factors on the intramolecular cycloaddition.[26] Failure to detect the corresponding cyclonucleosides in the NMR study of Urd[8] is understandable in that a crude mixture was used to obtain a poorly resolved 100-MHz spectrum. However, while this manuscript was being revised, observation of cyclonucleosides on irradiation of Urd in methanolic solution was reported.[31] Both the photocycloaddition and photohydration processes require an intermediate with a relatively positive center at C(6). The possible significance of such a zwitterionic intermediate in a biochemical environment was pointed out as early as 1958,[2b] but has received little attention. The present finding serves as an important example that nucleophiles other than water may be added to this intermediate in a biological milieu. Such an occurrence could result in cross-linkages or adducts between nucleic acids and other biomolecules, and be of greater biological consequence than the well-known photohydration reaction.

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The Fischer Indole Synthesis and Pinacol Rearrangement in the Mass Spectrometer

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Abstract: The gas-phase acid-catalyzed elimination of NH_3 from protonated phenylhydrazones is shown to be analogous in detail to a solution reaction, the Fischer indole synthesis. This is demonstrated using new mass spectrometric procedures based on mass-analyzed ion kinetic energy (MIKE) measurements in conjunction with isotope labeling. Specifically, (a) the product of the rearrangement sequence is shown to be identical with the protonated indole while (b) selection of compounds labeled to various extents from a single mixture—a uniquely facile procedure in MIKES—gives the expected label incorporation patterns in the ammonia elimination products. Formation of 3*H*-indole from suitable substituted ketones represents a variant on the Fischer synthesis which is also observed in the gas phase. The present methodology for characterizing gas-phase analogues of solution reactions should be generally applicable. This is further demonstrated by applying it to the pinacol rearrangement, where correspondence between the isolated and condensed phase chemistry is again observed.

Studies on ionic chemistry in the gas phase have borrowed heavily from both the concepts and the mechanistic probes of condensed-phase physical organic chemistry. This is evident in studies on gas-phase substituent effects,[1] steric effects,[2] ortho effects,[3] anchimeric assistance,[4] and tautomerism,[5] and in the gas-phase analogues of nucleophilic substitution,[6] electrophilic aromatic substitution,[7] transesterification,[8] and the Beckmann rearrangement.[9] Mechanistic probes used in the gas phase have included kinetic isotope effects[10] and orbital symmetry studies[11] among other traditional methods. It is noteworthy, however, that with rare exceptions, product analysis in the gas phase has been limited to mass measurement

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supplemented by observations on labeled analogues. Chemical ionization,[12] ion cyclotron resonance,[13] and flowing afterglow experiments[14] have widened the scope of possible correspondences between the two phases by turning the focus from intramolecular to more familiar bimolecular chemistry. Product characterization has, however, remained difficult.

With the development of mass-analyzed ion kinetic energy spectrometry (MIKES)[15] and collisional activation[16] new techniques of ionic product analysis are available. These tools have been proven in numerous studies on ion structure;[17] we here turn them to a study in which the focus of attention is the mechanistic analogy between condensed and isolated phases. The MIKE spectrometer is a reverse geometry mass spectrometer, i.e., the magnetic sector precedes the electric sector. Hence, any single mass may be selected with the magnetic sector and its fragments (metastable or collison induced) can be observed by scanning the electric sector voltage. The energy of the fragment ion is directly proportional to its mass.

Two reactions are studied with emphasis on the Fischer synthesis of indoles from protonated arylhydrazones.[18] We show that the indole is indeed the reaction product in the chemical ionization source and that there exists at some depth a mechanistic correspondence between the gas-phase and solution reactions. These studies are facilitated by another feature of MIKES, the fact that selected components of complex mixtures can be studied without prior sample treatment.[19] This made it unnecessary to isolate the hydrazone; the reagents (ketone and hydrazine) were simply mixed and introduced into the chemical ionization source or, alternatively, they could be introduced by separate inlet lines with in situ product formation. It also allowed a series of labeled analogues to be studied by using partially labeled reagents, a particularly unique and powerful capability.

