mational flexibility of the CPA method is that the best fit may be obtained with conformers that do not represent energetic minima. By constraining their search to just two Gaussian distributions, Noggle et al. obtain better matches of torsion angles between force field and NOE results but poorer populations.

IV. Summary

Conformer population analysis is a method for the elucidation of solution structures from NOE data that is an alternative to building structures from interproton distance constraint matrices. Compared with distance-constrained methods, CPA has the advantage of permitting multiple conformations in the fitting solution and, hence, is capable of removing biases inherent in the assumption of a single structure. Another advantage is that the method directly works with NOE intensities, an important distinction because NOE experiments produce intensity data, not distances.

The most significant disadvantage of the CPA technique is that it does not direct the construction of a molecular structure; a group of predefined conformations is required. Although molecular modeling techniques are capable of generating large sets of energetically reasonable conformations, it is obvious that the structures produced will reflect the inaccuracies of the force fields utilized (this is true of constrained methods as well, although the constraints commonly are less rigid). For large molecules (e.g. protons) the CPA method may be impractical because the generation of adequate trial structures using internal coordinate randomization techniques alone is unreasonable. However, other techniques, such as homology modeling, could be used to generate trial structures for proteins.

Because large sets of trial structures are required to sample conformational space thoroughly, data reduction techniques are needed. The clustering algorithms used in CPA accomplish data reduction by sifting through large data sets and identifying a smaller subset of representative structures. Refinement occurs by identifying clusters that are promising and focusing the fitting procedures on the members of those clusters. Convergence in this process is not assured, but in practice the NOE is sufficiently sensitive to structure that the method readily compresses its search to a small number of conformers. A final phase of significance testing produces the most parsimonious solution justified by the data itself.

Perhaps the most useful application of a multiconformational technique is in concert with molecular construction methods based on the one cross-relaxation:one distance approximation. A reasonable procedure may be to use the structure built by a molecular construction technique as a starting point for conformation searching by modeling techniques.

In the current implementation, the CPA technique is deficient in not considering the influence of conformer exchange and rapid internal motions, such as methyl group rotation. However, these limitations are imposed principally by the numerical integration schemes employed; a subsequent version will use more versatile matrix methods and will include the influence of conformer exchange and internal rotation kinetics. Future work will focus on the inclusion of symmetry and torsional information into the fitting procedure and on the experimental examination of solution structures of molecules having multiple conformations.

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Gas-Phase Formation of Four Isomeric $C_4H_4^{\bullet+}$ Ions. Ionic Isomer Quantitation with Neutralization–Reionization Mass Spectrometry

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Abstract: Four $C_4H_4^{\bullet+}$ isomers yield characteristic mass spectra by neutralization, dissociation of these neutrals, and product reionization to both positive ($^+NR^+$ spectra) and negative ($^+NR^-$ spectra) ions. Such measurements show that a wide variety of unsaturated/cyclic hydrocarbon ions, and of ionized 2,4,6-cycloheptatrien-1-one, yield $C_4H_4^{\bullet+}$ of the composition ~68% methylenecyclopropene ($\mathbf{c}^{\bullet+}$) and ~32% vinylacetylene ($\mathbf{a}^{\bullet+}$). This appears to result from the dissociation of a common intermediate, the excited benzene ion; for the $C_6H_8^{\bullet+}$ precursors, this is consistent with an early proposal of Franklin. This most stable $\mathbf{c}^{\bullet+}$ isomer can also be formed in even higher purity from ionized 6,7-benzo-3-methylenetricyclo[3.2.2.$0^{2,4}$]nona-6,8-diene, while the isomer $\mathbf{a}^{\bullet+}$ can also be formed by loss of H_2O from ionized 3-butyn-1-ol. Butatriene ions ($\mathbf{b}^{\bullet+}$) are formed by Br_2 loss from ionized 1,4-dibromo-2-butyne, but in addition the $C_4H_4^{\bullet+}$ products contain ~44% cyclobutadiene ions ($\mathbf{d}^{\bullet+}$). The $\mathbf{d}^{\bullet+}$ ions also result from dissociation of ionized 7,8-benzotricyclo[4.2.2.$0^{2,5}$]deca-3,7,9-triene and 3,4-diiodocyclobutene, and $\mathbf{d}^{\bullet+}$ is the main $C_4H_4^{\bullet+}$ product from 1,2- and 1,4-benzoquinone; with both, $\mathbf{d}^{\bullet+}$ is accompanied by ~14% $\mathbf{a}^{\bullet+}$, suggesting a common cyclopentadienone$^{\bullet+}$ intermediate. For each of 13 precursors the values for the $^+NR^+$ and $^+NR^-$ measurements differ from their average by 1.8 ± 2.6% (absolute), consistent with relatively high accuracy for NR isomeric analysis of gaseous ions.

