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AUTOMATED HETEROATOMIC PLOTTING AS AN AID TO THE PRESENTATION AND INTERPRETATION OF HIGH RESOLUTION MASS SPECTRAL DATA*†

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Abstract—A method for presentation of complete high resolution mass spectral data is discussed. This method, termed heteroatomic plotting, presents the data in the form of plots of relative intensity versus elemental composition. This method has the advantage of data presentation in an easily interpretable form that is similar to the bar graph presentation of low resolution mass spectral data. Elemental composition of all peaks and an indication of fragmentation processes leading to these peaks are provided by plotting the data in this manner.

THE utilization of the vast amount of accurate mass and ion abundance data contained in a high resolution mass spectral fragmentation pattern is only recently being realized through the development of sophisticated data acquisition and reduction facilities.[1-5] Since the high resolution mass spectrum of a complex organic molecule may consist of several hundred ions of differing elemental composition and relative abundance, there arises the task of their mental assimilation and subsequent interpretation in terms of salient features of molecular structure and stereochemistry.

It is imperative, therefore, that these data be manipulated so that they can be presented in a format which allows visual grasp of these salient features. This format should be chosen to accomplish the goal of providing the maximum amount of information concerning correlations of the fragmentation pattern with features of molecular structure and stereochemistry. One approach to data presentation has been suggested by Biemann *et al.* and has been termed "element mapping".[6] Another approach, termed "topographic element mapping", has recently been proposed by Venkataraghavan and McLafferty.[7]

An alternative method of data presentation has been developed in the course of our work with high resolution data obtained from photographic plates.[1] This method preserves the visual advantages of the bar graph used in displaying conventional low resolution mass spectra, while presenting all available information contained in the high resolution data. This method assumes "increasing" importance when used in

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† This technique was first presented at the 13*th Annual Conference on Mass Spectrometry and Allied Topics*. St. Louis, Mo., 16–21 May (1965) and at the *EUCHEM Conference* on Mass Spectrometry, Sarlât, France, 7–12 September (1965).

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the presentation of high resolution data from a recently developed real-time digital data acquisition system.[3,4] This system, which employs high speed digital data acquisition from a rapid magnetic scan of a mass spectrum, has indicated that greatly improved relative ionic abundances can be obtained as compared to photoplate methods. The bar graph seems most suited to present this intensity data for ready viewing and interpretation.

The method, termed heteroatomic plotting, consists of the following operations. The output from the mass calculation program, consisting of the nominal mass, calculated accurate mass, elemental composition and relative intensity of all lines on the photoplate—or all peaks in the real-time data—from the organic compound studied, is sorted by computer according to heteroatom content. For a particular compound this results in several groups of data differing in the number and kind of heteroatoms. These data are then directly input to a computer,* programmed to drive a high speed incremental plotter,† and a plot is drawn for each set of data. In addition to being quite rapid and inexpensive, this method enjoys great programmable versatility. The line drawing that is the net result can be made any convenient size. One is able to specify the various sizes and types of lettering and scales used, to specify the base peak of the relative intensity scale (taken to be the base peak of the entire spectrum unless otherwise specified), and to multiply any region of the spectrum times any scale factor (for example, to make high mass, low intensity features of the spectrum observable).

The results of this treatment may best be demonstrated by the use of four examples. The first two examples are the spectra of n-octadecane (**1**, obtained from the real-time system) and 2,11-dodecanedione (**2**, obtained from photoplate data). Since for **1** there are no heteroatoms, one obtains one plot (Fig. 1), the hydrocarbon ions. This spectrum, although a trivial example of the application of high resolution techniques, serves the purpose of clarifying the following discussion. The spectrum of **2** (Fig. 2) consists of three plots, one containing peaks determined by accurate mass measurement to contain only carbon and hydrogen (C/H plot), one for those peaks containing one oxygen atom (C/H O plot), and the last plot for those ions containing both oxygen atoms (C/HO2 plot). It is apparent from Figs 1 and 2 that one has immediately a clear, concise picture of the fragmentation pattern of these molecules. Equally important is the amount of information that can be obtained from this presentation through a proper choice of abscissa scales. It is important, initially, to realize that each heteroatomic group of ions are actually drawn with respect to nominal mass within each plot. Since the origin of the abscissas is the same *m/e* ratio for each plot, the same nominal masses from plot to plot, in Fig. 2, for example, lie directly above one another and thus a superposition of the plots would yield the low resolution mass spectrum of the compound. The horizontal scale is then adjusted accordingly to yield the actual C/H ratio.

The scale which has been chosen is based upon the C/H ratios (C_nH_{2n+1}) of fragments (even-electron ions) resulting from simple bond cleavages of a saturated aliphatic molecule (C_nH_{2n+2}). For example, the C/H ratios at the major divisions in a C/H plot represent the positions on the graph and the elemental compositions of the

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* Scientific Data Systems, Sigma 7.

