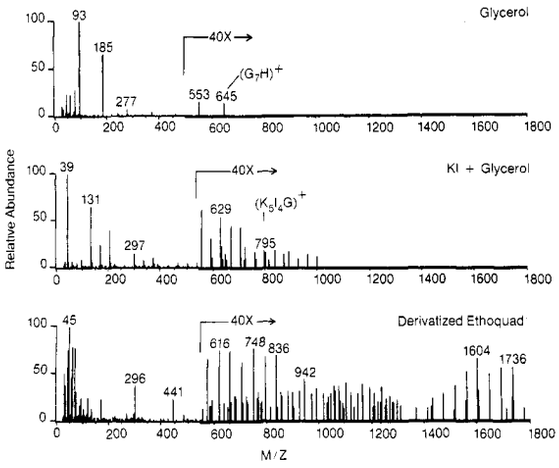

Figure 4. Comparison of the positive ion spectra of glycerol (top), KI plus glycerol (center), and Ethoquad 18/25 derivatized with perfluorooctanoic anhydride (bottom) under identical instrument conditions.

Ultramark did not exhibit any significant ions (≥1%) between m/z 313 and 766. We felt that such a large spacing between reference ions could cause problems in calibrating the spectrometer over the entire mass range. The high mass ions (1072, 1116, 1160, 1248, 1292, 1336, etc.) in the spectrum of derivatized Ethoquad (Figure 2) reached a maximum intensity of 6% (relative to the base peak at m/z 58) which is comparable to that of the high mass ions observed in the FAB spectrum of Ultramark 1621. Furthermore, there is a series of comparably intense ions between m/z 328 and 680 that facilitate computer calibration over the entire mass range.

Subsequent to this work, we have achieved a computerized nominal-mass calibration to ~2000 amu using NaI as the reference material (*9*) and an ion accelerating voltage of 6 kV. The lower accelerating voltage was necessary to circumvent magnet saturation effects which were causing the calibration to fail above ~1300 amu. This was in part due to the large spacing (150 amu) between ions in the calibration table. In addition, the ions observed above 1000 amu were approximately an order of magnitude less intense than those observed in the spectra of the derivatized Ethoquad 18/25. The derivatized Ethoquad 18/25 offers the advantages of higher sensitivity and closely spaced reference ions without the need to precisely mix a number of inorganic salts.

It is noteworthy that given a suitable lock-mass compound, these FAB-generated computer mass calibrations can be used in other ionization modes as well. We have used these calibrations to obtain spectra in the negative ion FAB, positive-ion EI, and positive and negative ion field desorption (FD) modes (*12*). These calibrations can be especially useful in the FD mode where fleeting signals and a different desorption behavior for each component in a mixture make the construction of a calibration mixture extremely difficult.

Registry No. Ethoquad 18/25, 28724-32-5.

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Differentiation of Monoepoxide Isomers of Polyunsaturated Fatty Acids and Fatty Acid Esters by Low-Energy Charge Exchange Mass Spectrometry

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Low-energy charge exchange mass spectrometry is a convenient technique for the unambiguous differentiation of monoepoxide isomers of polyunsaturated fatty acids, fatty acid methyl esters, and fatty acetates. This technique eliminates multistep derivatization procedures which are typically utilized prior to characterization of these isomers with conventional electron ionization mass spectrometry.

Arachidonic acid monepoxides have drawn recent attention due to successes in their regiospecific synthesis (*1-3*), conversion to biologically important HETE's (*3*), and identification as cytochrome P-450 oxidation products of arachidonic acid (*4*) that appear to have physiological importance (*5*, *6*). These monoepoxide isomers have been previously characterized on the basis of conventional 70-eV electron ionization (EI) mass spectrometry (*7*). However, the characteristic fragment ions observed in the EI spectra are extremely weak (relative abundance less than 5% of the base peak). Thus, multistep derivatization procedures have been utilized to convert the monoepoxide isomers into molecules which yield more definitive EI spectra (*4*, *7*). These derivatization procedures are difficult to apply to trace quantities of material isolated from biological sources since they typically involve several sample handling steps (such as catalytic hydrogenation of the carbon–carbon double bonds, epoxide ring opening, and derivatization) prior to characterization using GC/MS. We

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wish to report that low-energy charge exchange (CE) mass spectrometry (*8*) provides a simple, one-step method for the unambiguous differentiation of monoepoxide isomers of polyunsaturated fatty acids, fatty acid methyl esters, and fatty acetates. This technique completely eliminates the requirement of derivatization prior to mass spectral characterization.