A wide variety of studies over the past two decades[1-8] have sought the isomeric identity of the $C_4H_4^{\bullet+}$ radical cations found as a common product of the unimolecular dissociation of ionized unsaturated/cyclic hydrocarbons such as benzene.[5a,9] Although

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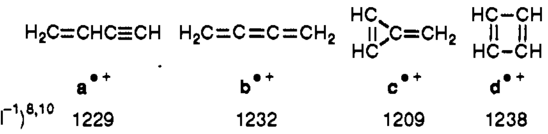
Table I. $^+NR^+$ and $^+NR^-$ Mass Spectra for $C_4H_4^{\bullet+}$ Ions from Various Precursors[a]

precursors	26^+	27^+	36^+	36^-	38^-	48^-	49^+	51^+	51^-	52^+	structure[b]
vinylacetylene	12.4	4.7	13.9	28	8.	50	51	55	94	31	**a**$^{\bullet+}$
butatriene	9.9	2.1	15.8	31	28	48	54	55	31	23	**b**$^{\bullet+}$
1	19.9	1.2	23	38	14.3	50	66	48	40	15.5	**c**$^{\bullet+}$
2	52	1.8	56	36	4.0	64	92	31	13	9.6	**d**$^{\bullet+}$
benzene	16.0	3.6	18.6	32	10.5	48	58	51	63	23	**a**$^{\bullet+}$ 33, 38; **c**$^{\bullet+}$ 67, 62
benzene (20 eV)[c]	15.9	3.6	18.4				58	51		23	**a**$^{\bullet+}$ 32, –; **c**$^{\bullet+}$ 68, –
benzene (CI)[d]	18.9	3.0	21	35	13.1	49	63	49	45	18.6	**a**$^{\bullet+}$ 13, 15; **c**$^{\bullet+}$ 87, 85
1,5-hexadiyne	15.0	3.2	16.5	33	10.4	49	55	52	56	23	**a**$^{\bullet+}$ 32, 31; **c**$^{\bullet+}$ 68, 69
2,4-hexadiyne	14.5	3.3	15.3	33	11.6	49	54	52	54	23	**a**$^{\bullet+}$ 30, 30; **c**$^{\bullet+}$ 70, 70
1,4-cyclohexadiene	14.0	3.4	15.3	31	10.8	48	54	52	62	23	**a**$^{\bullet+}$ 29, 40; **c**$^{\bullet+}$ 71, 60
1,3-cyclohexadiene	15.4	3.6	18.6	33	9.8	50	58	50	54	22	**a**$^{\bullet+}$ 28, 29; **c**$^{\bullet+}$ 72, 71
1,3,5-hexatriene	14.2	3.4	16.0	33	10.9	50	56	53	64	23	**a**$^{\bullet+}$ 29, 39; **c**$^{\bullet+}$ 71, 61
cyclooctatetraene	14.5	3.7	15.8	32	10.5	48	54	51	51	22	**a**$^{\bullet+}$ 29, 29; **c**$^{\bullet+}$ 71, 71
2,4,6-cycloheptatrien-1-one	16.0	4.0	18.2	32	10.2	49	58	52	59	24	**a**$^{\bullet+}$ 34, 40; **c**$^{\bullet+}$ 66, 60
3-butyn-1-ol	12.2	4.7	12.9	28	7.9	50	50	56	95	31	**a**$^{\bullet+}$ 100, 100
1,4-dibromobut-2-yne	22	1.9	26	34	22	53	63	49	27	19	**b**$^{\bullet+}$ 54, 57; **d**$^{\bullet+}$ 46, 43
1,4-benzoquinone	42	3.0	46	34	5.0	61	82	36	34	15	**a**$^{\bullet+}$ 11, 14; **d**$^{\bullet+}$ 89, 86
1,2-benzoquinone	39	3.0	44	32	5.8	56	81	37	34	16	**a**$^{\bullet+}$ 11, 16; **d**$^{\bullet+}$ 89, 84
3,4-diiodocyclobutene[e]				*e*	*e*	56			43		**a**$^{\bullet+}$ –, 50; **d**$^{\bullet+}$ –, ~50

[a] Abundances (peak areas) relative to that of the largest peak. [b] Isomeric composition based on the first four entries as reference spectra and measured $^+NR^+$ ($^+NR^-$) cross sections (30% transmittance, [base peak]/[precursor peak]) of **a**$^{\bullet+}$, 1.9×10^{-3} (3.1×10^{-5}), **b**$^{\bullet+}$, 1.9×10^{-3} (3.1×10^{-5}), **c**$^{\bullet+}$, 1.0×10^{-3} (1.9×10^{-5}), **d**$^{\bullet+}$, 7.8×10^{-4} (1.4×10^{-5}). [c] Ionizing electron energy at which the primary $C_4H_4^{\bullet+}$ abundance is 10% of its value using 70-eV electrons. [d] Ion source pressure 1×10^{-4} Torr. [e] $^+NR^-$ experimental error including isotopic correction is approximately double the usual error for m/z 48 and 51 and larger than the $C_4H_4^+$ contribution for m/z 36 and 38.