In the study of acid-catalyzed reactions in the isolated phase it is important to distinguish rearrangements associated with eliminations from simple isomerizations. The former give rise to ionic products which differ in mass from the reagent whereas the latter do not. Thus, prima facie evidence for the occurrence of the first type of rearrangement is the presence of a fragment ion resulting from the elimination; the occurrence of simple rearrangements may on the other hand be difficult to detect in the presence of large excesses of the unreacted species. This difficulty is compounded in chemical ionization where collisional stabilization can further increase the unreacted ion population at the expense of reactive ions. For these reasons the present study focuses on rearrangements which are accompanied by the elimination of neutral molecules.

Experimental Section

The MIKE spectrometer, CI source, and typical operating conditions have been described elsewhere.[15,20] Isobutane was used as reagent gas; the elimination reactions of interest were relatively more important at low source pressures (this decreases the extent of collisional stabilization of the protonated molecule) but absolute intensities were low unless a source pressure of ~0.5 Torr was used. MIKE spectra were routinely taken in the presence of nitrogen collision gas at 2×10^{-5} Torr indicated pressure (2 mTorr estimated collision cell pressure).

Samples consisting of pure compounds or a mixture of ketone and phenylhydrazine (ratio 1:1) were introduced into the CI source using the direct insertion probe. As a check that the desired elimination product was not being formed in solution, separate introduction of phenylhydrazine (direct probe) and acetone (septum in reagent gas line) was effected. This did not change the results including the MIKE spectrum of the acetone phenylhydrazine elimination product.

Results and Discussion

Fischer Indole Synthesis. The chemical ionization (CI) mass spectrum (isobutane reagent gas) of acetone phenylhydrazine shows a prominent ion due to the protonated molecule, *m/z* 149, as well as peaks at *m/z* 109 (protonated phenylhydrazine), 148 (ionized phenylhydrazone), and 59 (protonated acetone). In addition to these peaks an ion of low abundance (0.9% of $(M + H)^+$) occurs at *m/z* 132. The presence of this ion in the mass spectrum provides necessary but not sufficient evidence that an NH_3 elimination reaction, which may be analogous to the Fischer indole synthesis (Scheme I), may be occurring in the gas phase.

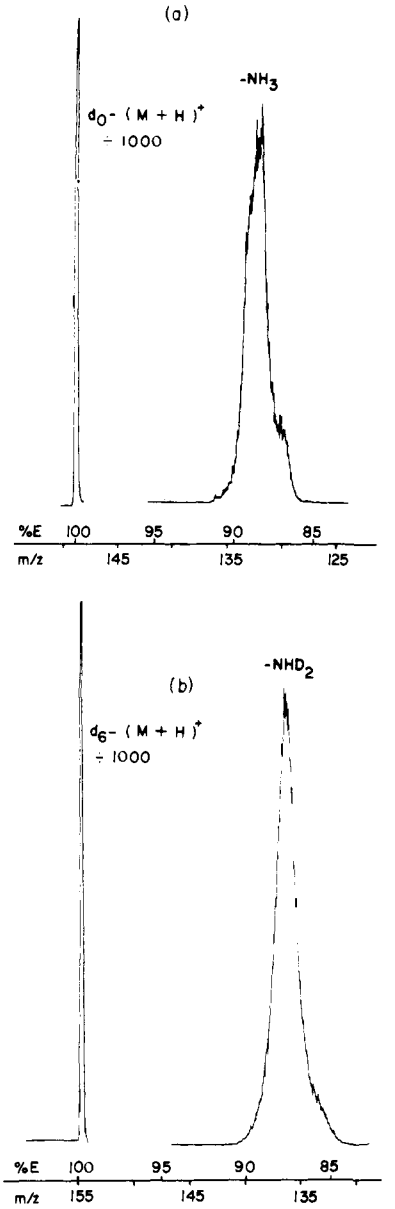


Figure 1. Elimination of ammonia from protonated acetone phenylhydrazone studied by recording the appropriate region of the MIKE spectrum in the presence of collision gas. The abscissa is calibrated in terms of both the measured ion kinetic energy (main beam 100% E) and the fragment ion mass calculated therefrom. The loss of ammonia from the unlabeled hydrazone (a) is replaced by specific loss of d_2 ammonia from the d_6 hydrazone as expected for Fischer indole synthesis (Scheme I).

