

$$R_i(\nu) = \frac{\sum a_{i,n} f_n(\nu)}{\sum f_n(\nu)} \quad (5)$$

The set of  $f_n(\nu)$ 's can now be obtained by matrix inversion of the set of spectral arrays. Here we use the set of coefficients  $a_{i,n}$  plus a line of 1's (corresponding to  $M(\nu)$ ) in the denominator and substitute a sliding row of spectral arrays  $M_i(\nu)$  in the denominator to obtain the set of component spectra.

In addition to  $\leq 0.01 \text{ cm}^{-1}$  spectral registration (forcing the use of a laser referenced interferometer type spectrometer), and a very good S/N ratio plus a built-in computer (again favoring the use of Fourier systems), an array oriented software is required to make this procedure convenient. (To the author's best knowledge, these requirements are met only by the Digilab FTS spectrometers).

The procedure used would then typically be: 1) The sample's spectrum is measured, the mixture left to evaporate for a while, and the measurement repeated. 2) The spectra are array ratioed, the number of flat regions of different height counted ( $N$ ), and their height measured ( $a_n$ ). 3) Assuming  $N = 2$ , one obtains the pure component spectra via the operations

$$f_1(\nu) = M_1(\nu) \frac{1}{a_1 - a_2} - M(\nu) \frac{a_2}{a_1 - a_2}$$

$$f_2(\nu) = M_1(\nu) \frac{1}{a_2 - a_1} - M(\nu) \frac{a_1}{a_2 - a_1}$$

and plots them under automatic scale expansion to avoid

scaling trail and errors.

This procedure for "unmixing" the spectra of mixtures requires enough memory space to store a minimum of  $N + 1$  spectra and a signal/noise ratio  $D\sqrt{N}$  times higher than desired in the final spectrum of the components, where  $D$  is the dynamic range between the absorption of the component and that of the mixture at the frequency for which the noise is specified (first-order approximation).

To experimentally demonstrate the procedure, a toluene-cyclohexane-hexane mixture was used. Figure 1 shows the separate reference spectra of the components, and Figure 2 that of their mixture. Figure 3 shows the ratio of the absorbances of this and another one where the more volatile constituents have become depleted, with only the flat regions shown. The insert shows in more detail the appearance of the complete plot and that of one such flat region.

The end result of the procedure described here shows the three separate spectra obtained for the components, Figure 4. Minor cross-contamination is evident in the spectra, but the components could readily be identified through them.

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## Identification of Characteristic Chromophores in Gas-Phase Ions by Photodissociation Spectroscopy

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**Photodissociation spectra are shown for parent ions of a number of hydrocarbons containing various patterns of unsaturation, and it is shown that the spectra are highly characteristic of structural features. Classes of ions readily identified include saturated hydrocarbons, compounds containing single (or isolated) double bonds, conjugated dienes, and benzene derivatives. Spectral differences arising from substituents and cyclic geometry are smaller but still significant. For all except the saturated hydrocarbon ions, the spectra are well understood in terms of previous theoretical work on  $\pi \rightarrow \pi^*$  transitions in radical ions.**

Optical spectroscopic approaches to characterizing gas-phase ionic species, of which the new technique known as ion photodissociation spectroscopy is notable, are interesting in their potential applicability to analytical problems. Photodissociation spectroscopy has the extremely low sample-quantity requirements and the automatic discrimination of components of mixtures which are common to mass spectrometric analytical methods; at the same time, it possesses the cardinal advantage of optical spectroscopic analysis in that the spectra are characteristic of the optical chromophores of the molecule, and therefore of its chemical structure. This approach thus unites attractive features

of mass spectrometry and optical spectroscopy in a way which may be complementary to both. The actual instrumental capability for achieving this is very recent, and it is the purpose here to describe the principles and techniques involved, and to illustrate with some examples the kinds of analytically useful information which are now available.