Organic molecules (M) are ionized with near collision efficiency (*9*) by charge exchange (eq 1) if the ionization potential of M is less than the recombination energy of the

$$R^{+\cdot} + M \rightarrow R + M^{+\cdot} \qquad \Delta H < 0 \qquad (1)$$

reactant ion ($R^{+\cdot}$). Charge exchange offers a simple method for varying the amount of internal energy initially deposited in $M^{+\cdot}$ (*8*). Thus, the fragmentation behavior of $M^{+\cdot}$ can be controlled by varying the exothermicity of eq 1. Furthermore, molecular cations possessing relatively low internal energies can be produced without the reduction in sensitivity that accompanies the use of low-energy electron ionization (*10*). Given the potential advantages of this technique and the fact that low-energy CE reactions can be conveniently studied in conventional high-pressure ion sources, it is surprising that CE has been only occasionally used for selective ionization applications (*11–13*) and has been infrequently used as a molecular structure probe (*14*, *15*).

EXPERIMENTAL SECTION

Instrumentation. All mass spectra were obtained by direct insertion into a Hewlett-Packard 5985B GC/MS system operating at an ion source temperature of 200 °C.

Reagents. Reagent-grade carbon disulfide was obtained from MCB Manufacturing Chemists, Inc., Cincinnati, OH, while reagent-grade *m*-chloroperoxybenzoic acid was obtained from Aldrich Chemical Co., Milwaukee, WI. Both chemicals were used without further purification. The starting polyunsaturated alcohols or methyl esters were obtained from NuChek Prep., Inc., Elysian, MN, except for the 5,8,11,14,17-icosapentaenoic acid which was obtained from Sigma, St. Louis, MO. The purity of these latter materials was verified by TLC prior to use.

Procedure. In all cases, the requisite polyunsaturated alcohol or methyl ester was epoxidized with *m*-chloroperoxybenzoic acid (1 equiv, CH_2Cl_2) and the reaction product flash chromatographed (*16*) to separate the monoepoxides from unreacted starting material and diepoxides. The monoepoxide mixture was then resolved into individual components by HPLC (the alcohols were first acetylated with Ac_2O/pyridine) by using 9:1 hexanes/ether on a μ-Porasil column. The order of elution of the epoxides generated from tri- and tetraenes was 11,12-epoxide, 14,15-epoxide, 8,9-epoxide, and, lastly, the 5,6-epoxide (if present). The elution order of the monoepoxides derived from the *all-cis*-5,8,11,14,17-eicosapentaenoic acid (as the methyl ester) was 14,15-epoxide, 11,12-epoxide, 8,9-epoxide, 17,18-epoxide, and, lastly, 5,6-epoxide. NMR spectrometric characterization of the recovered products (^1H and in some cases ^{13}C NMR) was consistent with the structures assigned.

The individual isomers were characterized by CS_2 CE mass spectrometry. The major reactant ion (eq 1, $R^{+\cdot} = CS_2^{+\cdot}$) has been previously produced by electron ionization of N_2/CS_2 mixtures (*9*). The $CS_2^{+\cdot}$ ion (IP ~ 10.1 eV) (*17*) has a recombination energy which is ~0.7 eV above that of $C_6H_6^{+\cdot}$, a species that has already been shown to ionize methyl arachidonate (*11*).