By mass selecting the protonated molecule, *m/z* 149, its reactions can be studied free of interference from those of other ions. When this is done and the resulting product ions, formed by collision of $(M + H)^+$ with nitrogen, are analyzed, one obtains the spectrum shown in part in Figure 1a. Loss of 17 amu (NH_3) from the protonated molecule is a major process. Such a reaction is not expected for protonated acetone phenylhydrazone and a rearrangement of the ion, either prior or subsequent to collision, is indicated. It should be noted that the major reactions of this species, the formation of *m/z* 93, 92, 77,

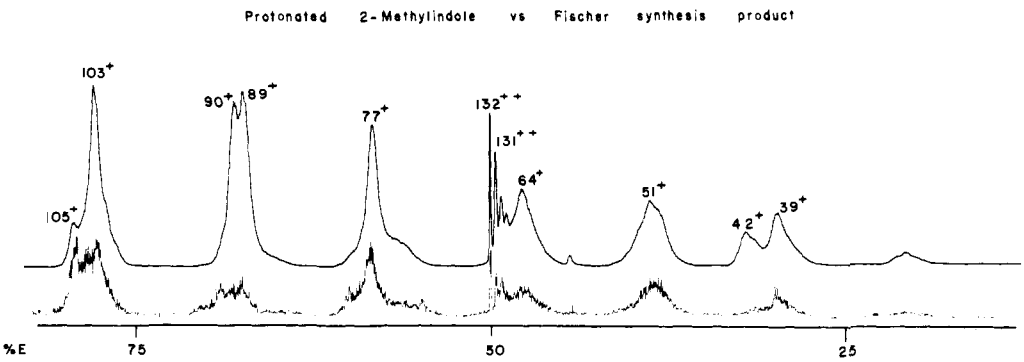
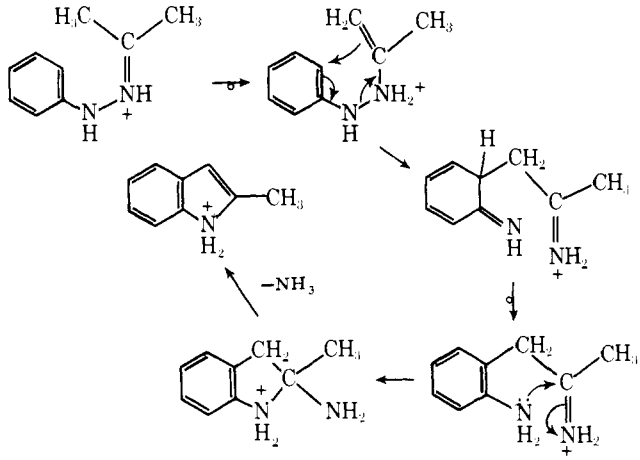


Figure 2. Fischer indole synthesis in the gas phase yielding protonated methylindole from acetone phenylhydrazone as shown by a comparison of the MIKE spectrum of the deamination product, *m/z* 132 (lower spectrum), with that of protonated 2-methylindole (upper spectrum). The MIKE spectra are recorded in the presence of collision gas. The presence of a second component in the deamination product is indicated by the peak at 105^+ which showed unique temperature characteristics.

Scheme I. Fischer Indole Synthesis from Protonated Acetone Phenylhydrazone Showing the Mechanism Advanced for Both the Solution and the Isolated Phase



65, and 58, are expected for the protonated hydrazone structure.[21] Apparently only a minor proportion of the ions undergo the rearrangement.[22] Further confirmation that the elimination of 17 mass units does indeed correspond to ammonia loss comes from the MIKE spectrum of the hydrazone formed from acetone-d_6 (Figure 1b). A clean loss of 19 mass units is observed, i.e., NHD_2 is lost.

