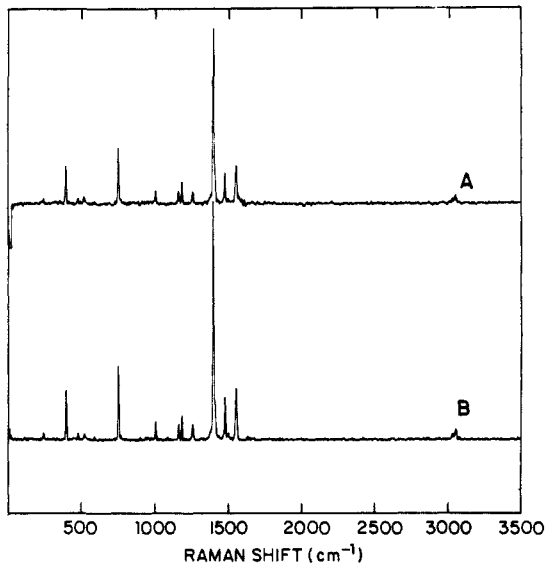

Figure 11. (A) Subtraction result from Figure 10. (B) FT-Raman spectrum of anthracene.

Fluorescein, respectively. As a further example, the material whose Raman specrum is shown in Figure 3 has a measured fluorescence quantum yield of 1% at 5145 Å. The ability to use higher power levels in the near-infrared coupled with the multiplex and throughput advantages of the interferometer compensates for the loss in scattering cross section. This approach offers a universal solution to the problem of fluorescence in Raman spectroscopy.

An additional advantage offered by interferometric Raman spectroscpy is the possibility of spectral subtraction. The frequency precision of a laser referenced interferometer is quite high, approximately 0.01 cm^{-1}. This high precision ensures that two spectra can be arithmetically combined without introducing artifacts or features due to a lack of frequency registration. This process of spectral subtraction has been done previously in conventional Raman spectroscopy, but great care must be taken in instrument alignment and calibration. Figure 10A shows the FT-Raman spectrum obtained on a physical mixture of anthracene and bis(phenylimino)terephthaldehyde (30:70). The spectrum shown in Figure 10B is that of pure bis(phenylimino)terephthaldehyde.

By adjusting a subtraction factor to remove features in the composite spectrum which are due solely to component A, the spectrum shown in Figure 11A was obtained. Figure 11B is a reference spectrum of pure anthracene. The agreement between the subtraction result and the reference is excellent.

Conclusions

It has been shown that by using a multiplexing spectrometer and a near-infrared laser, high-quality Raman spectra can be obtained in reasonable measurement time. The problem of fluorescence background is totally circumvented with this approach, and the problem of thermal decomposition is minimized since the only absorption mechanism involves excitations of vibrational overtones and combinations. The high frequency precision of the interferometric instrument allows accurate spectral subtractions to be performed. The problem of effective Rayleigh scatter removal can be addressed by the use of multistage dielectric filters, and Raman data can be obtained down to within 250 cm^{-1} of the exciting line.

Registry No. BPT, 14326-69-3; tantalum cyclopentadienyl tetrachloride, 62927-98-4; anthracene, 120-12-7; poly(*p*-phenylene terephthalamide), 24938-64-5; rhodamine, 12676-92-5; fluorescein, 2321-07-5.

Transmethylation Reactions of L-Carnitine in Energized Condensed Phase[1]

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Abstract: The FAB spectra of L-carnitine negative ions provide unique data concerning the chemistry of the sampled molecule in energized condensed phase which can be correlated to some extent with those obtained in vivo or under conventional laboratory conditions. Demethylation of the ammonium moiety and Hoffman degradation are the base induced processes preferentially populated, while the relative intensity of $(M - H)^-$ is negligible. The chemistry of the demethylated anions in the gas phase has been investigated by MS/MS techniques.

Results and Discussion

The use of mass spectrometry in analyzing large involatile molecules[2-5] has been facilitated by the introduction of desorption ionization techniques such as FAB[6]. Since the formation of secondary ions depends on the chemistry of the analyte/solvent system in the energized condensed phase[7], a desorption ionization spectrum displays not only protonated or deprotonated molecules but also ionic species formed both by unimolecular processes in the gas phase and by bimolecular interactions in the condensed layers.[7-9] Because of the widespread use of desorption ionization methods in analyzing polar materials it has become essential to