Direct determination of the optical spectrum of a gas-phase cation by observing its absorption spectrum is a straightforward and obvious approach, but, in practice, the difficulty of building up a usable concentration of gas-phase ions has restricted such measurements to a handful of very simple ionic species (1, 2). However, it has been increasingly recognized that a more indirect, and more tractable, approach to this problem through ion photodissociation gives spectra which are for many purposes equivalent to direct absorption spectra (3-10). The principle involved is that the dissociation of an ion by photon absorption, which can be readily observed, necessarily implies that the photon wavelength corresponds to an optical absorption of the ion. Thus the wavelength dependence of photodissociation is expected to show peaks corresponding to some or all of the allowed optical transitions, and this expectation is borne out in practice in a very satisfactory way.

Numerous other approaches have been described to the problem of identifying and distinguishing gas-phase ions in a more selective and powerful way than inspection of 70-eV

fragmentation patterns. Among those which have attracted recent attention are appearance potential measurements, gas-chromatographic pre-separation, collisional activation, metastable peaks, and selective ion-molecule reactions. The technique described here seems potentially attractive as a capability complementary to one or more of these other approaches; in particular, as an ICR technique, it would be fully compatible with and complementary to an analytical ICR facility based on high-resolution Fourier-transform ICR (11) with selective ion-molecule reaction capabilities (12). (See Ref. 12 for one of numerous illustrations of the possibilities of ion discrimination by reactivity.)

## EXPERIMENTAL

While other methods have been described, the techniques of ion cyclotron resonance (ICR) have dominated the study of ion photodissociation processes (13). The techniques of ICR spectroscopy have often been described and are widely known (14-16). For the present purpose, it is chiefly important that, under conditions where the trapping voltage and magnetic field are substantial and drift voltages are appropriate, the ICR cell functions as a very efficient ion trap. This phenomenon has been used in several geometries, all of which end up trapping ions for times very long compared with their rate of collision with neutral molecules; in our experiments, trapping times of several seconds to several minutes are normally used at pressures of the order of  $3 \times 10^{-8}$  Torr. The marginal oscillator detector of the ICR spectrometer monitors the relative abundance of the different ion masses, and, in particular, when light shines into the cell, the detector shows and measures the decrease in abundance of the parent ion and the increase in abundance of the photodissociation products. The *photodissociation spectrum* of a given ion is the spectrum obtained as a function of light wavelength by measuring the decrease in parent-ion ICR signal due to photodissociation as the wavelength of the irradiating light varies.

Although pulsed modes of operation have been used and have attractive features (17, 18) we have found it convenient to obtain spectra in a steady-state mode, in which ion production in the source is continuous and ions are observed continuously with a low rf level in the analyzer; presence of the rf observing field is found to have little effect on ion trapping times. Solution of the steady-state equations involving ion production, loss, and photodissociation indicate that the quantity  $W = (I_0 - I)/I$ , where  $I$  is the parent ion signal in the presence of light and  $I_0$  is the signal in the dark, is an appropriate measure of photodissociation rate (4).  $W$  is directly proportional to light intensity and also directly proportional to the ion trapping time, which is measured for each ion examined. Using measurements of the relative photon flux as a function of wavelength, and of the trapping time of each ion, the  $W$ 's measured can be converted to relative cross sections for photodissociation; reference to a known standard (toluene cation in our work) then gives absolute photodissociation cross sections vs. wavelength, and this is the form in which data will be presented.

The ionizing electron energy is always kept as low as practicable, usually about 1-2 eV above the reported appearance potential of the parent ion. In a typical experiment without special precautions, an amount of sample of the order of  $10^{-7}$ - $10^{-8}$  g is consumed, nearly all of which is pumped away by the ion pump. With special precautions against contamination, much slower pump rates allow much lower sample consumption. A Hg/Xe arc is used as the light source, and the wavelength is selected with a monochromator (200-600 nm, 13-nm bandwidth) or interference filters (400-810 nm, 10-nm bandwidth). Data come from the spectrometer as shown in Figure 1 for a portion of the 2,5-dimethyl-2,4-hexadiene cation spectrum. Correction for light intensity and trapping time and reference to the standard (the cross section for  $C_7H_8^+$  at 400 nm is taken (19) as  $5.2 \times 10^{-18}$  cm<sup>2</sup>) gives spectra such as those shown in Figures 2-5.