This result shows that interchange (scrambling) of hydrogen atoms between the ring, the amino hydrogens, and the methyl hydrogens does not occur in the $(M + H)^+$ ion. Not only is the reaction specific, but it also occurs with precisely the label retention expected if its mechanism were to parallel that of the solution reaction (Scheme I). A slightly different mechanism is feasible with a nitrenium ion intermediate and two 1,2-sigmatropic shifts (orbital symmetry allowed) instead of two 1,3-sigmatropic shifts (orbital symmetry disallowed if concerted[23]). In either case, it should be noted that the elimination of ammonia must proceed faster than intramolecular proton transfer which is essentially irreversible.

The MIKES methodology is uniquely suited to the examination of reaction mechanisms by isotopic labeling. The labeled compound can be introduced in both a chemically and isotopically impure form, and the desired ion selected from the resulting mixture by mass analysis. This was done in order to obtain the results shown in Figure 1b. In addition, this feature allows several other labeled variants to be selected from the same mixture and their spectra may provide complementary mechanistic information. This feature of MIKE spectrometry was used here in examining the d_5 and d_4 phenylhydrazones. The relative contributions of these compounds depends upon the extent of exchange occurring in the CI source, but in a typical case the d_4:d_5:d_6 ratio was 1:6:30. The d_4- and d_5-protonated phenylhydrazones gave MIKE spectra which show that (1) only the methyl hydrogens are exchanged with those of the reagent gas and (2) ammonia loss occurs with label retention which is in agreement with that predicted for the Fischer reaction mechanism. The site of labeling, point (1), is further indicated by the fact that *m/z* 93, which contains all the nonmethyl hydrogens, is not shifted while *m/z* 58, which contains the methyl and amino hydrogens, is quantitatively shifted to *m/z* 62 in the d_4 compound and to *m/z* 63 in the d_5 compound. The second point (2) follows from the ammonia loss labeling pattern in the d_5 ion which was NH_2D:NHD_2 4:7 while that calculated was 4:8. The d_4 ion gave measured ratios of NH_3:NH_2D:NHD_2 of 1:5:4 while the calculated ratio is 1:5:4.

The foregoing results provide evidence for the correspondence between the gas-phase and the solution reactions in which ammonia is eliminated from phenylhydrazone on acid catalysis. However, the key step in solution chemistry, product analysis, has not yet been examined in the gas phase. Analysis of the structure of the product ion $(M + H - NH_3)^+$ was therefore undertaken by examining its MIKE spectrum. Comparison with authentic material, in this case the protonated indole, forms an essential part of the analysis. The MIKE spectra of authentic protonated 2-methylindole and the acetone hydrazone elimination product are compared in Figure 2. The good correspondence observed points to the presence of protonated 2-methylindole in the *m/z* 132 ion beam and thus confirms the overall reaction sequence shown in Scheme I. The agreement between the narrow charge stripping peaks seen in the region of 50% E is especially noteworthy since these electron stripping processes giving the double charged ions are uniquely sensitive to different isomers.[24]

The slight differences which occur in Figure 2 indicate the presence of a minor component with some other structure or elemental composition. Given the low abundance of the $(M + H - 17)^+$ ion and the complexity of the sequence leading to the indole the occurrence of competitive reactions is not unexpected. This interpretation was confirmed by the fact that the peaks associated with the extraneous component (e.g., 105^+) showed a different temperature profile than the rest of the spectrum.

To further test the hypothesis that a Fischer indole synthesis can occur in the gas phase a number of other substrates were examined. 2-Butanone provided a straightforward extension of the acetone results: the MIKE spectrum of the authentic Fischer rearrangement product, protonated 2,3-dimethylindole, corresponding to that of the $(M + H - NH_3)^+$ elimination product derived from the hydrazone. In addition, the MIKE spectrum of the protonated hydrazone itself showed a prominent peak due to loss of NH_3, as required for indole