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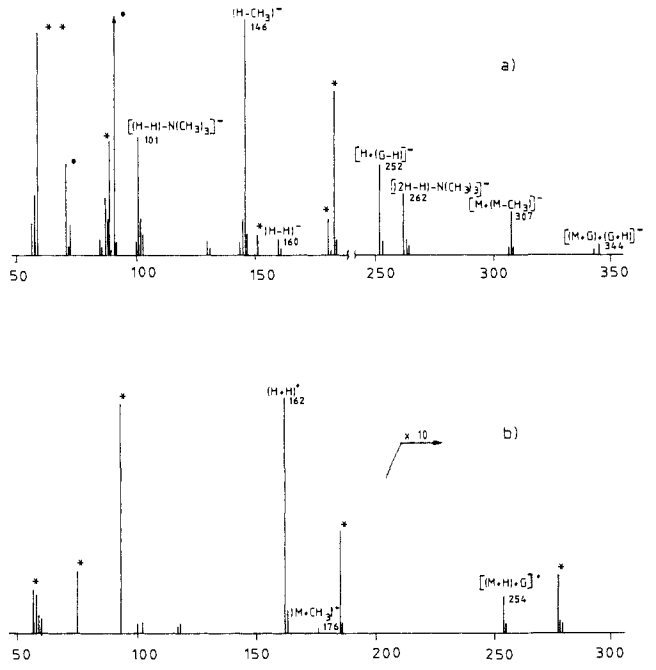


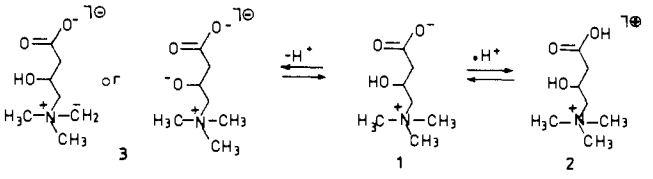
Figure 1. FAB spectra of L-carnitine: (a) negative ions (v^-); (b) positive ions (v^+). *, glycerol background; **, m/z isobaric ions.

recognize those reactions occurring in other than the gas phase. In order to carry out a systematic study of these processes it is therefore necessary to determine the mechanism whereby ions formed in collisional sputtering are ejected. The correlation between surface properties and FAB mass spectral response provides some experimental evidence concerning the origin of the ionic species observed.[10] A recent theoretical model which interprets sputtering as a surface effect[11] has proved to be in good agreement with both experimental evidences[12] and computer-simulated processes.[13] It has been established that the characteristic depth of origin of the sputtered species is equal to the elastic collisional mean-free-path of the species moving toward the surface.[11] It is now clear that those species which leave the target have their origin in the first monolayer and that their charge is determined at the point when they are released into the gas phase.[14,15] It can therefore be excluded that a concentration of ionic species can be built up on the matrix. This means that it is necessary to use an on-line system to determine the structure of the charged particles which leave the surface: a mass spectrometer can provide reliable and exhaustive data concerning the structure of the ions thus produced.

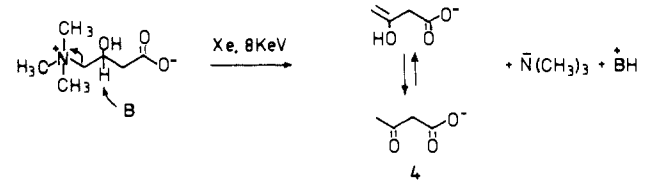
The chemical processes which occur in the condensed phase and lead to well-defined products are not therefore to be considered a limitation of desorption ionization techniques but rather a source of additional reactivity data whenever those reaction paths populated can be interpreted by means of the classic tools of synthetic organic chemistry.

L-Carnitine (**1**) or γ-trimethylammino-β-hydroxybutyrobetaine, known also as vitamin B_T, is a biologically important molecule involved in many methabolic pathways. Evidence from X-rays[16] and NMR studies[17] both suggest interesting correlations between

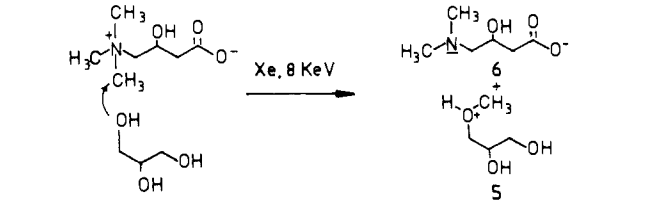
Scheme I



Scheme II



Scheme III



the structural specificity of L-carnitine and its biological activity. Furthermore, in vivo experiments have clearly shown that **1** acts as a donor of "labile" methyl groups, while no acceptor activity has been detected.[18]

