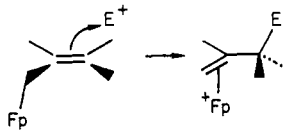
tonation of this substance regenerates the cationic olefin complex (**4a**), alkylation of **6a** with $Me_3O^+BF_4^-$ results in the exclusive formation of the methoxy diene complex (**7**, 24%).

The high reactivity of Fp(vinyl ether) complexes ensures stereospecific and regiospecific alkylation of kinetically generated enolates, free of complications due to equilibration of the enolate or to proton exchange.[5,13] Thus, enolate **1b**[14] gave adduct **3b** (90%) as a single stereoisomer[6] on reaction with **2** (THF, −78 °C, 1 h). This substance is assigned trans C-2,6 stereochemistry, assuming preferential axial attack of the vinyl cation on the enolate anion.[15] Treatment of **3b** with $HBF_4{\cdot}Et_2O$ (CH_2Cl_2, −78 °C, 0.5 h), followed by decomposition of the salt **4b** with NaI, gave *trans*-2-vinyl-6-methylcyclohexane **5b**[9,16] in 79% overall yield from **1b**. The sequence thus allows the isolation of the isomer, which is thermodynamically disfavored on conformational as well as on structural grounds.

Surprisingly, only partial isomerization of **4b** to the cis isomer **8** may be achieved through deprotonation (Et_3N, CH_2Cl_2, 25 °C, 2 h) to **6b**,[17] followed by reprotonation ($HBF_4{\cdot}Et_2O$, CH_2Cl_2, −78 °C, 0.5 h). The product, 78% yield after demetalation (NaI, acetone, 25 °C, 0.5 h), is a mixture of *cis*- and *trans*-2-vinyl-6-methylcyclohexanones (**9** and **5b**) in a ratio of 1:2.[18] Protonation, unlike the alkylation of **6a**, may take place preferentially at C-2 rather than at the carbonyl oxygen, since in the latter circumstance tautomerization of the resulting dienol complex (**7**, H in place of Me) would be expected to give the more stable cis-2,6-disubstituted cyclohexanone. The ratio of cis and trans isomers may instead reflect stereochemical control of kinetic protonation at C-2 through the spatial orientation of the Fp group, which is known to direct electrophillic attack in (η^1-allyl)-[19] and (η^1-propargyl)Fp[20] complexes trans to the Fp–C bond (eq 4).



(4)

The reactivity of **2** is sufficiently great that reaction is not measurably impeded by full substitution at the enolate carbon center. Thus, the enolate **1c**[21] reacts smoothly with **2** (THF, −78 °C, 1 h) to give the adduct **3c** in 90% yield as a 2:3 mixture of stereoisomers.[22] Protonation of this product ($HPF_6{\cdot}Et_2O$, −78 °C, 0.5 h), followed by treatment of the resulting salt **4c** with NaI (acetone, 25 °C, 0.25 h), gave the free enone **5c**[7a] as a colorless oil (88%).[9,23]

Finally, the introduction of a *trans*-propenyl group at C-2 in 2-methylcyclohexanone is readily achieved by using the electrophile complex **10**. This substance is prepared from 2-bromopropionaldehyde diethyl acetal by metalation with NaFp, followed by treatment of the product with HBF_4,[4] and is obtained exclusively as the cis isomer.[24] Reaction of enolate **1c** with cation **10** (THF, −78 °C, 1 h) gave the adduct **3d** as a yellow oil. This was converted to the salt **4d** ($HBF_4{\cdot}Et_2O$, CH_2Cl_2, −78 °C, 0.5 h) and then to the free enone (NaI, acetone, 25 °C, 0.5 h). Kugelrohr distillation gave the product in 78% overall yield as a colorless oil.[9,27] Spin decoupling shows the product to be entirely the *trans*-propenyl ketone, a stereochemical outcome expected for preferential trans addition of the nucleophile to the Fp(olefin) cation[28] and trans elimination of ethanol from the adduct.[11]

We are currently examining some further elaborations and synthetic applications of these reactions.