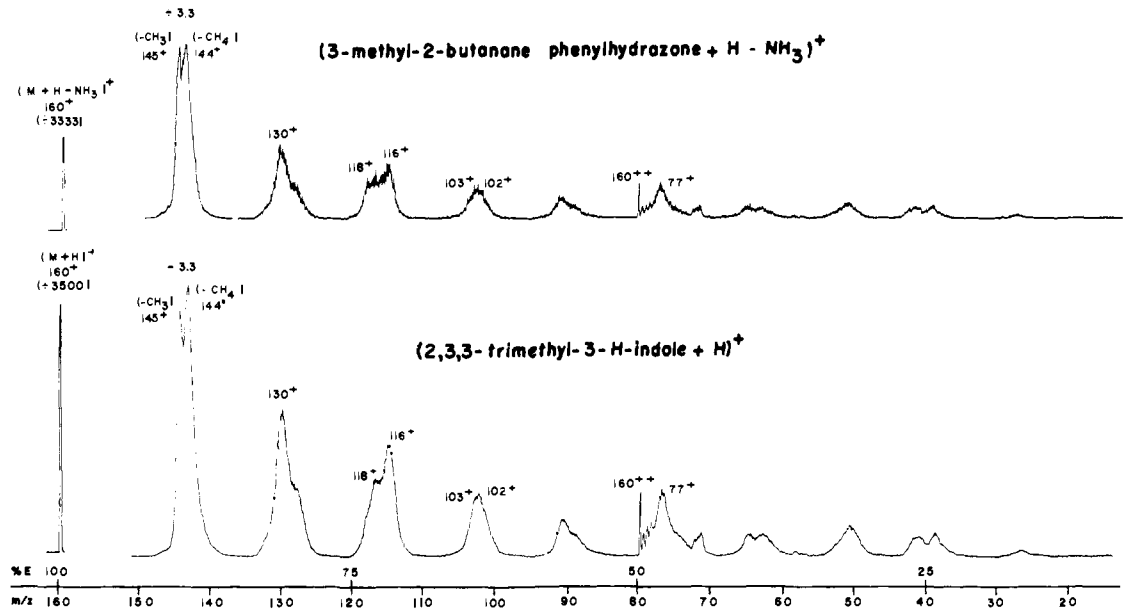


Figure 3. Variant on the gas-phase Fischer indole synthesis leading to 3*H*-indole. The formation of this compound is established by comparison of the MIKE spectrum of the reaction product (a) with that of the authentic 3*H*-indole (b).