The spectrum of positive ions (v^+) obtained in SIMS experiments[7] from the hydrochloride salt of compound **1** displayed the intact cation at m/z 162, some fragments at low mass unit which were mainly formed by degradation of the ammonium moiety, and a peak at m/z 176 (13%) produced by intermolecular methylation. When glycerol solutions of zwitterionic L-carnitine were analyzed under FAB conditions in the positive mode, it was found that at different glycerol-to-sample (G/S) ratios,[10] the intensity of the transmethylated product $(M + CH_3)^+$ ranged from 0.9 (G/S = 0.5) to 13% (G/S = 0) of the base peak which was always represented by the $(M + H)^+$ cation at m/z 162. Under the same experimental conditions, the spectrum of the negative ions (v^-) showed m/z 146 (base peak) and abundant fragments at m/z 101, 87, and 59, whose relative intensity was to some extent influenced by the G/S value. The deprotonated anions at m/z 160 corresponded to 6–7% of the base peak for different G/S values, while it reached 12% in the absence of a matrix.

MS/MS experiments (v scans) confirmed that m/z 146 was not formed from m/z 160, at least when long living species were sampled. It would be in any case difficult to devise a reasonable mechanism to drive a 14 mass unit loss unimolecularly from gaseous $(M - H)^-$ precursors. The v^- spectrum recorded at high G/S values also displayed many structurally distinct clusters such as $(M + nG)^-$, $[(2M - H) - N(CH_3)_3]^-$, $(2M + nG)^-$, and $[M + (M - CH_3)]^-$. The latter was still present in the spectra obtained when the glycerol matrix was absent.

The v^+ and v^- FAB spectra of carnitine are therefore considerably different in so far as the Brønsted and Lewis type acid–base equilibria are concerned. To obtain stable quasimolecular ions from zwitterionic compound **1** would require a proton-transfer process leading either to one of the two opposite charges being neutralized or to a new one being created. The very close similarity between the v^+ spectra of **1** and that of its hydrochloride would seem to suggest that in the first case a straightforward neutralization of the carboxylate moiety takes place (Scheme I). On the other hand, the $(M - H)^-$ deprotonated molecule of carnitine must contain one positive and two negative sites. MS/MS applications on FAB-induced gaseous oligonucleotides have shown

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that multiple charged species, where $(n - 1)$ positive sites balance n negative ones, can be produced and released into the gas phase.[5] Therefore the low intensity of $(M - H)^-$ ions observed must be connected to the particular chemistry of the molecule under investigation.

Some of the possible base-induced processes undergone by carnitine in the condensed phase are shown in Scheme I. The position of the equilibria involving species **1**, **2**, and **3** is determined by the reactivity of the system under the particular experimental conditions adopted. Simple thermodynamic considerations suggest that the energy profile leading to species **2** must be much easier to obtain than that leading to ions of type structure **3**. The action of a base on substrate **1** in the same environment could also populate a classic Hoffman-type 1,2 elimination leading to a degradation of the molecule into trimethylamine and acetoacetate anions **4** (Scheme II). A very abundant peak ranging from 60% (G/S = 2.7) to 27% (G/S = 0.03) was in fact present at m/z 101 in the v^- spectrum of **1** which was formed neither unimolecularly nor by collision-induced decomposition from gaseous $(M - CH_3)^-$ ions at m/z 146. The same species was also formed (31%) when carnitine was analyzed in the absence of the glycerol matrix. Of all the possible reaction channels driven by proton abstraction from compound **1**, that leading to a Hoffman degradation is preferentially populated with the result that **4** is afforded at m/z 101 (Scheme II). The antiperiplanar configuration preferred between the leaving amine group and the approaching base can be much more easily obtained in dilute solutions (G/S = 2.7).

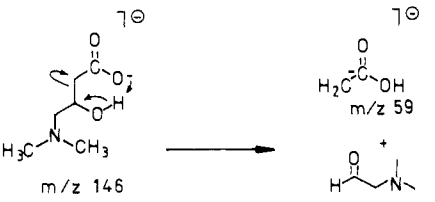
These results indicate that of all the possible Brønsted type equilibria induced in the energized condensed phase, those actually populated reflect the expected chemistry of the sampled molecule.