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(16) NMR ($CDCl_3$) δ 6.3–5.8 and 5.2–4.8 (ABX multiplets, CH=CH_2), 1.02 (d, J = 6 Hz, CH_3).
(17) NMR ($CDCl_3$) δ 7.15 (t, 1, J = 9 Hz, CH=).[6b]
(18) Methyl doublet; δ_{cis} 1.07, δ_{trans} 0.97. Compare the C-2 methyl-doublet resonances in 2,3-dimethylcyclohexanone; δ_{cis} 0.93, δ_{trans} 0.97: Pfeffer, P. E.; Osman, S. F. *J. Org. Chem.* **1972**, *37*, 2425.
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(22) The isomers were separated by TLC: NMR (major isomer, $CDCl_3$) δ 4.80 (s, 5, Cp), 3.75 (m, 4, CH_2O, CHO), 2.40 (m, 2, CH_2CO), 2.2–1.5 (m, 8, CH_2), 1.17 (t, 3, J = 7 Hz, CH_3), 1.06 (s, 3, CH_3); (minor isomer, $CDCl_3$) δ 4.87 (s, 5, Cp), 3.70 (m, 3, OCH_2, OCH), 2.45 (m, 2, CH_2CO), 1.70 (m, 8, CH_2), 1.22 (t, 3, J = 7 Hz, CH_3), 1.15 (s, 3, CH_3).
(23) NMR ($CDCl_3$) δ 6.02 (q, 1, $|J_{Ax} + J_{Bx}|$ = 28 Hz, CH=), 5.1 (t, 2, $|J_{AB}|$ = 1 Hz, CH_2=), 2.4 (m, 2, CH_2CO), 1.21 (m, 6, CH_2), 1.18 (s, 3, CH_3).
(24) The formation of the cis isomer in this sequence is most likely the result of thermodynamic rather than kinetic control as had earlier been proposed.[4] The rotational barrier about the putative double bond in these vinyl ether complexes is relatively low (<25 kcal/mol),[25] and hence, cis–trans isomerization should be facile. Moreover, cis olefin complexes of several transition metals are generally found to be more stable than their trans isomers.[26]
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A New Soft Ionization Technique for Mass Spectrometry of Complex Molecules

Sir:

In a number of areas of organic and biochemical research, there is a growing need for high mass, high sensitivity mass spectrometry applicable to thermally labile molecules of low volatility.[1] In particular, it is often very important to obtain easily identifiable ions characteristic of the intact molecule so that the molecular weight can be determined. In response to this need, several new techniques have been developed. These include field desorption,[2] chemical ionization,[3] plasma desorption,[4] laser desorption,[5] and organic SIMS.[6] All of these techniques show some promise for at least partially fulfilling the above-stated need, but only the first two have as yet been widely applied. We have recently been engaged in developing a new combined liquid chromatograph–mass spectrometer (LC–MS) system suitable for application to involatile molecules. In the course of this work, we have discovered a new ionization technique which appears widely applicable to mass

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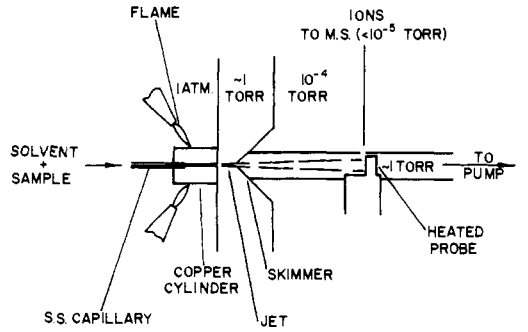

Figure 1. Simplified schematic diagram of the experimental apparatus. The approximate pressures in the differentially pumped vacuum chambers are indicated on the diagram.

spectrometry of involatile molecules. The purpose of this communication is to present the first results from this new ionization technique.