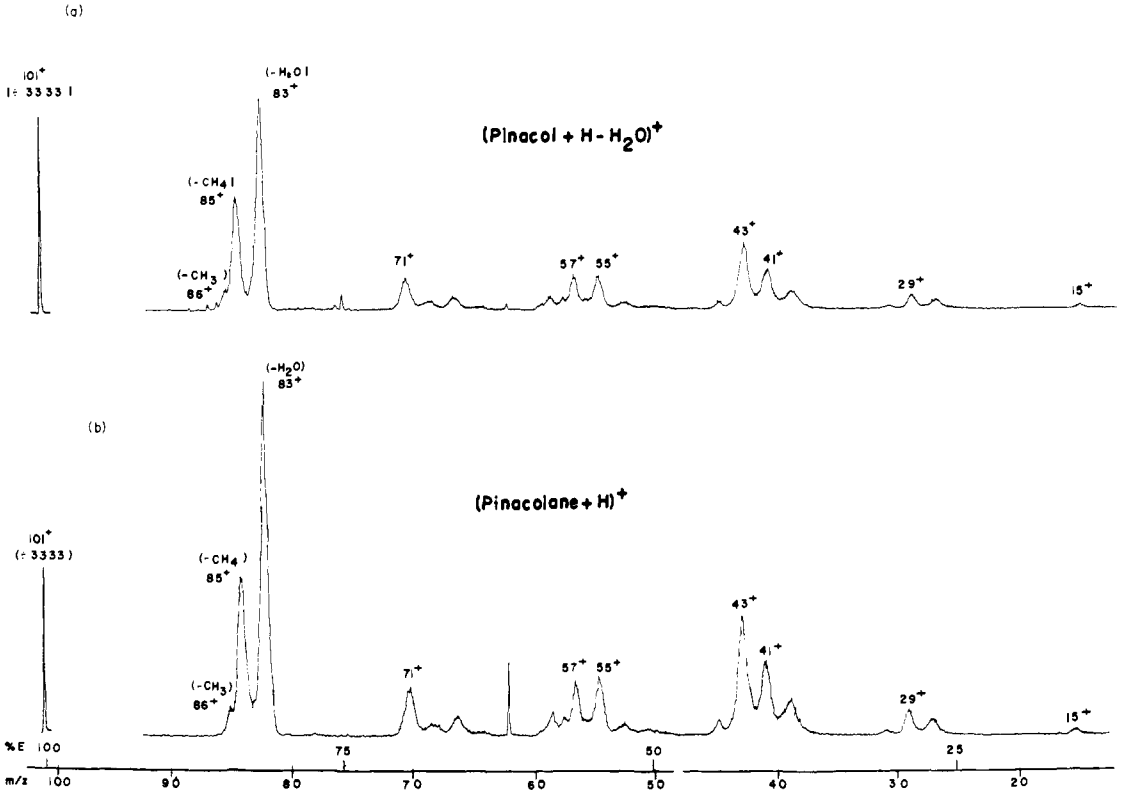


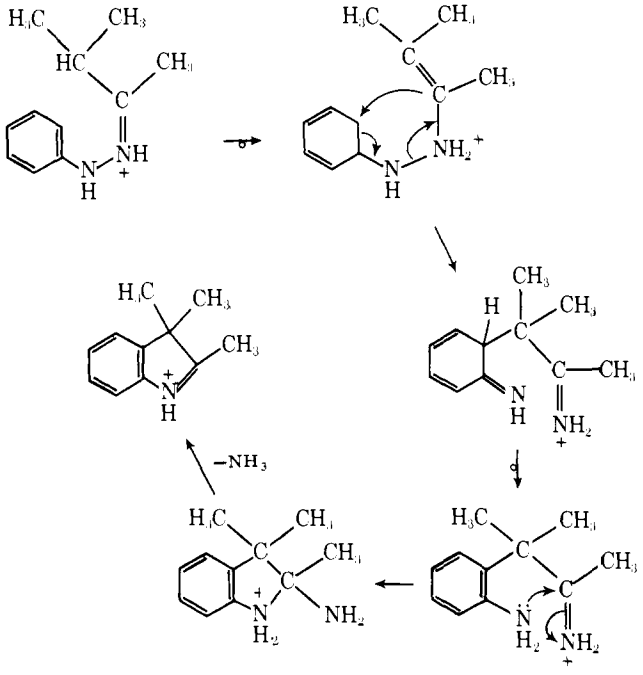
Figure 4. Comparison of the MIKE spectrum of protonated pinacolone (b) with that of the dehydration product of protonated pinacol (a). The identity of the two spectra confirms the occurrence of a gas-phase pinacol–pinacolone rearrangement. The sharp peak at 63% E is due to a first field-free region reaction of an ion other than *m/z* 101.

synthesis. Acetaldehyde also apparently underwent the Fischer indole synthesis in the gas phase, although no product has been observed in the reaction in solution.

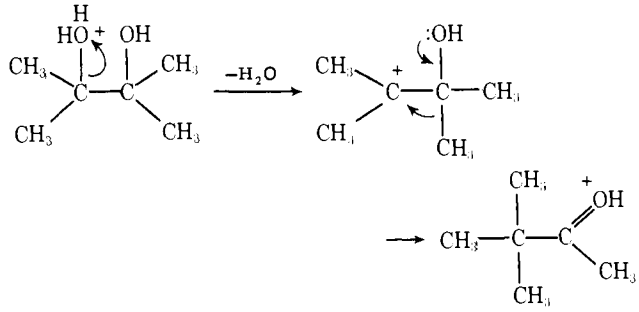
When methyl isopropyl ketone is employed as the carbonyl compound the indole synthesis is blocked and formation of the 3*H*-indole occurs in solution (Scheme II).[18] This substrate was therefore of particular interest in the gas-phase experiments. The MIKE spectra of the $(M + H - NH_3)^+$ product ion and authentic protonated 3*H*-indole are compared in Figure 3. The agreement is perfect[25] and the solution/gas phase analogy is thus seen to extend even to variants on the reaction. The foregoing results, if they do not prove that the gas-phase mechanism is exactly analogous to the solution reaction, at least require a detailed similarity. (It is noteworthy that the details of the electron transfers in the solution mechanism itself remain a topic of continuing debate[26].)