A similar argument can be applied to the transmethylation mechanism where the formation of $(M - CH_3)^-$ and $(M + CH_3)^+$ is driven by bimolecular nucleophilic displacement processes in the form of Lewis-type equilibria. Interestingly enough, $(M - CH_3)^-$ anions, at m/z 146, always represent the base peak of the v^- spectra, while the corresponding $(M + CH_3)^+$ species at m/z 176 can hardly be distinguished from the background of the v^+ spectra recorded under similar experimental conditions (G/S = 0.5; m/z 176 = 0.9%). The formation of the methylated cations requires interactions between two carnitine molecules, as clearly indicated by the enhancement of m/z 176 in concentrated solutions or in the absence of a matrix. On the other hand, the demethylation process seems to be almost independent of the concentration of the analyte. The only source of a "labile" methyl group can be the trimethylammonium moiety of **1**. But the competitors for this electrophile are all those functional groups of the molecules exposed to bombardment which can carry on a nucleophilic activity. Mass spectrometric evidence suggests that the most probable site of methylation is the carnitine hydroxyl group:[7] under the same experimental conditions, the hydroxyl group of a glycerol molecule could interact with **1** thus releasing both $(M - CH_3)^-$ and the methylated species **5** which undergoes proton exchange in the same environment (Scheme III).

The data obtained from v^+ and v^- spectra are therefore complementary and together provide a complete picture of the reactivity of the sampled molecule under the particular experimental conditions adopted. A methyl group can easily be transferred from carnitine to a suitable acceptor whereas the methylation of the same substrate corresponds to a less competitive reaction channel than that of other processes with lower activation energy. Similar results have been obtained in vivo with use of nicotinammide as a nucleophile in the presence of vitamin B as catalyst. A similar action is also exhibited by tetramethylammonium iodide, choline, and other classic biologically methylating agents.[19]

From the data discussed above it follows that the reaction conditions experienced by a solvated species exposed to fast-atom-bombardment are quite similar to those typical of a conventional laboratory experiment. Therefore, a kinetic energy atom beam of a few keV can be used to induce bimolecular reaction on model compounds which are similar to their thermally allowed processes. Furthermore, a full exploitation of the facilities of a "mass spectrometer laboratory"[20] enables the structure of the ionic products thus formed to be determined on-line. The demethylated carnitine anions sputtered from the target have in fact been characterized by MS/MS experiments on the m/z 146 species.

Scheme IV



The metastable ion (MI) spectrum of m/z 146 showed a single daughter ion at m/z 59 which may have been formed by a formal retro-Claisen condensation process (Scheme IV). The same reaction path is also populated by the fast-reacting species (Figure 1a). However, the fragment thus produced overlaps with isobaric species formed from the $(M - H)^-$ quasimolecular ions of glycerol. Although the contribution of the second transition could easily be determined, it is nonetheless clear from the above reported MIKE experiments that the reaction reported in Scheme IV must certainly occurs from the $(M - CH_3)^-$ precursors in the gas phase.

The collision-induced dissociations (CID) of the parent m/z 146 have given low yields of m/z 130 and 87 and abundant daughter ions at m/z 59 which overlap with the unimolecular reaction products. The low reactivity of the $(M - CH_3)^-$ anions as shown by MI, CID, and v^- stable ions spectra is in good agreement with the expected chemistry of these species.

Conclusions

In order to correctly interpret the mass spectra of the desorption ionization process it is essential to understand the chemical changes undergone in the energized condensed phase. The formation of quasimolecular ions is always accepted as a straightforward acid–base process since the gaseous species thus formed which are extremely useful in analytical applications show structural similarity with the neutral precursors. The behavior of carnitine, a zwitterionic molecule otherwise intractable by mass spectrometry methods,[21] indicates that chemical reactions competing with the classic proton-transfer process can be preferentially populated. However, those processes must not be considered as a limitation of desorption ionization techniques but rather as a source of valuable information to better understand the chemistry of the molecules being analyzed. In the particular case examined here, the v spectrum displays an unexpected feature which, though not particularly useful for analytical purposes, does provide chemical evidence enabling possible correlations between the in vivo activity of carnitine and its chemical reactivity in the energized condensed phase.

Experimental Section

L-Carnitine, inner salt, and hydrochloride were all obtained from SIGMA-TAU, Italy. L-Carnitine inner salt (lot no. 998/3, 98% pure, enzimatically) gave a single spot (R_f 0.56, I_2) by TLC on silica gel plates with as eluant a mixture of $CHCl_3$ (46%), MeOH (31%), HCOOH (11.5%), and H_2O (11.5%). The samples were mixed in glycerol–water (1:1) at different concentrations. The G/S values corresponded to the ratio of the relative intensities of the base peak of the sample (S) and of the glycerol (G) clusters at m/z 183 (v^-) and 185 (v^+). The mass spectra were obtained on a ZAB-2F instrument operated at an accelerating potential of 8 keV by using the standard FAB gun. A neutral Xe beam of 8 keV energy and a neutral current of approximately 1 mA were employed. The spectra were recorded at 1000 resolution by scanning the magnetic field. CID spectra were obtained by admitting air into the 2nd FFR collision cell thus lowering the intensity of the primary ion by a factor 3. v scan spectra have been obtained by scanning the accelerating

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voltage from 4 to 8 kV while the magnetic and electrostatic fields were kept constant at the values for the transmission of species having 4 keV of translational energy.