## RESULTS AND DISCUSSION

Photodissociation spectra were measured for representatives of several classes of hydrocarbons: acyclic olefins (Figure 2), acyclic dienes (Figure 3), cyclic olefins (Figure 4), and  $C_6$  saturated hydrocarbons (Figure 5). In general, the reported absolute values of the cross sections are probably good within a factor of two, higher accuracy being unne-

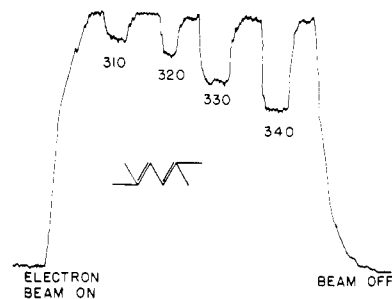


Figure 1. Typical data showing a small portion of the  $C_8H_{14}^+$  photodissociation spectrum.

Following the recorder trace from left to right: initially the electron beam is off and the marginal oscillator detector puts out its baseline level. The beam is turned on, and the level rises to the steady-state  $C_8H_{14}^+$  signal level. The decrease in steady-state  $C_8H_{14}^+$  signal is then determined for several wavelengths. Finally, the beam is turned off, and the rate of decay back to the baseline gives the ion residence time in the cell

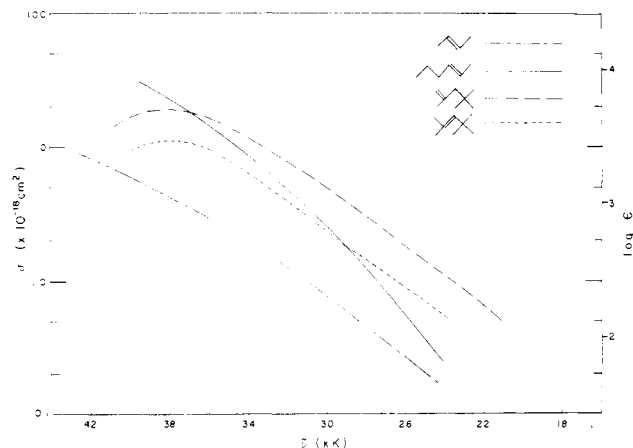


Figure 2. Photodissociation spectra of parent cations of several acyclic olefins

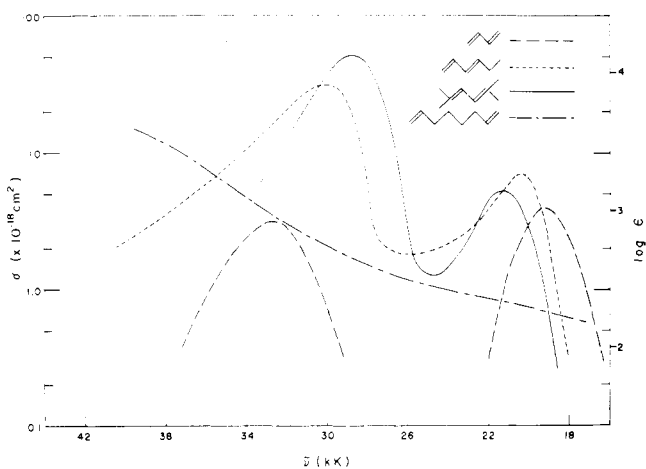


Figure 3. Photodissociation spectra of acyclic diene parent ions.

sary for the kind of qualitative comparison contemplated here. It should be stressed that what is obtained is a cross section for *photodissociation*; however, it may be illuminating to compare the numbers obtained with absorption data. For this purpose, there is indicated on the figures the values of molar absorptivity  $\epsilon$  which would correspond to the observed cross sections if the photodissociation and absorption spectra were equivalent. Since the photodissociation cross section must always be less than or equal to the optical absorption cross section, these  $\epsilon$  values are in fact *lower limits* to the true ion absorptivities.