Pinacol Rearrangement. This reaction was studied in less detail than the Fischer indole synthesis, in part because the system is much less complicated. Thus given that the same elimination (H_2O loss) is observed in the gas phase as in solution the chances of this occurring with alkyl group migration to give the pinacolone seemed excellent (Scheme III). This was

Scheme II. Variation on the Fischer Indole Synthesis Where Formation of the Indole Is Blocked and the 3*H*-Indole is Formed



Scheme III. The Elimination/Rearrangement of Protonated Pinacol to Give Protonated Pinacolone



confirmed; H_2O loss is a major reaction of protonated pinacol in chemical ionization. Moreover, the product ion clearly has the same structure as protonated pinacolone as the exact correspondence between the MIKE spectra given in Figure 4 shows.

It is of note that this MIKE spectrum can be interpreted from first principles based on a knowledge of the fragmentation patterns of protonated ketones.[27] In this case, at least, the authentic product is not necessary except to confirm the assignment. Thus, the primary fragmentations undergone by the protonated molecule are loss of 18, 16, and 15 mass units. These correspond to three of the four primary cleavage modes of protonated ketones, viz., loss of water, an alkane molecule, and an alkyl radical.[27] The fourth general reaction type, alkene loss, is predicted not to be important since it would require transfer of a methyl hydrogen but it probably contributes to the observed peak at *m/z* 43. The lower mass ions are all readily accounted for as the result of predictable further fragmentations from the primary reaction products of protonated ketones.[28]

Conclusion

This paper uses methodology for product analysis as the basis for detailed comparisons of gas-phase and solution reactions.[29] The case emphasized here, the Fischer synthesis, is an acid-catalyzed reaction, but the CI/MIKES methodology is not limited to the study of such reactions. This case also provided a stringent test of the methodology since the possible range of product structures is great. The occurrence of the complex Fischer sequence, involving multiple bond-forming steps and hydrogen transfers within the few microseconds the ion survives, is noteworthy but not unexpected. Ions of low internal energy typically undergo complex bond reorganization sequences in fragmenting by low activation energy pathways.[23] It is also worth noting that the complexity of the Fischer synthesis results in a much slower reaction rate and hence a peak of lower abundance in the CI mass spectrum than is the case for the simple pinacol-pinacolone reaction. This accounts for the somewhat better match with the MIKE spectrum of authentic material in the latter case.

It is worth reiterating that the principles used here to investigate gas-phase mechanisms are identical with those employed in studies on solution reactions, even though techniques differ greatly. In particular, the gas-phase Fischer indole synthesis has been studied using the classical principles of (1) product identification (MIKE spectra of product compared to authentic compounds (ions)), (2) isotopic substitution, and (3) structural variations in the reagents to divert the reaction (3*H*-indole).

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(25) Ring closure giving the alternate isomeric indole, 2-isopropylindole, does not occur in solution.[18] However, should this product be formed in the gas phase, it would be expected to exhibit a much larger loss of 43 mass units and less loss of 15 and 16 than observed. These expectations are based on the general observation that the $(M + H)^+$ ions of alkyl-substituted indoles undergo prominent alkyl radical losses.
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(28) For example, the ion resulting from loss of 15 mass units is formulated as the methyl isopropyl ketone molecular ion.[27] This ion is expected and observed to fragment further by loss of methyl and isopropyl radicals giving *m/z* 71 and 43.
(29) A referee has emphasized that the MIKES methodology stands in contrast to solution chemistry in that its selectivity might cause attention to be focused on minor side reactions.

Structure of a Hydrated Sodium-Lasalocid A (X-537A) Dimer: an Intermediate in Complex Formation

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Abstract: A sodium-lasalocid A-water (2:2:2) complex, crystallized from 95% ethanol, appears to be an intermediate in the monomer to dimer transition that accompanies ion capture and transport. The complex crystallizes in the monoclinic space group $P2_1$ with cell dimensions a = 12.148 (2) Å, b = 27.589 (3) Å, c = 11.802 (3) Å, β = 110.25 (1)°, and Z = 2. The structure consists of two sodium ions and two water molecules enclosed by two lasalocid A ions. Six of the seven oxygens coordinated to one sodium ion are contributed by both lasalocid A ions; a water molecule provides the seventh site. The other sodium ion is coordinated to four oxygens of a single lasalocid A ion and both water molecules.

