Mass Spectroscopy: A Tool Now Available to the Fatty Acid Chemist[1]

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THE NUMBER OF FATTY ACID and lipid chemists who attended the symposium on "The Applications of Mass Spectroscopy to Fatty Acid and Lipid Chemistry," held as part of the 39th Fall Meeting of The American Oil Chemists' Society, as well as members of the Society who have been following the increasing number of reports of research involving mass spectroscopy appearing in the *Journal*, have undoubtedly become aware that the mass spectrometer has finally become a tool for the solution of practical problems.

The application of mass spectroscopy to fatty acid chemistry is not, of course, due to the fact that mass spectroscopy is a new science. The basic principles of mass spectroscopy, including the separation and registration of atomic masses, were illustrated by Wien in 1898 (1) and demonstrated in proof of the existence of isotopes, by Thomson in 1912 (2), some 55 to 65 years ago. The then-sophisticated mass spectrometers were described by Dempster (3) and by Aston (4) in 1918 and 1919, 46 to 47 years ago. Thus, mass spectroscopy can be considered as being just about 50 years old.

Nor were fatty acid chemists unaware of mass spectroscopy. Weinhouse, Medes and Floyd (5) over 20 years ago reported on application of mass spectroscopy to settle an important question regarding the mechanism of fatty acid metabolism. At that time, there were three proposed mechanisms of fatty acid metabolism: (1) the β-oxidation-condensation theory, which assumed that the ketone bodies are formed by the condensation of some 2-carbon intermediate resulting from β-oxidation of fatty acids; (2) the multiple alternate oxidation, which assumed that oxidation occurs at alternate carbon atoms throughout the fatty acid chain; and (3) the classical theory of β-oxidation, which assumed successive β-oxidation along the fatty acid chain. Weinhouse and his colleagues reasoned that if the carboxyl carbon of normal octanoic acid is labeled with C^{13} and incubated with liver slices from fasted rat, the newly synthesized acetoacetic acid could be decomposed into acetone and CO_2, according to the procedure of Van Slyke; and the distribution of the C^{13} between these two fractions determined by mass spectroscopy could be compared with that which would be predicted for this distribution according to the three theories. The β-oxidation-condensation theory would predict equal distribution of the excess C^{13} (above natural abundance) between the carboxyl and carbonyl groups of the acetoacetic acid formed. By the multiple alternate oxidation theory, the acetoacetic acid resulting would contain excess C^{13} in its carboxyl group only. Finally, the classical theory of β-oxidation would predict no excess C^{13} (above natural abundance) in the acetoacetic acid formed. The CO_2 liberated as a result of the decomposition of the acetoacetic acid by the Van Slyke procedure was analyzed for its excess C^{13} content by mass spectroscopy. The acetone was precipitated as the mercury complex and further degraded. The two end carbons of the acetone, isolated from the mercury complex, were differentiated from the central carbon by chemical means and were then oxidized to carbon dioxide and analyzed by mass spectrometry for excess C^{13}. The results revealed equal amounts of excess C^{13} in both the carbonyl and carboxyl fractions leading the authors to conclude: "When n-octanoic acid labeled by the incorporation of C^{13} in the carboxyl group was incubated in vitro with liver slices from fasted rats, the resultant acetoacetic acid contained all excess C^{13} equally distributed between the carbonyl and carboxyl carbon atoms. The results offer unequivocal evidence that the ketone bodies are formed by condensation of a 2-carbon intermediary resulting from β-oxidation of the fatty acid," a first triumph for mass spectroscopy in the field of fatty acid chemistry.

However, it should be noted that all of the mass spectroscopic examinations in the experiments of Weinhouse et al. were made by the analysis of CO_2. This was in accord with the mass spectroscopy of twenty years ago, that it was a tool for the analysis of gases (or low boiling readily vaporized liquids). Mass spectroscopy was limited to molecules of low molecular weight. It was not ready as a tool which the fatty acid or lipid chemist could use to investigate his long-chain fatty acids, esters, or glycerides or their many derivatives. It was not until 1951 that O'Neal and Wier (6) described a mass spectrometer with a heated inlet system and provision for determination of the mass spectra of compounds of low volatility. We entered a period in which major emphasis was placed on instrumentation to permit measurements at higher and higher mass numbers and at the higher resolutions required to adequately separate the more complex mass fragments from larger molecules.

In 1959, Ragnar Ryhage, with the Laboratory for Mass Spectrometry, I Karolinska Institutet in Stockholm described a mass spectrometer which made use of an inlet system which could be operated at temperatures up to 400C, permitting measurements of compounds with molecular weights up to 619 (n-tetratetracontane). Ryhage collaborated with Einar Stenhagen from the Institute of Medical Biochemistry, University of Gothenburg, and with Erik von Sydow from the Institute of Chemistry, University of Uppsala, in a systematic survey of the mass spectra of pure compounds of interest to the fatty acid chemist. With colleagues from their respective institutions, these three Swedish research workers, during the next five years, published a remarkable series of approximately 40 research papers dealing with fatty acid compounds, mostly as methyl esters. These papers were mainly of the survey type, containing mass spectra of pure compounds and analyses of the spectra including identification of the base (most intense) peak, the parent (molecule-ion) peak, most of the more intense peaks and explanations for the formation of characteristic peaks from fragment rearrangements. These papers constitute a comprehensive survey of the mass spectra of numerous fatty acid materials, contain much information of considerable value regarding the interpretation of the mass spectra of these materials, and open up the potentials of this tool to research investigations of natural commodities in the areas of fatty acid and lipid chemistry. The fatty acid or lipid chemist contemplating the use of mass spectroscopy in his research is well advised to become familiar with them. An excellent review of the work of the Swedish research group has been published

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by Ryhage and Stenhagen (7).