A simplified schematic diagram of the apparatus is shown in Figure 1; a more detailed description of the LC–MS system and its performance is given elsewhere.[7] Briefly, the effluent from the LC enters the vaporizer through a stainless-steel capillary tube (0.015-cm i.d.) which is partially immersed in a copper cylinder heated to ca. 1000 °C by four small oxy–hydrogen flames. As a result of rapid heating, a jet of vapor and aerosol is produced near the exit from the stainless-steel tube. The jet is further heated as it passes through the 0.075-cm diameter channel in the copper; it then undergoes an adiabatic expansion, and a portion passes through the skimmer to the ion source where the beam impinges on a nickel-plated copper probe which is electrically heated to ca. 250 °C. The ion source is equipped with an electron gun for producing ions for normal CI operation, but for this work it was turned off. Under typical operating conditions, about 95% of the liquid is vaporized, and the remainder is in the form of a highly collimated particle beam which is accelerated to approximately sonic velocity by the rapid expansion of the vapor. In our present geometry, these particles typically have a mass of about 10^{-7} g and carry a charge of ca. 5×10^{-12} C each; we estimate their velocities at about 10^5 cm/s. Involatile samples appear to be carried preferentially by the particles, and our preliminary results indicate that at least 80% of an involatile solute, e.g., adenosine, is transmitted to the ion source with about 5% of the solvent. Both positively and negatively charged particles are produced, and both the magnitude of the charge and the relative numbers of positive and negative particles depend on the properties of the solvent and on the vaporization conditions. The best conditions for the new ionization technique in our preliminary studies involve the use of 0.2 M formic acid as the solvent at input flow rates in the range of 0.5–1 mL/min. Under these conditions, the current measured to an electrically isolated probe inside the ion source and at source potential is typically $+10^{-8}$ A average; the current is modulated at a frequency of about 2000 Hz, which apparently corresponds to the arrival rate of individual particles.

When the charged particle undergoes a high-energy impact with the heated probe, it is wholly or partially vaporized, and some of the resulting molecules are ionized. The ions observed by using a quadrupole mass filter are primarily the protonated or cationized species produced by chemical ionization; however, it appears that much less energy is transferred to the ions in the present technique as compared to the more conventional CI technique. For example, the spectrum of adenosine 5′-monophosphate (AMP) obtained by the new technique is shown in Figure 2. The protonated molecular ion is not observed by conventional chemical ionization, but spectra very similar to that shown in Figure 2 are obtained by field desorption.[8] The spectrum shown in Figure 2 was obtained in a single scan from 10 to 600 amu in 4 s; the peaks below

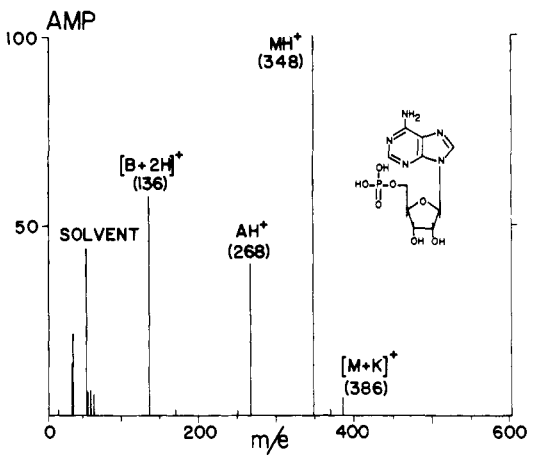

Figure 2. Positive ion mass spectrum of AMP obtained by the new technique. The sample of AMP (20 μL of a 0.1 mg/mL solution) was injected by using the LC without column as an inlet system. Liquid phase was 0.2 M formic acid at a flow rate of 0.5 mL/min, and the spectrum was scanned from 10 to 600 amu in 4 s.