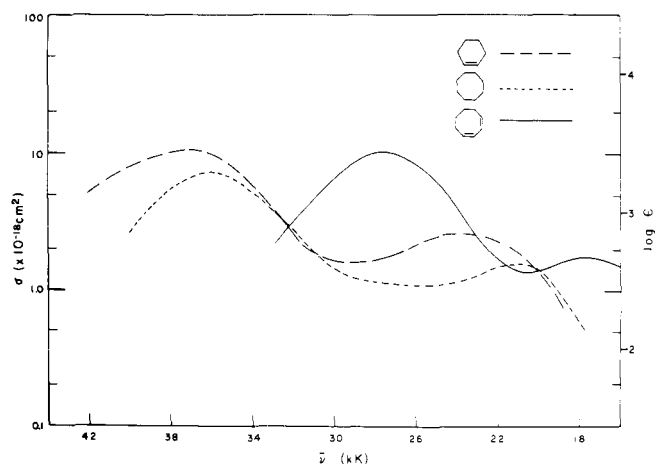


Figure 4. Photodissociation spectra of cyclic olefin parent ions

Most notable is the similarity between the spectra of ions containing similar chromophoric groups, and the dissimilarity of the spectra of the different classes of ions. The alkenes photodissociate only very weakly in the visible, but rise gradually to very strong peaks above  $36\,000\text{ cm}^{-1}$ . The conjugated dienes show a well defined peak of moderate intensity in the visible and a very strong peak around  $30\,000\text{ cm}^{-1}$  (in fact, the cross section measured for 2,5-dimethyl-2,4-hexadiene cation at  $28\,000\text{ cm}^{-1}$  is by far the largest photodissociation cross section yet measured). The two  $\text{C}_6$  saturated hydrocarbons have strikingly similar double-peaked spectra in the visible and no observed uv peaks. The cyclic compounds show spectra which are entirely consistent with the spectra of their acyclic analogs, (with, for the unsaturated compounds, some evidence for a red shift of the photodissociation peak). For the unsaturated species, our theoretical understanding of these spectra is on a satisfactory basis, and this is discussed in the Appendix.

### CONCLUSION

In terms of analytical utility, photodissociation would seem to have the possibility of immediate identification of certain characteristic functional groups in the ions comprising the mass spectrum of a complex compound: obviously, the capability illustrated here for the parent ion is applicable as well to fragment ions. There are two cases in the present set of spectra which illustrate well the potential for distinguishing isomeric structures which are difficult to distinguish on the basis of mass spectral fragmentation alone. One case is the contrast of the cyclohexane and hexene cation spectra, which are highly characteristic of their respective structural types, and show no evidence of interconversion of the isomers following ionization. The other case is the three isomeric ions of cyclooctene, 1,7-octadiene, and 2,5-dimethyl-2,4-hexadiene. The conjugated diene spectrum is totally distinct from the other two spectra; the positions of the peaks in the two isomers containing isolated double bonds are similar, although there is an observable red shift in the cyclic species, and with refinement of the instrument in the far uv, such differences could be utilized in an analytical context.

### APPENDIX

**Theoretical Interpretation of Radical Cation Photodissociation Spectra.** The discussion in the text is put on a phenomenological basis, and indeed it seems likely that much of the analytical usefulness of photodissociation spectra will be based on matching spectra of an unknown ion with a previously characterized species. However, for all of the ions considered except the saturated species, the interpretation of the positions of the peaks (and perhaps their intensities) can be put on a solid footing, as