In this study, primary ionization of CS_2, at an indicated source pressure of 0.1–0.2 torr, was effected with a beam of high-energy electrons (200–250 eV) emitted from a heated rhenium filament. The observed reagent ion spectrum at 0.1 torr was as follows: $S^{+\cdot}$ (5), $S_2^{+\cdot}$ (9), $CS_2^{+\cdot}$ (100), $CS_3^{+\cdot}$ (3), and $(CS_2)_2^{+\cdot}$ (4). The most significant change in the reagent ion spectrum, as the source pressure was increased from 0.1 to 0.2 torr, was a dramatic increase in the relative abundance of $(CS_2)_2^{+\cdot}$. At 0.2 torr, the intensity of this ion is nearly equal to that of $CS_2^{+\cdot}$. The extent of reaction of $(CS_2)_2^{+\cdot}$ with polyunsaturated monoepoxide isomers is unknown. This ion has a recombination energy that is lower than that of $CS_2^{+\cdot}$ by the binding energy of the dimeric species. Thus, if $(CS_2)_2^{+\cdot}$ is energetic enough to ionize these unsaturated epoxides, it will produce molecular ions with less internal energy than those

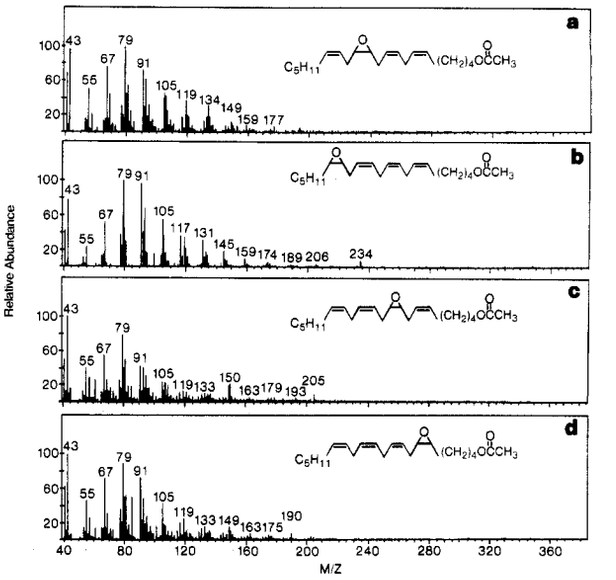


Figure 1. Electron ionization (70 eV) mass spectra of the monoepoxide isomers of arachidonyl acetate.

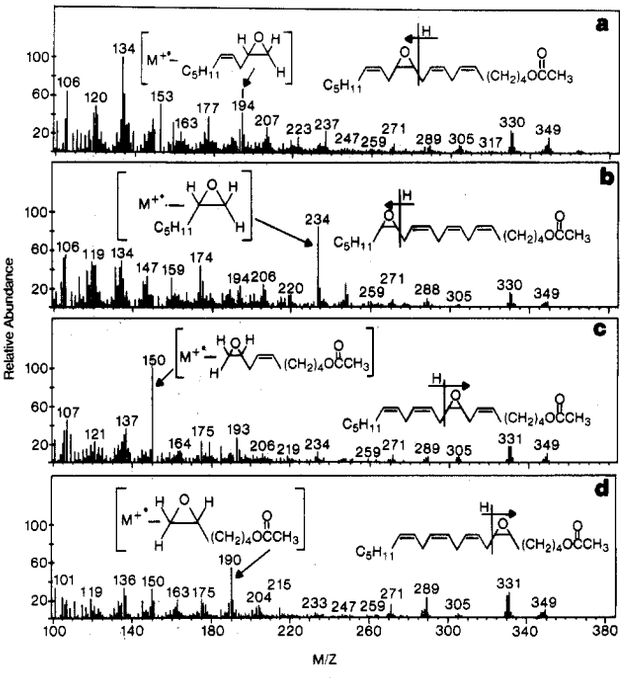
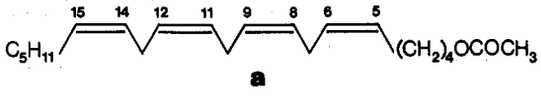


Figure 2. Carbon disulfide charge exchange mass spectra of the monoepoxide isomers of arachidonyl acetate.