† California Computer Products, Inc.

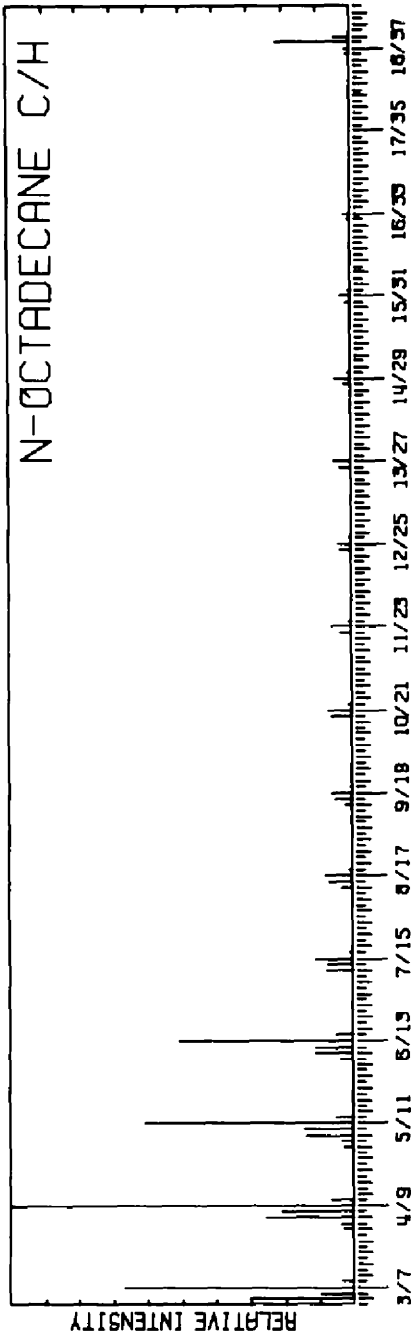


FIG. 1 High resolution mass spectrum of n-octadecane.