In the past several years lasalocid A (X-537A) (Figure 1) has been the subject of a great many biochemical investigations because of its ability to transport ions across natural and synthetic membranes.[1-3] It has also been extensively studied by X-ray crystallographic techniques in order to determine the structure of this ionophore complexed to a variety of monovalent and divalent ions. Results of the crystallographic studies have shown that the free acid and complexed forms can exist as both monomers and dimers. Dimeric structures have been observed for the 1:2 barium-lasalocid A,[4] the 1:2 water-lasalocid A,[5] and two distinctly different 2:2 sodium-lasalocid A[6] complexes. Differences in the torsion angles in the backbone of the 12 independent X-537A molecules or anions present as monomers or dimers are not more than 26°.

The structures of all of the dimeric forms consist of a "sandwich"-type complex in which the complexed molecule or ion resides in a cavity between the two ionophores. Two classes of dimers have been observed, those in which there is head to tail association of the molecules forming the dimer (observed in the barium and silver salts and one form of the sodium complex) and head to head dimers (observed in the water complex and the second form of the sodium complex). The surfaces of both types of dimeric complexes are composed primarily of hydrophobic groups and it seems clear that one or both of these dimeric forms are responsible for transport through nonpolar media.

Observed monomeric species, a free acid form and a sodium ion complex,[7] contain a methanol molecule which forms hydrogen bonds to the ionophore; in addition, the oxygen of the methanol molecule fills out the coordination sphere of the sodium ion.

It has been suggested that metal uptake and release in polar environments involve monomeric forms, while transport in nonpolar media takes place by means of a lasalocid dimer. In addition, the results of NMR studies in nonpolar solvents have indicated the presence of a dimeric structure similar to but not identical with that observed for the sodium ion complexes in the solid state.[6] We report here the crystal structure of a dimeric form of this ionophore complexed to both water and sodium ion.

Experimental Section

Single crystals of the dimeric 2:2:2 sodium-lasalocid A-water complex were grown from 95% ethanol. Unit cell data are given in Table I. The intensities of 3896 independent reflections ($\sin\theta_{max}/\lambda$ = 0.497 Å^{-1}) were measured on an Enraf-Nonius CAD-4 diffractometer using Ni-filtered Cu Kα radiation. No significant changes were observed in the intensities of two standard reflections which were measured after every 96 intensities were recorded. Intensities were corrected for Lorentz and polarization (Lp) factors but not for extinction or absorption. Real and imaginary dispersion corrections were applied to the atomic scattering factors.[8] On the basis of a $2\sigma(I)$ test, 2942 data were considered observed. The variance of each F was calculated according to the method of Stout and Jensen[9] [$\sigma^2(F) = k/4(Lp)I[\sigma^2(I) + (0.06I)^2]$; $w(F) = 1/\sigma^2(F)$]. Unobserved data were given zero weight and not included in the refinement.

The structure was solved through the use of the direct methods program QTAN[10] and refined by full-matrix least squares, treating the vibration of carbon atoms isotropically and that of sodium and oxygen anisotropically, to a residual of 0.078 ($R = \sum||F_o| - |F_c||/\sum|F_o|$) for the observed data and 0.105 for all data.[11] The weighted residual ($R_w = [\sum w(|F_o| - |F_c|)^2/\sum wF_o^2]^{1/2}$) was 0.106. Hydrogen atom contributions were included in the final two cycles of least-squares refinement by calculating their positions on the basis of the heavy-atom positions at the end of each cycle; methyl groups were assumed to have a staggered conformation. No contribution was included for the hydrogen of the hydroxy groups or the methyl attached to the benzoic acid group since these positions cannot be calculated unambiguously.

Discussion

The overall structure of the dimer, illustrated in Figure 2, consists of two sodium ions and two water molecules enclosed in a cavity constructed from the two lasalocid A ions. While

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