears. In a typical GC-MS analysis of a natural fatty acid mixture of 12 peaks, about 36 spectra are usually obtained. It takes

ca. 3 hr to manually work up the data from one mass spectra. Masses are assigned, intensities of peaks determined and normalized. It must be determined if peaks are overlapping. It does not take much imagination to realize one would need 100 hr to analyze this one chromatogram. What if the chromatogram has 50 or 100 peaks? This brings us to our last gas chromatographic subject, the application of data processors or computers to handle the large amount of GC-MS data. As the GC-MS analysis is being made, it normally is being stored on tape or floppy discs. This data can then be worked over by a computer program at your leisure. The kind of information that can be obtained from a typical GC-MS analysis, using a computer program is: (a) mass spectra of all peaks; (b) breakdown plus molecular ion in CI mode; (c) scan for selective ions; (d) quantitation of peaks; (e) intensities of ions within peaks; (f) detection of two or more compounds in one GC peak; (g) comparison of reference library spectra with unknowns to get best fit for identification; (h) recreation of structures and empirical formulas.

Some libraries that are available for scanning mass spectra data are NIH/EPA with spectra for 23,000 compounds; the ISC in Michigan with spectra for 25,576 compounds; and Cornell University with 41,424 spectra.

As one can see, gas chromatography has come a long way since James and Martin first separated volatile fatty acids using a titrimetric detector.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Long chain fatty acids and their nitrogen derivatives do not readily lend themselves to analysis using commercial HPLC instruments. One reason is that the commercially available detectors are not very effective without derivatizing these compounds. The refractive index (RI) detector is somewhat insensitive. The long chain fatty acids, particularly the saturated ones, do not show strong ultraviolet absorption for detection with UV detectors. Attempts to overcome this problem, using flame detectors and other approaches, have not been too successful. In any event, the most commonly available sensitive commercial detectors are the UV detectors. The fact that gas chromatography has been such an outstanding success for the analysis of fatty acids and their derivatives has not made HPLC a compelling area of research. However, this attitude is now changing.

There have been several main thrusts of research for the HPLC of the fatty chemicals. One is the development of strong UV absorbing derivatives (11). These include phenacyl, benzyl, p-bromophenacyl 2-naphthacyl, p-nitrobenzyl esters of the fatty acids, methoxyanilides of fatty acid, and 3,5-dinitrobenzoyl derivatives of the amines. Another area of interest is column research. Chain length and isomers can be separated on reverse phase columns (12). Gel permeation columns also give interesting separations based on size and structure of the fatty compound. However, probably one of the most useful separations of the fatty acids and related derivatives is the ability of HPLC to separate compounds on the basis of functional group instead of chain length. This type of separation can easily be accomplished on silica gel columns. An example of this type of analysis is to determine small amounts of fatty acids or other functional group compounds in the presence of high molecular weight materials of low volatility. Essentially one can get a HPLC fingerprint of a complex mixture. The compounds of interest can also be isolated by preparative chromatography for further characterization. This technique can be a powerful and useful analytical tool.

THIN LAYER CHROMATOGRAPHY (TLC)

Thin layer chromatography is an important and convenient technique for the analysis of fatty acids and fatty derivatives. It is a marvelously simple yet powerful and inexpensive tool for the fatty compounds. Essentially TLC employs a glass plate coated with an absorbent layer (usually silica gel). A small sample of from 10-100 micrograms is spotted near the bottom part of the plate. The plate is then placed in a closed vessel or developing tank. The developing tank can be a simple jar of some type. A developing solvent is placed in the jar, and the solvent rises up the plate by capillary action. As the solvent rises, the components in the sample will also usually move. The distance the components rise depends on solubility, polarity, functional groups, and other factors. The plate is removed when the solvent reaches the top. It can then be sprayed with visualizing reagents or charred with sulfuric acid and heat. It is usually quite easy to find solvent systems that can give you the separations you require.

In our laboratory, we often use microscope slides dipped in a chloroform slurry of silica gel. The developing chamber is an 8 oz. screw cap jar. The developing time with most solvent systems is ca. 5-10 min. The plate is removed and sprayed with 50% sulfuric acid. It is then placed on a hot plate at low heat. The separated compounds will then char and show up as black spots. The system works extremely well with fatty compounds because of their low volatility and ease of charring. Compounds of low volatility often will evaporate with the developing solvent. TLC is a beautiful system for determining the purity of compounds. It can give information in five minutes that cannot be obtained with wet analysis or many expensive instruments. Several typical useful solvent systems are: fatty esters and fatty acids – petroleum ether/ethyl ether/acetic acid, 90:10:1, benzene/methanol 80:20; fatty amines and diamines – chloroform/methanol/ammonia 95:5:1, benzene/methanol/ammonia 70:30:1.