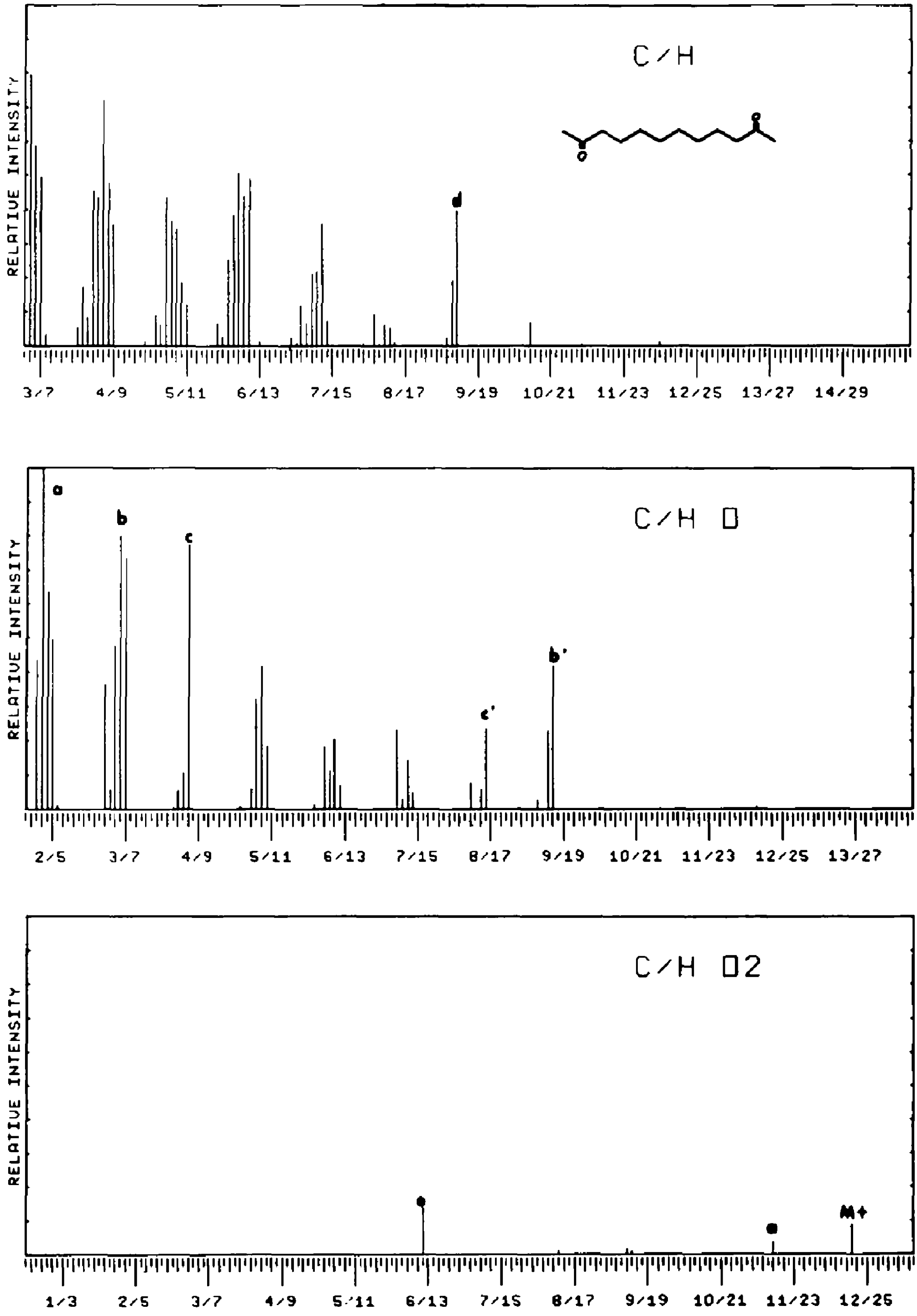
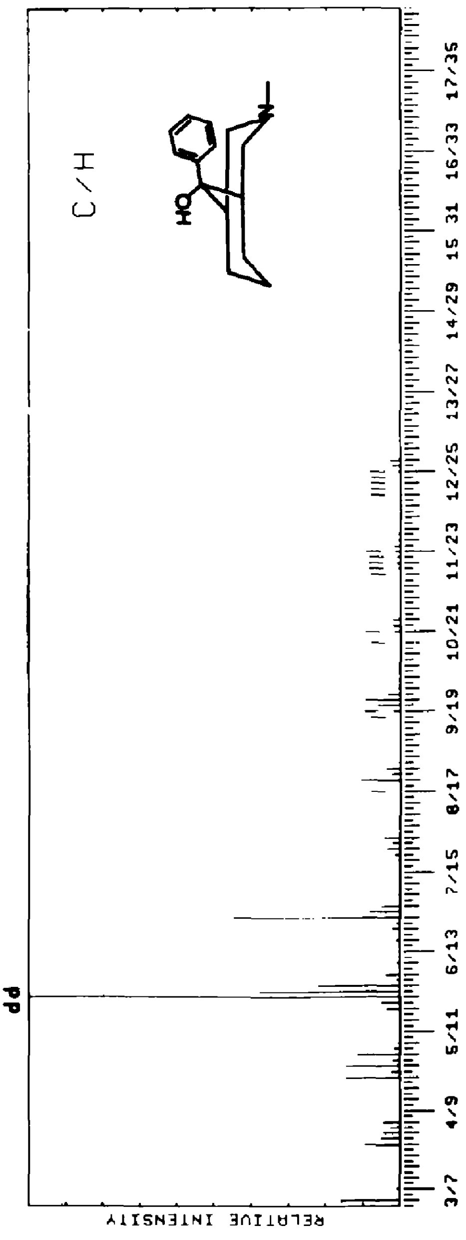


FIG. 2 High resolution mass spectrum of 2,11-dodecanedione.

fragments (C_nH_{2n+1}) resulting from simple cleavages of a saturated hydrocarbon molecule. This point is best illustrated in Fig. 1, where the major fragments, arising from simple bond cleavages of a saturated hydrocarbon molecular ion, fall at the major divisions and have the elemental compositions indicated by the C/H ratios. For a C/H O plot, the major divisions represent the positions and compositions for oxygen containing ions resulting from simple cleavages of a saturated aliphatic alcohol or ether—and so forth for other heteroatomic plots. From this scale the elemental composition of all ions can be immediately obtained. The distance between major divisions (differing by CH_2) is divided into 14 divisions (14 mass units). Since each division represents one H atom, and since fragment ions containing less than 2n + 1 H atoms will lie *below* the major divisions, one need only count down from the number of hydrogens in a major division to determine the elemental composition. For example, in the C/H O_2 plot in Fig. 2, the molecular ion (M^+) lies three small divisions below the major division 12/25. Its elemental composition is thus $C_{12}H_{22}O_2$. Similarly, the peak one small division below the major division 6/13 in this same plot must have the composition $C_6H_{12}O_2$ (peak *e*).