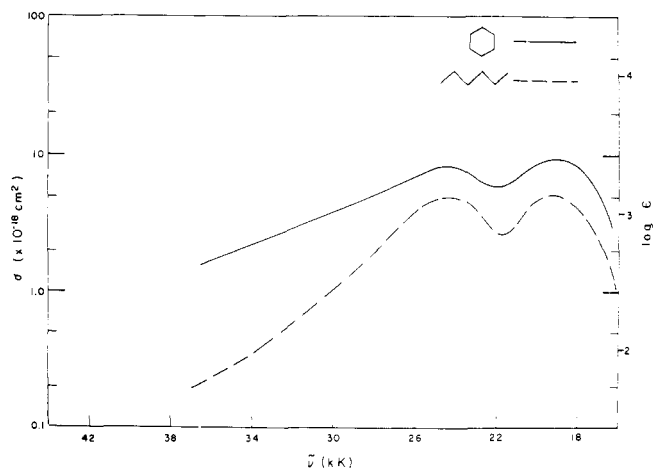


Figure 5. Photodissociation spectra of two saturated hydrocarbon parent ions

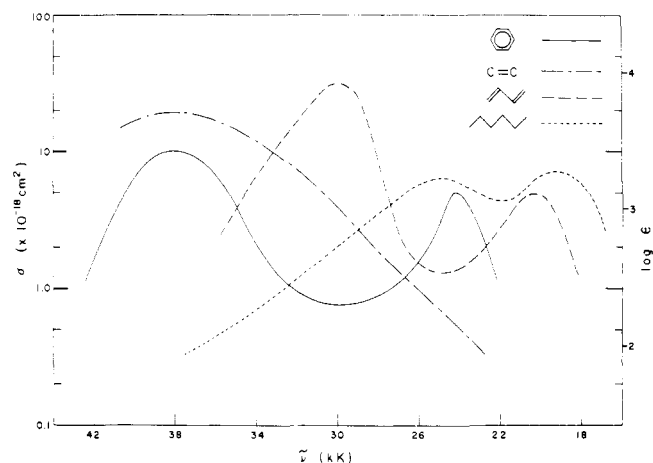


Figure 6. Typical photodissociation spectra for each of the classes of radical ions for which systematic data now exist

These spectral patterns and intensities can be substantially affected by substituents and cyclization, as is evident in Figures 2-5

briefly discussed below.

In all of the unsaturated ions, the ion ground state is a  $\pi$  state corresponding to removal of an electron from the highest occupied orbital, which is always a  $\pi$  orbital. Since there is no reason to suspect that  $\pi \rightarrow \sigma^*$  transitions will have intensities comparable to  $\pi \rightarrow \pi^*$  transitions, it is appropriate to apply to these ions the well developed theoretical understanding of  $\pi \rightarrow \pi^*$  spectra in radicals. For the ions containing only isolated double bonds, this is straightforward, since only one  $\pi \rightarrow \pi^*$  transition is possible in an isolated two-center, singly-occupied  $\pi$  orbital. In the neutral alkenes, the corresponding transition occurs above  $50\,000\text{ cm}^{-1}$ . In the cation, the photodissociation spectra indicate a single very broad, fairly intense absorption in the uv; in those cases where the data extend beyond the peak wavelength, the peak is in the vicinity of  $37\,000\text{ cm}^{-1}$ ; while in the other cases, the peak is an unknown higher energy. (The spectra do not suggest a peak above  $50\,000\text{ cm}^{-1}$  for any of the ions.) The very substantial red shift of the peak wavelength in going from the neutral alkenes ( $\lambda_{\text{max}}$  near  $60\,000\text{ cm}^{-1}$ ) to the radical cations is entirely in accord with theoretical expectation: in a Huckel-type calculation (20) for the parent ethylene system, the transition energy in the cation is  $2\beta$  ( $47\,000\text{ cm}^{-1}$ ), while configuration interaction and electron repulsion raises this value to  $61\,000\text{ cm}^{-1}$  in the neutral molecule. Thus, qualitatively, it is expected that the alkene radical cations should have  $\lambda_{\text{max}}$  below  $50\,000\text{ cm}^{-1}$ ; the quantitative aspects of substituent effects and the effects of cyclic constraints remain to be investigated.