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Registry No. 1, 541-15-1.

Stereochemical Effects in the Gas-Phase Pinacol Rearrangement of *cis*- and *trans*-1,2-Dimethylcyclopentane-1,2-diol

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Abstract: The pinacol rearrangement of *cis*- and *trans*-1,2-dimethylcyclopentane-1,2-diol, promoted by the gaseous Brønsted acids D_3^+, $CH_5^+/C_2H_5^+$, and $t\text{-}C_4H_9^+$, was studied by mass spectrometric and radiolytic methods. Dehydration of the protonated substate is rate limiting, and competitive experiments with pinacol, carried out at high pressure (760 torr), showed that the cis rearranges more rapidly than the trans isomer, indicating participation of the migrating methyl group to the leaving water molecule. The results are compared with those concerning the same substrates in solution, where no evidence of anchimeric assistance was found.

The impressive number of studies in solution regarding the pinacol rearrangement rendered it a classic in any organic chemistry textbook. The interpretation of its mechanism, however, is complicated by the interference of undesired competitive pathways promoted by the rather drastic conditions required, e.g., strong-acid catalysis in protic solvents.[1] Such problems have been clearly enumerated,[2] and, in particular, the direct observation of a bona fide steric effect in pinacol rearrangement seemed questionable,[3] under the experimental conditions prevailing in solution.

Mass-analyzed ion kinetic energy (MIKES) and collision-induced dissociation (CID) spectrometry showed that the ions produced by water loss from protonated pinacol rearrange to the protonated pinacolone structure in the gas phase,[4] and allowed the study of related acid-catalyzed rearrangements in the chemical ionization (CI) source of a mass spectrometer.[5]

Occurrence of the gas-phase pinacol rearrangement at atmospheric pressure was also indepedently demonstrated by radiolytic methods,[6] via the actual isolation and structural characterization of the neutral end products.

In this study, a combination of radiolytic and mass spectrometric techniques was applied to provide direct evidence of stereochemical effects in the course of the pinacol rearrangement of isomeric 1,2-dimethylcyclopentane-1,2-diols; the reactions were carried out in the gas phase under experimental conditions where the factors hampering the interpretation of solution data (cis-trans isomerization, solvent interactions, etc.) are largely eliminated.

cis- and *trans*-1,2-cyclopentanediols were used as the substrates in the present experiments for the following reasons: (i) their symmetry makes protonation on either of the oxygen atoms perfectly equivalent; (ii) the presence of the five-membered ring establishes a definite steric relationship between the migrating methyl group and the leaving water molecule, and allows comparison with related rearrangements occurring in freely rotating glycols, such as the pinacol itself; (iii) the system has been independently studied in solution,[7-9] where the results strongly depend upon the experimental conditions; and (iv) under suitable conditions, the gas-phase reaction of interest is not complicated by other undesired processes.

Experimental Section

Materials. Hydrogen, deuterium, methane, isobutane, oxygen, and trimethylamine were high-purity gases from Matheson Co., used without further purification. Tetramethylene glycol and methyl *tert*-butyl ketone were chemicals from Fluka A.G. 1,2-Dimethylcyclopentene, used as starting material for the synthesis of the substrates, was prepared by dehydration of 1,2-dimethylcyclopentanol (K & K) with phosphoric acid[10] and purified by distillation at 104 °C, 760 torr.

cis-1,2-Dimethyl-1,2-cyclopentanediol (*cis*-**1**) was synthesized from the olefin and osmium tetroxide;[11] after distillation, the product was further purified by several successive crystallizations from ethyl acetate at −60 °C (mp 24 °C; bp 92 °C at 10 torr).

trans-1,2-Dimethyl-1,2-cyclopentanediol (*trans*-**1**) was prepared by addition of freshly distilled 1,2-dimethylcyclopentene on peroxyformic acid;[12] the crude glycol was purified by treatment with periodic acid and recrystallizations from benzene–cyclohexane (mp 105 °C). 2,2-Dimethylcyclopentanone (**2**) was prepared by the method of Wilcox and Mesirov.[13]

All the substrates and products were further purified by preparative GLC on the same columns employed for the analysis of products. Their final purity, as checked by GLC, exceeded 99.9 mol %.

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[†] Università di Camerino.

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