The second purpose achieved by this scale is involved in the location of the peaks relative to the major divisions (C_nH_{2n+1}). The number of divisions below a major division is directly related to the degree of unsaturation (rings plus double bonds) of the ion and reflects the fragmentation process involved in the formation of an ion. There are two different sized tic marks between the major divisions to aid in mental assimilation. For example, in the C/H O plot in Fig. 2, the peaks at $C_9H_{17}O$ (peak *b'*) and C_2H_3O (peak *a*) fall at *medium*-sized tic marks, two divisions below the major division where the saturated fragment would fall, indicating a two hydrogen deficiency, or one degree of unsaturation. Peaks falling four divisions below the major division must then possess two degrees of unsaturation (rings plus double bonds), and so forth. In conjunction, peaks falling at major divisions or medium tic marks are even-electron ions arising from fragmentation processes involving simple bond cleavages followed by elimination of neutral molecules from the fragment ion. Peaks falling at the smallest tic marks are odd-electron ions representing either molecular ions, ions resulting from an odd number of rearrangement processes, or elimination of neutral molecules from the molecular ion. An example of this would be the ion *b*, C_3H_6O (Fig. 2), which results from a rearrangement process (McLafferty rearrangement) in the molecular ion.

The third example is illustrated in the high resolution mass spectra of an epimeric pair of amino alcohols (Figs. 3a and 3b).[8] These spectra are presented alternately to illustrate the power of this method in a comparison of spectra of closely related compounds. A quick glance at the two pairs of C/H and C/H O plots (Fig. 3a) is sufficient to determine that they are essentially identical as far as ion types[9] are concerned, with only one prominent relative intensity difference noted (peak *dd*). A comparison of the pairs of C/H N and C/H NO plots (Fig. 3b), however, indicates several important peaks present in one heteroatomic plot that are absent in the corresponding plot of the other epimer (peaks *d*, *e*, *f* and *p*, *q*, *r*), peaks that must be a result of the effect of stereochemistry on the fragmentation of these epimers. From this simple information the elucidation of the fragmentation mechanisms involved becomes an easy task, a task that would be more difficult with the two alternative methods of data presentation mentioned above.


PP
C/H
HO
N
RELATIVE INTENSITY
3/7
4/9
5/11
6/13
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8/17
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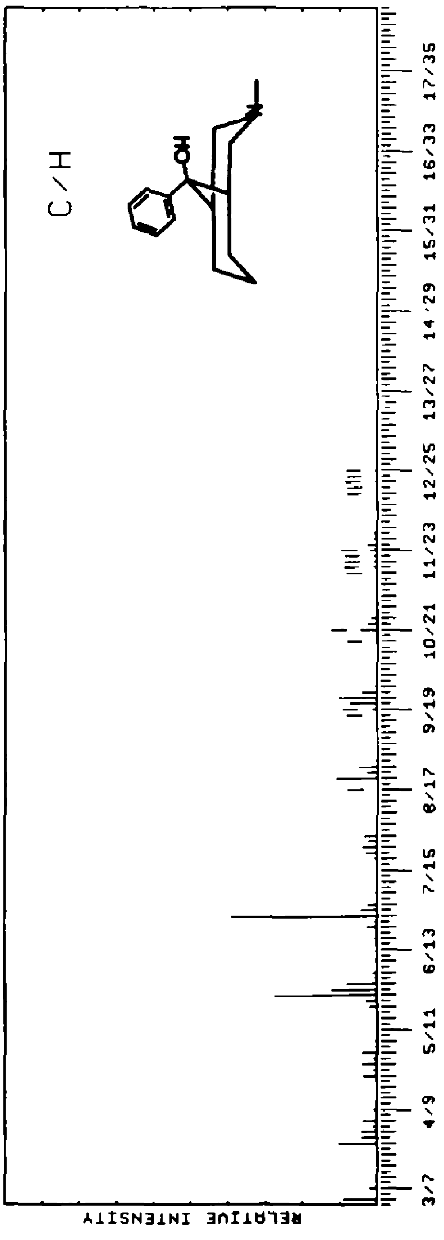
16/33
17/35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14 29
15/31
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17/35