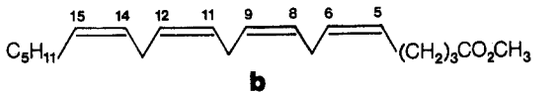
produced by reaction with $CS_2^{+\cdot}$. The spectra shown in Figures 1, 2, and 3 have been background subtracted. This is particularly important for the CE spectra which exhibit a prominent $(CS_2)_2^{+\cdot}$ ion at m/z 152.

RESULTS AND DISCUSSION

Figure 1 compares the 70-eV electron ionization mass spectra of four monoepoxide isomers of arachidonyl acetate (a) while Figure 2 compares the spectra of these isomers ob-



a



b

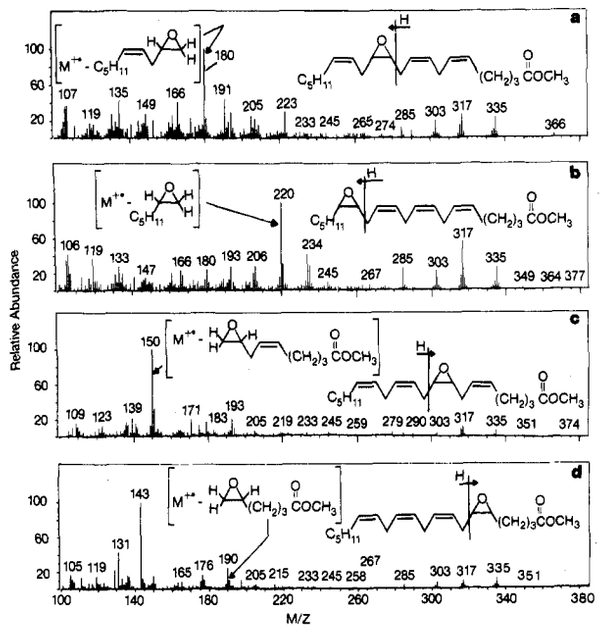


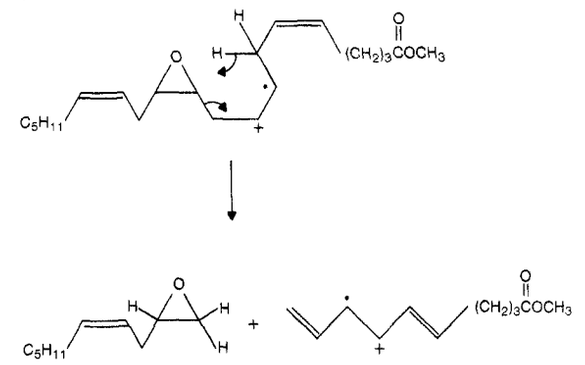
Figure 3. Carbon disulfide charge exchange mass spectra of the monoepoxide isomers of methyl arachidonate.

tained with charge exchange ionization using pure CS_2 as the reagent gas. The electron ionization spectra, which are very similar, do not contain significant molecular ions (m/z 348) and are dominated by structurally insignificant low weight fragment ions. The CS_2 CE spectra, on the other hand, are quite distinct and can be readily correlated with molecular structure. The CE spectra all exhibit a weak $(M + H)^+$ adduct ion at m/z 349 which confirms the molecular weight. Protonation occurs in the high-pressure source as a result of residual hydrogen containing impurities or as a result of sample ions protonating sample molecules. More importantly, each spectrum also contains an intense ion corresponding to elimination of an intact epoxide molecule (as shown). This ion is diagnostic of the original position of the epoxide group within the molecule and can be used to differentiate the various isomers.

Close reexamination of EI spectra of the epoxy fatty acetate isomers (Figure 1) reveals the presence of the structurally diagnostic fragment ions formed by loss of an intact epoxide molecule from the molecular ion. However, these fragment ions are of much lower relative abundance than the corresponding ions produced by CS_2 CE ionization. These fragment ions are most clearly evident in the spectra of the 14,15 (Figure 1b) and 8,9 (Figure 1c) epoxy isomers and are less prominent in the spectra of the 11,12 (Figure 1a) and the 5,6 (Figure 1d) epoxy isomers. For unknown samples, it would be difficult to assign these weak ions produced by EI. Without confirmatory molecular weight information, these odd electron ions could be easily misassigned as intact molecular ions. Furthermore, the origin of these relatively weak signals would be uncertain unless the purity of each component was clearly established. Structure assignment is facilitated by use of CS_2 CE since the relative abundance of the structurally diagnostic fragment ions is significantly enhanced and the spectra also contain direct molecular weight information.