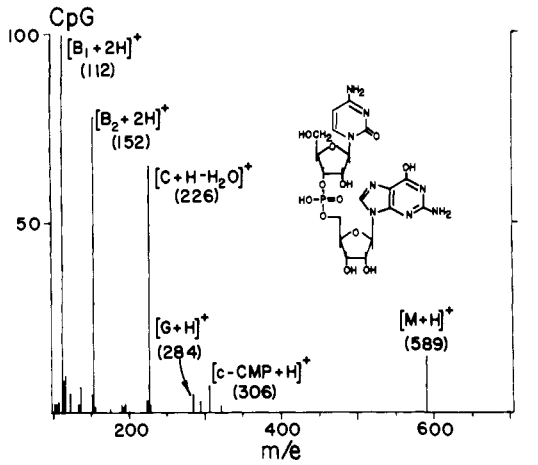

Figure 3. Positive ion mass spectrum of the dinucleotide CpG obtained by using the new ionization technique and scanning from 100 to 700 amu in 4 s. Inlet conditions as given in Figure 2.

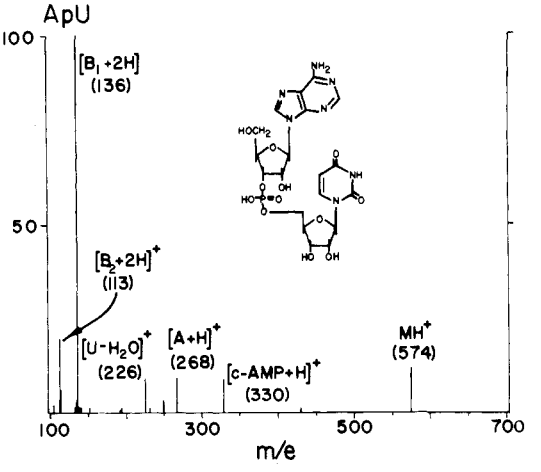

Figure 4. Positive ion mass spectrum of the dinucleotide ApU under the same conditions as in Figure 3.

mass 100 are background ions due to the solvent. When one considers that the concentration of the sample was about 10 ppm relative to the solvent, the ratio of sample ions to background ions is amazingly high. The behavior of AMP by this new ionization technique is fairly typical of all of the nucleosides and nucleotides investigated to date. In all cases, intense MH^+ ions are observed together with a few structurally significant fragments.

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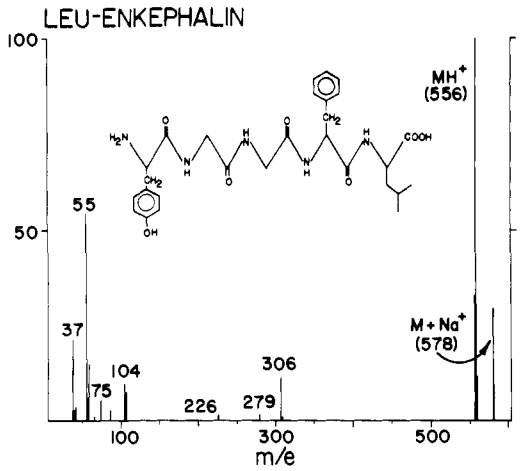

Figure 5. Positive ion mass spectrum of the pentapeptide leucine enkephalin by the new ionization technique.

Spectra obtained on two dinucleotides are shown in Figures 3 and 4. These spectra are very similar to those obtained by Schulten with field desorption.[9] As shown in the FD work, these spectra are sufficient to uniquely determine the structure of the dinucleotide. In particular, the protonated cyclophosphates (mass 306 for CpG and mass 330 for ApU) are observed for the nucleoside in position 1, but the corresponding cyclophosphate containing the other nucleoside (mass 346 for CpG and mass 307 for ApU) is not detected.

The new ionization technique has been applied to a number of other interesting classes of compounds. All of the common amino acids have been investigated as well as several di- and tripeptides and one pentapeptide. A mass spectrum of the pentapeptide, leucine enkephalin, is shown in Figure 5. This spectrum is typical of all the amino acids and peptides investigated in that the protonated molecular ion is the base peak in the spectrum; a small amount of alkali addition also occurs, but very little fragmentation is observed. The di- and trisaccharides which have been studied give only $M + Na^+$ and $M + K^+$ with very little fragmentation and no protonated molecular ions. Preliminary studies have been conducted on a number of other interesting systems, including antibiotics, vitamins, and fatty acids. In general, protonated and/or cationized molecular ions are observed as major peaks in the spectra. More detailed results will be presented later.