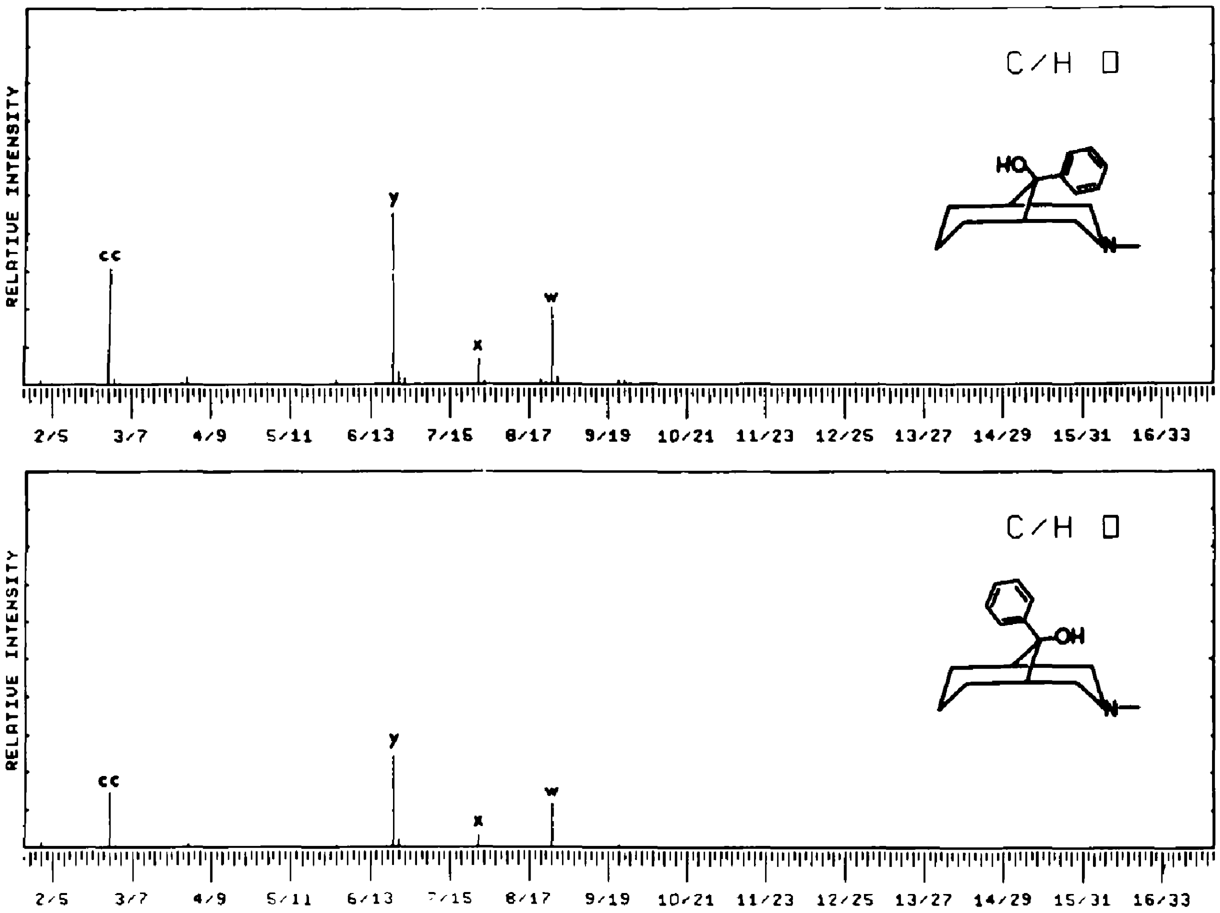
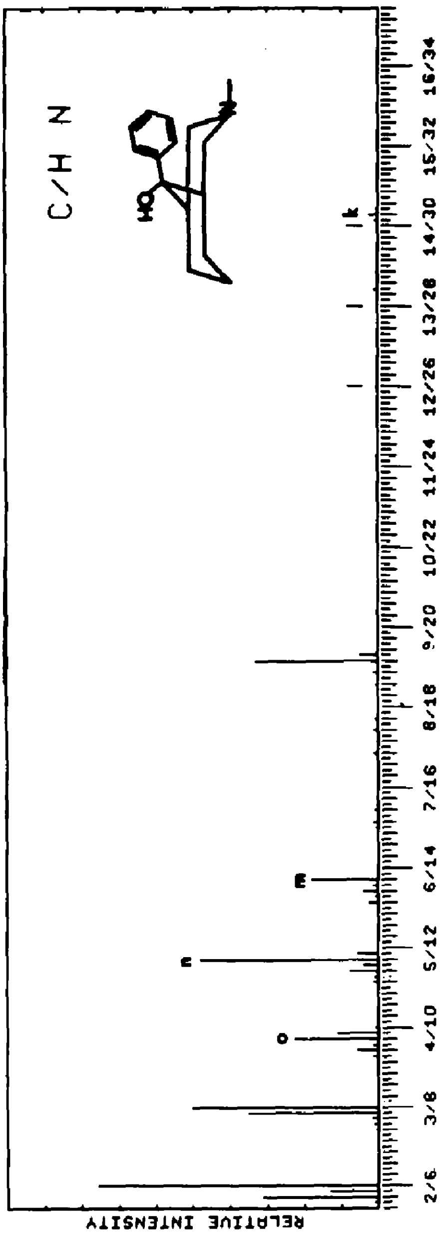


FIG. 3a Amino alcohol epimers, C/H and C/H O plots.