The least satisfactory part of TLC has been the difficulty of quantitating the spots. The uses of densitometry and reflectance have been reported. The problem is reproducibility, since spot intensities are not always proportional to carbon content. Extraction of the spots for weighing or for use with other analytical techniques is not very convenient. TLC is a simple, yet powerful analytical technique that only costs about a dollar or so to get involved with. Such a situation has not gone unnoticed by the instrument makers. How can they make a $10,000 instrument to do this? The chromatogram itself is difficult to improve on. However, any instrumental emphasis would be best made in the area of quantitation. An instrumental technique has been developed that appears most interesting in that it can be applied to the TLC analysis of fatty acids and other fatty compounds (13).

This instrument, called the Iatroscan (Iatron Laboratories, Inc., Tokyo, Japan), is used in conjunction with a TLC rod called the Chromarod. This is a quartz rod that is coated with a sintered silica gel or alumina coating. A 0.1 to 10 μg sample is spotted on the rod, and a chromatogram is developed in the usual manner. The rod is then passed through the flame ionization detector (FID) of the analyzer. The organic components pyrolyze, and a signal is generated in much the same manner as with an FID detector in gas chromatography. Quite acceptable chromatograms are obtained. The quantitative results obtained are not as good as those obtained with a gas chromatograph. However, they appear to be as good as results obtained with much HPLC work being performed. With more development, this instrument undoubtedly will be a useful tool to complement both GC and HPLC in fatty acid and fatty derivative analysis.

INFRARED SPECTROSCOPY (IR)

Infrared spectroscopy is one of the most useful tools for the identification and characterization of organic compounds. Like most analytical instrumentation, the greatest

advances in infrared spectroscopy have been in the refinement of the instrument. The IR instruments have been combined with microprocessors for ease of operation. The use of computers to search files, to interpret spectra, and to generate structures from IR data is certainly a great advance in instrumental analysis. The computer can also be used to enhance spectra obtained from very small samples.

Of course all of these instrumental advances can be applied to all organic chemicals and not just fatty acids and their derivatives. One of the most common uses of IR spectroscopy in the analysis of fats and fatty acids is the determination of trans-unsaturation (AOCS tentative method Cd 14-61). Recently, an interesting procedure for trans-unsaturation using IR Attenuated Total Reflectance (ATR) Spectrophotometry was described (14). The samples do not have to be weighed or diluted. A special ATR cell is used to make readings at 10.3 um. The system can be used to monitor a continuous flow system. The presence of catalyst does not affect the readings. Results obtained using this IR probe compared well with the AOCS method. This development is very interesting in that the prototype ATR probe described in this publication worked well in the initial experimental work. However, the later commercial exploitation of the idea failed because the probes could not be produced to give reproducible results. Nevertheless, the idea is certainly an interesting one and could be an important advance in IR spectrophotometry.

NUCLEAR MAGNETIC RESONANCE (NMR)

Nuclear magnetic resonance spectroscopy is another powerful tool that can be utilized for the elucidation of the structure of long chain chemicals. It can be used to complement gas chromatography and infrared spectrophotometry to develop a wealth of information on an individual or mixture of compounds. Average chain length, primary, secondary, tertiary amine using derivatization techniques can be determined.

Quaternary ammonium compounds (15) can be determined in the presence of amines. Ethoxylated and propoxylated chains can be analyzed. Even iodine value and molecular weights can be estimated on fatty compounds (16). Different types of esters such as methyl, ethyl, butyl and isopropyl stearates can be distinguished from each other in the same sample.

Fourier transform NMR has helped increase the sensitivity of the technique. ^{13}C NMR with Fourier transform has tremendously advanced the field of NMR spectroscopy. For example, it has been used to determine the distribution of fatty acids in a triglyceride (17). The techniques of ^{13}C NMR spectrometry can give a fingerprint type of identification that can be used in a way similar to infrared spectroscopy.

ENVIRONMENTAL ANALYSIS

The final topic of this discussion is the environmental analysis that may be encountered in dealing with fatty chemicals. There is a tremendous amount of regulatory interest in nitrosamines because they are powerful carcinogens. Amine-fatty acid soaps are often used as emulsifiers in cosmetic and other formulations. Nitrosamines have been reported in these products. There is also interest in whether nitrosamines occur in fatty nitrogen compounds since these are often used as emulsifiers in pesticide formulations.

The most recent development for determining nitrosamines in organic compounds is the Thermal Energy Analyzer (TEA) (18). This device employs a technique in which the N-NO bond is catalytically cleaved. The released nitrosyl (NO) radical is reacted with ozone to give an electronically excited nitrogen dioxide. The excited NO_2 decays back to its ground state with the emission of light energy. The light emitted is detected with a photomultiplier system. The intensity of the energy emitted is proportional to the N-nitrosyl radical concentration, and this is directly related to the N-nitroso compound that is present in the original sample. TEA is usually interfaced with a gas chromatograph. In this way, specific nitrosamines can be detected at extremely low levels (ppb).

Another common environmental analysis connected with fatty acids is the determination of chlorinated dibenzodioxins or "chick edema factor" (19). This analysis is done by going through a cleanup procedure followed by electron capture gas chromatography. In this way, as little as 0.1 ppb of the very poisonous dioxins can be detected.

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