In the more extended radical-ion  $\pi$  systems, transitions of several types are possible. Following the terminology of Shida and Iwata (21), the transitions which promote an electron from a filled orbital into the highest occupied orbital (half-filled in the ground-state ion) are denoted I transitions; these have no counterpart in the

neutral molecule. The transition promoting the single electron from the half-filled orbital to the lowest empty orbital is the A transition, and is analogous to the lowest-energy transition in the neutral. The remaining transitions involve several types of excited state configuration, and are all classed as B transitions; they lie at energies too high to be of concern here.

The conjugated dienes are expected to exhibit two peaks, corresponding to the lone I transition (in the visible), and the A transition (in the uv) (21, 22). (Note that within the  $\pi$ -electron approximation methods, the radical anion and the radical cation of alternant hydrocarbons should have identical spectra by the well known "pairing theorem.") As has been discussed in detail (8), the observed spectrum of butadiene cation agrees excellently with calculation in the position of these two bands. It is apparent from Figures 3 and 4 that substituents (and possible cyclic constraints) have a substantial effect on the peak positions, and again these effects remain to be studied in detail. Calculation on the conjugated diene system suggests that the uv peak should be substantially more intense than the visible peak (21, 22), and while this is not true for butadiene cation, it is obviously true for the substituted butadiene ions. While it has been seen that the intensities of photodissociation peaks need not correlate with absorption intensities this observation offers some slight encouragement in thinking that the observed intensities may be qualitatively meaningful.

It is known that saturated hydrocarbons with several carbons have a multitude of occupied molecular orbitals within a few eV of the highest occupied orbital (23); since all of these can give rise to I-type transitions in the cation, with no immediately apparent selection rules, the interpretation of spectra such as those in Figure 6 is likely to be a difficult task. The spectra are included here to illustrate the possibly surprising fact that the saturated hydrocarbon cations are quite strongly colored.

The two peaks in the spectra of benzene cation derivatives arise again from the lowest I transition (visible) and the A transition (uv; this degenerate configuration splits to give several predicted peaks, of which only the lowest is observed in this work.) These spectra agree very well with calculations, as has been discussed in detail for toluene cation (8).

#### ACKNOWLEDGMENT

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## Mass Fragmentographic Assay for 11-Hydroxy- $\Delta^9$ -tetrahydrocannabinol from Plasma

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**A mass spectrometric assay for 11-hydroxy- $\Delta^9$ -tetrahydrocannabinol is presented. It is sensitive to 3 ng/ml of plasma and has a relative standard deviation of 4%. The technique relies on the derivatization of the phenol moiety by extractive alkylation. This reaction is novel to the cannabinoid series. Since all cannabinoids are phenols, this procedure may have wide utility. It was possible to monitor 11-OH- $\Delta^9$ -THC in dog plasma for 2 h after its administration. However, no 11-OH- $\Delta^9$ -THC was detected in dog plasma after oral or intravenous administration of  $\Delta^9$ -THC.**

The understanding of cannabis pharmacology is difficult because of the unresolved question of the existence and/or the nature of an active metabolite. A metabolite proposed to be active is 11-hydroxy- $\Delta^9$ -tetrahydrocannabinol (11-

OH- $\Delta^9$ -THC) (1). The elucidation of the biochemical pharmacology of a drug has been predicated upon the availability of assays capable of measuring the drug in plasma and/or serum (2, 3). Assays based on mass fragmentography for  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) have been reported by Agurell et al. (4) and Rosenfeld et al. (5) These were found to be sufficiently sensitive for use in pharmacokinetics in human studies. To date, there has been no reported mass fragmentographic assay for 11-OH- $\Delta^9$ -THC.

The mass fragmentographic assays reported for  $\Delta^9$ -THC differed in the methodology of the incorporation of deuterium for use as an internal standard as well as in the extraction procedure. Agurell synthesized  $\Delta^9$ -THC labeled at the 1', 2' positions and used an LH-20 column to purify the sample. The McMaster group utilized 1-O-perdeuteriomethyl- $\Delta^9$ -tetrahydrocannabinol (1- $\text{OCD}_3$ - $\Delta^9$ -THC) and derivatized their extract using *N,N,N*-trimethyl anilinium