C/H N
HO
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2/6
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5/12
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11/24
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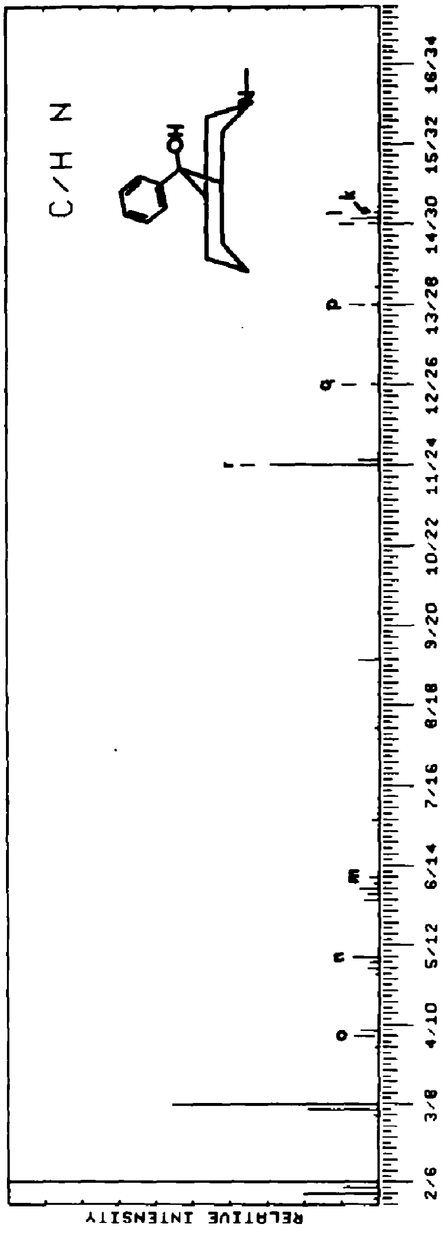
14/30
15/32
16/34
RELATIVE INTENSITY


C/H N
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16/34
RELATIVE INTENSITY

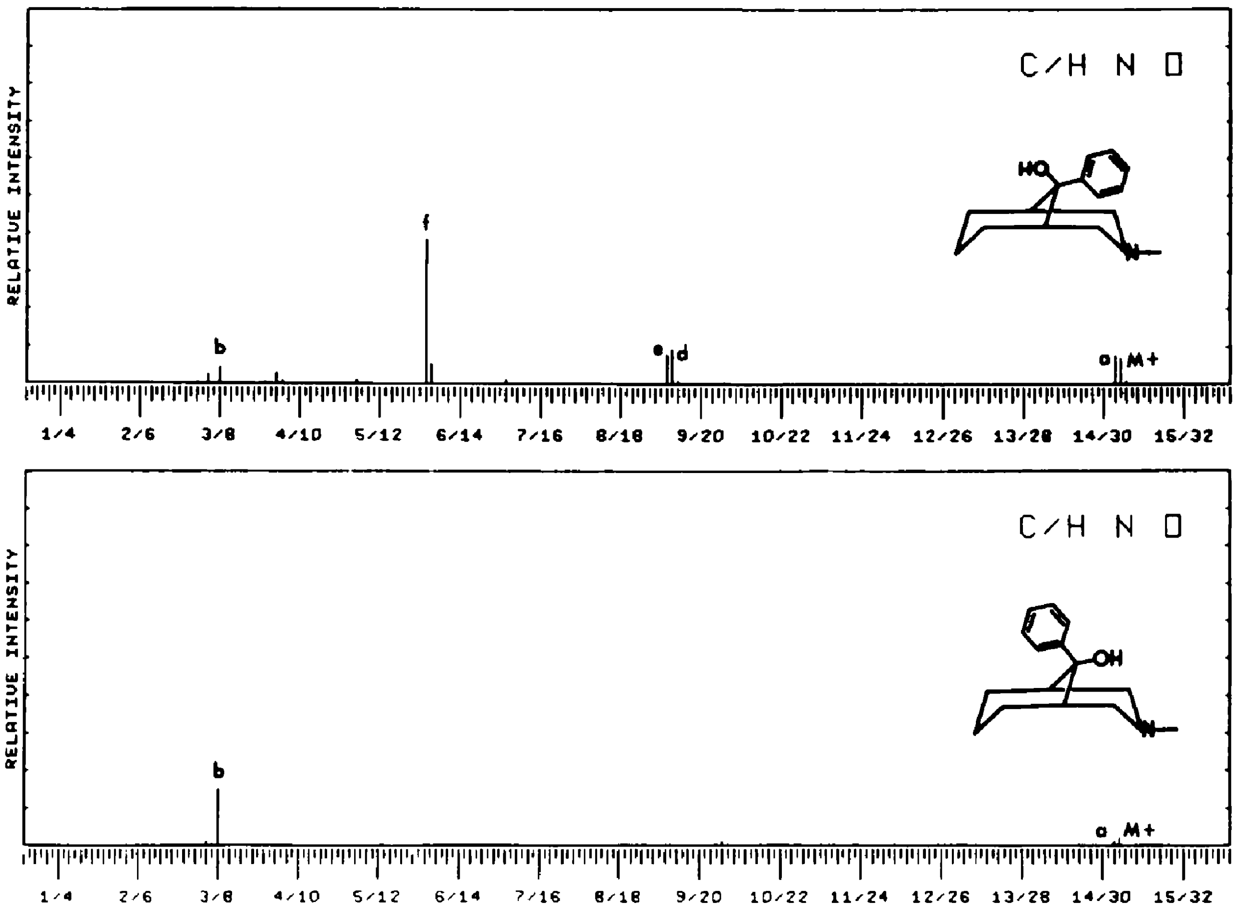
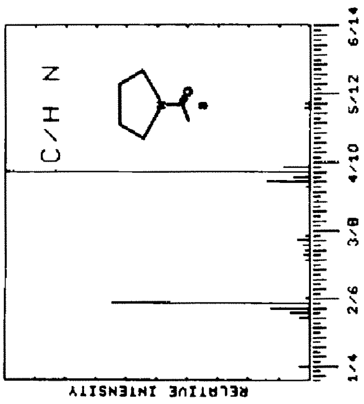
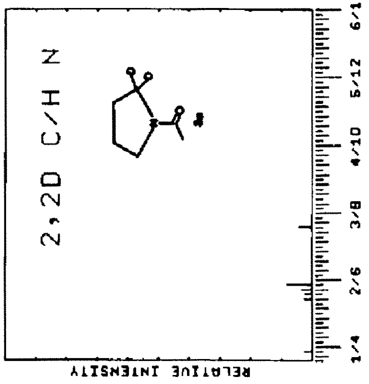
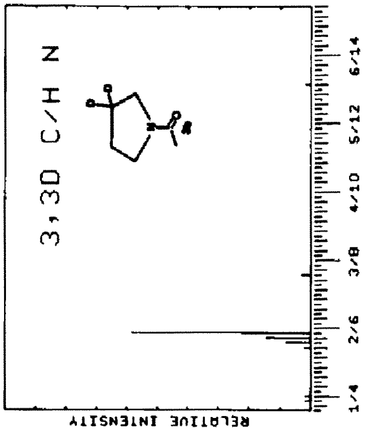
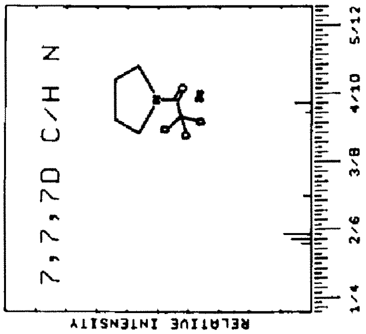