The carbon disulfide CE spectra of the set of monoepoxides derived from methyl arachidonate (b), Figure 3, were qualitatively similar to the spectra of the acetates. Weak $(M + H)^+$ adduct ions at m/z 335 confirm the molecular weight of each isomer while loss of an intact epoxide molecule verifies the original position of the epoxy group. Furthermore, comparison of the CE spectra of the fatty acetates with the spectra of the corresponding fatty acid methyl esters enables the

Scheme I



identification of those fragment ions that contain the remote ester groups. For example, the spectrum of the 11,12 monoepoxy fatty acetate (Figure 2a) exhibits an abundant fragment ion at m/z 194 which shifts to m/z 180 in the spectrum of the corresponding methyl ester (Figure 3a). For these isomers, the fragment ion formed by elimination of the epoxide molecule contains the remote ester group. On the other hand, the masses of the corresponding fragment ions generated by CE of the 8,9 monoepoxy fatty acetate (Figure 2c) and fatty acid methyl ester (Figure 3c) are identical (m/z 150) since the remote ester group is eliminated as part of the neutral epoxide molecule. Comparisons of this nature confirm that the epoxide molecule is always eliminated from the side of the molecular ion that contains less than two methylene-interrupted double bonds.

The importance of the second double bond in directing the fragmentation of unsaturated monoepoxides was further explored by analysis of a series of monoepoxytrieneacetates (structure a with the 5,6 carbon–carbon double bond hydrogenated). The isomers with the epoxide group in the 14,15 and 8,9 positions fragmented as expected. However, the 11,12 isomer, which does not contain two methylene-interrupted double bonds on either side of the epoxide group, did not fragment by loss of an intact epoxide molecule.

The fragmentation behavior of unsaturated epoxides can be rationalized (Scheme I) by considering charge localization initially occurring at the double bond closest to the epoxide group. The adjacent methylene hydrogens participate in the rearrangement reaction which precedes (or accompanies) elimination of the epoxide molecule. The resulting product ion, a diallylically stabilized species, is expected to be quite stable.

The CS_2 CE spectra of the 14,15 and 5,6 monoepoxy isomers of free arachidonic acid have also been obtained. These spectra are similar to those obtained from the fatty acetates and fatty acid methyl esters. The 14,15 monoepoxy isomer fragments by loss of $C_7H_{14}O$ yielding the base peak (m/z 206) in the spectrum. The 5,6 isomer fragments by loss of $C_6H_{10}O_3$ yielding the structurally diagnostic ion (m/z 190) with a relative abundance of 47% of the base peak. Finally, the monoepoxides of the biologically interesting *all-cis*-5,8,11,14,17-eicosapentaenoic acid have also been successfully characterized by this low-energy charge exchange technique.

In conclusion, low-energy charge exchange mass spectrometry provides a simple, one-step method for the unambiguous differentiation of monepoxide isomers of polyunsaturated fatty acids, fatty acid methyl esters and fatty acetates.

Registry No. Arachidonyl acetate 11,12-epoxide, 90605-92-8; arachidonyl acetate 14,15-epoxide, 90605-93-9; arachidonyl acetate 8,9-epoxide, 90605-94-0; arachidonyl acetate 5,6-epoxide, 90605-95-1; methyl arachidonate 11,12-epoxide, 90693-45-1; methyl arachidonate 14,15-epoxide, 70219-56-6; methyl arachidonate 8,9-epoxide, 82835-56-1; methyl arachidonate 5,6-epoxide, 70219-59-9.