The spectra and the interpretations of the mass fragment patterns, as they appear in this review paper, or in the original publications will be (and in fact have already been) of considerable assistance to the fatty acid and lipid chemist using mass spectroscopy in the solution of his problems. Its usefulness in this regard has been acknowledged by several research workers, including some who will appear on the program of this symposium (8,9).

The papers which appeared on the applications of mass spectroscopy to fatty acid and lipid chemistry during about the period of the late 1950's into the first years of the 1960's were mainly survey papers. They are reminiscent of the survey papers in infrared absorption spectroscopy which appeared some ten to fifteen years ago which described the spectra of pure long-chain fatty acids, esters, glycerides and many derivatives, giving frequency positions of characteristic bands and correlations of these bands to organic functional groups which gave rise to them. This survey period, which in the history of the applications of mass spectroscopy to lipid chemistry was dominated by the contributions of the Swedish workers, is now coming to a close. We are now no longer reading papers concerned with *mass spectrometry* and its potential applications to fatty acid or lipid chemistry. We are beginning to see more and more papers dealing with *problems* in *fatty acid* and *lipid chemistry* which are being solved with the aid of the mass spectrometer.

This does not mean that additional survey-type papers will not appear. There is, of course, considerable overlap between the period of survey papers describing spectra of pure compounds and actual applications to practical problems. In a review written in 1961, Dutton (9) said, "Analytical applications of mass spectroscopy to lipids are largely yet to be made." We can now report that they are being made. When preparing the manuscript for the chapter in Markley's "Fatty Acids" (10) dealing with spectral properties, about 1957, we searched the literature for at least an isolated example of an application of mass spectroscopy to fatty acid chemistry, and abandoned the idea because there just were not any. In a proposed revision to up-date this material, a section on applications of mass spectroscopy contains some 50 or 60 references.

In these introductory remarks only a very few specific applications can be given as examples. Certainly the studies on phthiocerol by the Swedish workers (11–16) should be cited. The brilliant elucidation of the structure of this compound probably represents the first example of the establishment of structure of a lipid material principally by means of mass spectrometry. The Swedish workers are also responsible for a practical method for locating the position of the double bond in long chain compounds by deuteration with deuterium hydrazine and subsequent mass spectra analysis (17). In this country Paschke, Peterson and Wheeler have made practical use of mass spectroscopy in the elucidation of dimer acid structures (8,18,19). Rubenfeld et al. (20), in a study of straight-chain alkylbenzenes, has applied mass spectroscopy to detergents and Sonneveld and colleagues have applied the technique to animal fats in the analysis of butterfat (21).

This is the new era which is just getting underway, about which we are to hear much today. And, as the fatty acid and the lipid chemist appreciates more and more this valuable tool, the mass spectrometer, we predict we will see an ever increasing number of papers in the literature of fatty acid and lipid chemistry dealing with the applications of mass spectroscopy.

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Pioneer Member Dies

J. J. Ganucheau

J. J. GANUCHEAU (1918), Honorary Member of AOCS since 1964, died July 18, 1966, in New Orleans.

At the time of his death, he was serving as a consulting chemical engineer, having retired from the Wesson Company (Wesson Division of Hunt Foods) in 1962, after 44 years of service.

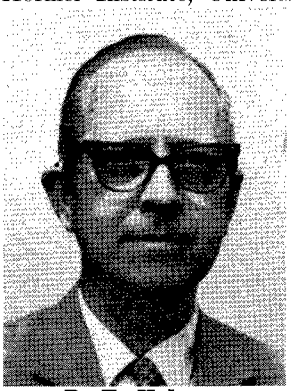
In a JAOCS Commentary, dated March, 1959, Mr. Ganucheau wrote: "The fact that the AOCS is one of the foremost scientific organizations in the world was not brought about by chance but by men."

He himself contributed the following committee service to AOCS: Crude Mill Operations, 1930–33, Chairman, 1933–34; Refining, 1930–34; Third Vice President, 1936–37; Advertising, 1938–42; Second Vice-President, 1942–43; Uniform Methods, 1942–62; Soap in Refining Oils, 1943–62; New Orleans Resident Representative 1954–; Governing Board, 1954–57; Soapstock, 1955–62; By-Laws Revision, 1959–60; Honorary Member, 1964.

His dedication to AOCS was recognized and deeply appreciated by his friends and co-workers, and his achievements will be remembered.

Borden Award to R. T. Holman

R. T. HOLMAN (1946), professor of biochemistry at Hormel Institute, University of Minnesota, Austin, Minnesota, has been named winner of a $1,000 Borden Award in Nutrition for 1966.



R. T. Holman

The award recognizes outstanding research on the nutritive significance of compounds of milk and was awarded by the American Institute of Nutrition. The award consists of an honorarium and a gold medal, provided by the Borden Co.

Dr. Holman was recognized for his research in the metabolism and quantitative requirement of essential fatty acids in animals and man.

Dr. Holman holds several editorial posts, including that of Associate Editor, *Lipids*. He organized the symposium on mass spectrometry of lipids (Cincinnati, 1965), and the symposium on essential fatty acids (Los Angeles, 1966).