At present, the overall ionization and detection efficiency for the new ionization technique is between one and two orders of magnitude lower than for conventional chemical ionization in our apparatus; however, the background ionization of the solvent is lower by 4 or 5 orders of magnitude in the new technique. This latter fact coupled with the reduced amount of fragmentation that is generally observed led to the surprising result that the detection limit for the protonated molecular ion of most of the involatile substances investigated is substantially lower with the electron beam turned off! Typically, sample input rates in the range of 1–10 ng/s are sufficient to obtain reproducible, "clean" spectra when scanning at rates on the order of 100 amu/s over the full spectral range. Higher sensitivities are obtained by using single-ion monitoring or scanning over a limited mass range. Some results obtained for adenosine by scanning over a 5-amu range centered on the MH^+ ion at mass 268 are shown in Figure 6. In these experiments, 200 pg injected gave an integrated response about five times the noise level.

Clearly, a large amount of additional work will be required before the full value of the new ionization technique can be determined. Some of the details of the ionization process are not yet fully understood, and it is unlikely that our present techniques and apparatus are optimum. Even at the present primitive stage of development, the new technique appears to provide many of the advantages of field desorption or plasma desorption without some of the disadvantages. In particular, the new technique is compatible with on-line LC; it is fast, sensitive, relatively simple, and inexpensive.

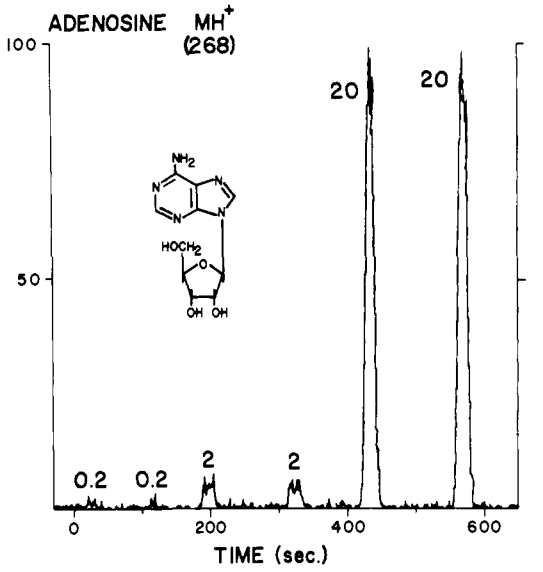

Figure 6. Response of the mass spectrometer by using the new ionization technique to a series of injections of adenosine. The plot gives the total ion current (arbitrary units) measured by scanning (1 s) over a 5-amu range centered on the MH^+ ion at mass 268. Each evolution peak is labeled with the quantity of adenosine injected in ng. The peaks are 20-s wide fwhm, and the noise level between peaks summed over the width of a peak corresponds to the extrapolated response for 40 pg.

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Time-Resolved Proton Magnetic Resonance Studies of Polynucleotides

Sir:

Examination of the proton magnetic resonance spectrum of yeast transfer RNA^{Phe} by the spin–echo sequence gives a simplified subspectrum with enhanced resolution. The normal proton magnetic resonance spectrum of biopolymers generally shows few resolved resonances because of the line widths of individual resonances and because of the very large number of proton resonances in the spectrum. As a result, much of the information intrinsically present in the proton magnetic resonance spectrum is unavailable. In essence, the problem is one of separability, how to separate and separately access information from single resonances. The 360-MHz proton magnetic resonance spectrum of yeast $tRNA^{Phe}$ shown in Figure 1 illustrates this problem.[1] Focusing, for example, on the 7–9-ppm region where resonances from the adenine A8 and A2, guanine G8, cytosine C6, and uracil U6 protons are found,[2] it can be seen that there are only a couple of resolved or partially resolved resonances at the high- and low-field extremes of the region plus some sign of structure throughout the main body of the feature, a poorly resolved spectrum. However, just as the

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