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Evaluation of a Dual Mass Spectrometer System for Rapid Simultaneous Determination of Hydrogen-2/Hydrogen-1 and Oxygen-18/Oxygen-16 Ratios in Aqueous Samples

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The commercial prototype of a twin mass spectrometer system (AquaSIRA, VG Instruments, Ltd.) has been evaluated for its linearity, accuracy, and precision in the measurement of $^2H/^1H$ and $^{18}O/^{16}O$ ratios in a series of water standards and urine samples. With an instrument correction, $(^2H/^1H)_{corrected} = [(^2H/^1H)_{measd} - 13.23]/0.9139$, the $^2H/^1H$ ratios (89–696 ppm) were determined with a mean precision of 0.5 ppm and a mean accuracy of 0.87 ± 0.67 ppm. The $^{18}O/^{16}O$ ratios (1894–2684 ppm) were determined to be linearly proportional to the true values with a mean precision of 0.6 ppm and a mean accuracy of 2.7 ± 2.9 ppm. The ability of the system to measure absolute ratios of $^2H/^1H$ and $^{18}O/^{16}O$ accurately and precisely in aqueous samples at high enrichment levels of ^2H and ^{18}O suggests that this system will be important in nutritional and clinical studies employing $^2H_2{}^{18}O$.

The measurement of $^2H/^1H$ and $^{18}O/^{16}O$ isotope ratios is done customarily in two separate mass spectrometers following the conversion of water to H_2 gas for $^2H/^1H$ values (*1*, *2*) and equilibration of the water oxygen isotopes with carbon dioxide to form $C^{16}O^{18}O$ for $^{18}O/^{16}O$ values (*2*). Each type of gas sample is introduced into a dual inlet isotope ratio mass spectrometer and its isotopic abundance is compared to that of a reference gas. Because the inlet pressures can be adjusted independently for both standard and sample gases, the ionization source pressure conditions and major ion beam intensities can be matched closely to minimize the measurement errors involved in the comparison.

The requirement for separate sample preparation and mass spectrometric analysis for ^2H and ^{18}O in a single sample is time-consuming, but until the need arose to analyze hydrological samples in large numbers, there was little incentive to develop an instrument to carry out the facile and rapid measurement of both isotopes in the same water sample. In order to eliminate the sample preparation step for the oxygen isotope measurement, Majzoub and Nief (*3*) developed the first mass spectrometer in which the determination of ^{18}O in water samples was carried out directly on water vapor. Subsequently, Hagemann and Lohez (*4*) described a twin mass spectrometer system in 1978 in which a furnace, for conversion of water to hydrogen gas, was added for the simultaneous determination of $^2H/^1H$ and $^{18}O/^{16}O$ in water samples. To date, however, water analyses with this instrument reportedly have been limited to those having natural abundances of ^2H and/or ^{18}O.

The design used by Hagemann and Lohez, which is the basis of the Aqua-SIRA instrument, has several radical elements which include a pronounced and significant memory of previous samples, the use of dynamic (vs. static) isotope ratio determination techniques, and, most importantly, the direct determination of absolute abundances in the place of differential measurements with reference standards. The latter was of particular concern because analysis of samples significantly enriched in ^2H and ^{18}O would be required, and the linearity of response at levels 2- and 3-fold above natural abundance had not been established. Moreover, it would be necessary to inject biological samples of salt-containing fluids, such as urine, directly into the inlet system and the effects of sample matrix on the isotope ratio measurements was unknown and could be resolved only after an extensive evaluation had been undertaken.

The description of the Aqua-SIRA instrument system, its characteristics and mode of operation, its performance, and the limits of its accuracy and precision are the subjects of this paper. Although this paper is limited necessarily to a specific commercial product, the question of interest is the following: Are the analytical results obtained from this type of mass spectrometer system adequate to support biomedical and clinical studies with ^2H and ^{18}O? On the basis of the performance levels obtained in the use of this system, a positive answer is justified.

EXPERIMENTAL SECTION

Physical Characteristics. Aqua-SIRA is a mass spectrometer system (VG Isogas, Limited, Cheshire, England) designed for

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