FIG. 3b Amino alcohol epimers, C/H N and C/H NO plots.


C/H N
RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
6/14


2,2D C/H N
RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
6/14


3,3D C/H N
RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
6/14


7,7,7D C/H N
RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12

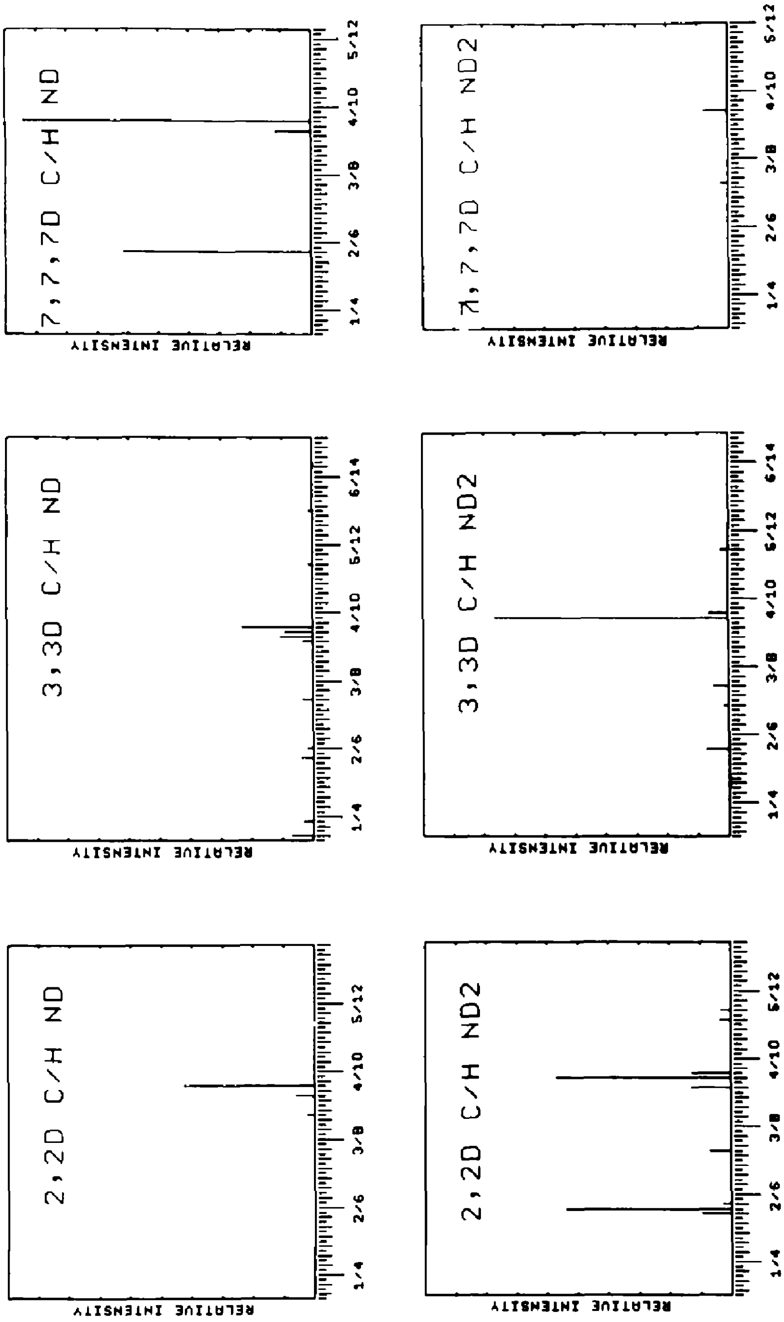
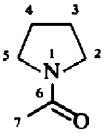


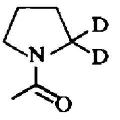
FIG. 4 C/H N plots of N-acetyl-pyrrolidine and deuterium labeled derivatives.

It may be pointed out that the graphs are in reality plotted against C_nH_{2n+1+N}, where N is the number of nitrogens in the elemental composition. A saturated amine, for example, contains one more hydrogen than its hydrocarbon analog, and its saturated fragment ions containing one nitrogen would fall at C_nH_{2n+2}.

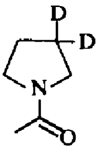
Another point of importance is the significance of the short vertical tic marks above certain of the peaks in presented spectra. This tic mark indicates a highly unsaturated ion (7 or more degrees of unsaturation) and is used where the peaks belonging to one major division interfere with the major division immediately below. For example, in Fig. 3b the peak falling at $C_{11}H_{24}N$ is marked with this tic mark. This merely indicates that the peak belongs to the C_{12} division and, since there are fourteen divisions between each major division, its composition is $C_{12}H_{12}N$. These interferences result because C versus H_{12} species have the same *nominal* mass. For compounds that would have both peaks present (11/24 N and 12/12 N in the above example), the highly unsaturated species can either be plotted separately or, in the case of a highly unsaturated molecule, plotted versus C_nH_n.



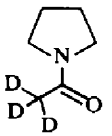
3



3a



3b



3c

The fourth example serves to indicate the powers of the combined methods of the heteroatomic plotting technique and the real-time data acquisition system[3,4] with its capability for extremely accurate mass and intensity measurement. In Fig. 4, the heteroatomic plots of N-acetylpyrrolidine, 3, and three deuterium labeled analogues, 2,2-d_2-N-acetylpyrrolidine, **3a**, 3,3-d_2-N-acetylpyrrolidine, **3b**, and 7,7,7-d_3-N-acetylpyrrolidine, **3c**, are presented.

Although the mass spectrometer employed (in the real-time mode) in these studies, Consolidated Electrodynamics Corporation 21-110B, is not capable of resolving the H_2–D doublet ($\Delta M = 0{\cdot}0015$ amu) at high masses, it is possible to measure the mass of single peaks with sufficient accuracy to determine the deuterium content of each peak. The plots (Fig. 4) presented (only the C/H N ions for this example) for the series of compounds 3, **3a**, **3b**, **3c**, were plotted considering deuterium as an additional heteroatom, with deuterium content assigned on the basis of calculated mass only. The two most intense peaks noted in this plot are at $C_4H_{11}N$, which formally represents the loss of the elements of C_2H_3O from the molecular ion, and C_2H_5N. The plots of the three labeled compounds allow an examination in some detail of the genesis of these peaks through the fragmentation of the molecular ion. The intensity basis for the plots for each compound is the sum of the contributions of all the ionic species yielding the peak corresponding to C_4H_8N in the unlabeled compound.

In conclusion, then, it is felt that a convenient method for presentation of the wealth of data contained in a complete high resolution mass spectrum has been developed. This method allows an easy visual appreciation of relative abundances in the fragmentation pattern, provides the degree of unsaturation (rings plus double bonds) of all the ionic species and information on the possible fragmentation processes leading to a particular ion, in addition to providing the elemental compositions of all the ionic species in the spectrum. Also, this method provides a standard format for the presentation of all high resolution mass spectral data, which will allow computer pattern recognition, inter-comparison of spectra and computer interpretation of data.

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