most found evidence for no more than two isomers,[1-6] recent experiments indicate that at least four isomers are stable.[8] On



the basis of appearance and ionization potential measurements, an early study[1a] found $C_4H_4^{\bullet+}$ ions from 1,3-cyclohexadiene gave data consistent with isomer **a**$^{\bullet+}$, but those from 1,4-cyclohexadiene and 1,3,5-hexatriene were not, suggesting isomer **d**$^{\bullet+}$. From similar ΔH_f values found for $C_4H_4^{\bullet+}$ from ionized benzene, 1,4- and 1,5-hexadiyne, and pyridine, Rosenstock et al. proposed that threshold decomposition forms cyclic $C_4H_4^{\bullet+}$ ions, presumed to be more stable than the linear isomers.[1b] Baer et al. suggested that this isomer is the methylenecyclopropene ion (**c**$^{\bullet+}$), as their kinetic energy release values were in excellent agreement with predictions of the quasiequilibrium theory for benzene → **c**$^{\bullet+}$ over the range 14.8–18.0 eV; a break in the $[C_3H_3^{\bullet+}]/[C_4H_4^{\bullet+}]$ plot at 15.6 eV indicated the formation of a second, probably linear, isomer.[2] Kinetic studies of benzene$^{\bullet+}$ dissociation at longer ion lifetimes by Lifshitz et al. indicated either that two $C_4H_4^{\bullet+}$ structures were formed or that the longer lived ions were collisionally de-excited.[3] Using trapped ion cyclotron resonance spectrometry with $C_4H_4^{\bullet+}$ from ionized benzene and pyridine, Ausloos concluded that one isomer, thought to be **a**$^{\bullet+}$, reacted with benzene and another did not, even after 300 ms.[4]

Although mass spectra from metastable ion and collisionally activated dissociation (CAD) of $C_4H_4^{\bullet+}$ ions from different precursors show similar product abundances and kinetic energy release values,[3-5c-f] Bowers et al. found that $[C_2H_3^{\bullet+}]/[C_2H_2^{\bullet+}]$ is relatively high in the CAD spectrum of $H_2C{=}CH{-}C{\equiv}CH^{\bullet+}$, consistent with its isomeric structure.[5c] Postulating that **c**$^{\bullet+}$ should yield the lowest value of this ratio, his measurements indicated that electron ionization of benzene ions produces 53% **a**$^{\bullet+}$ and 47% **c**$^{\bullet+}$. This is also consistent with cross section measurements of charge neutralization.[6] Formation of a third isomer **b**$^{\bullet+}$ was indicated by laser photodissociation studies of $C_4H_4^{\bullet+}$ ions,[7a] but remeasurements of van der Hart gave contradictory evidence.[7b]

An initial study[8] utilizing neutralization–reionization (NR) mass spectrometry[11] showed that the isomeric cations **a**$^{\bullet+}$–**d**$^{\bullet+}$ could be distinguished by converting these to the corresponding neutrals, followed by their dissociation and product ionization to both positive ions ($^+NR^+$ spectra) and negative ions ($^+NR^-$ spectra). This paper describes the application of these complementary techniques to the ion chemistry of several systems producing $C_4H_4^{\bullet+}$ and evaluates the accuracy of the NR method for ionic isomer quantitation[12] using comparison of the independent $^+NR^+$ and $^+NR^-$ results.

Experimental Section

Measurements were made with a tandem mass spectrometer[13] that contains a Hitachi RMH-2 double-focusing instrument as the first mass analyzer (MS-I) and an electrostatic sector as MS-II, using 70 eV electron ionization (unless noted otherwise) and 9.9 keV ion acceleration. For $^+NR^+$ mass spectra, the MS-I selected masses undergo charge exchange neutralization with Hg vapor (~90% transmittance) in the first collision cell (cls-I), the remaining ions are deflected electrostatically, and the resulting fast neutrals are dissociated and reionized by collision with He (~30% transmittance) in a second collision cell (cls-II). For charge-reversal experiments, a single collision target (benzene, ~30% transmittance) is used for neutralization, dissociation, and reionization in the same cell (cls-II).[11g-i] All collision cells are differentially pumped. Mass spectra are measured in MS-II under computer control[13b] for multiple (20–50) scan averaging and peak area determination. Calibrations of reference isomeric spectra and cross sections (alternating measurements of the base peak and the precursor ion) were done at least daily. The isomeric compositions reported in Table I were calculated from the corresponding ^+NR spectral data using regression analysis[14]

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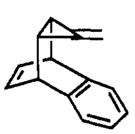
assuming linear superposition of component spectra, based on the $^{+}$NR spectra and relative cross sections of the reference ions $\mathbf{a}^{\bullet+}$–$\mathbf{d}^{\bullet+}$; the same approach has been used for isomeric quantitation from CAD mass spectra.[12b] The minor amounts of $^{13}CC_3H_3^+$ ions in the *m/z* 52 precursors were calculated from the $C_4H_3^+$ abundances, and their small contributions were subtracted from the measured NR spectra.

6,7-Benzo-3-methylenetricyclo[3.2.2.0^{2,4}]nona-6,8-diene (**1**), 7,8-benzotricyclo[4.2.2.0^{2,5}]deca-3,7,9-triene (**2**), and butatriene were synthesized as described earlier.[8,15] 1,2-Benzoquinone was prepared by oxidation of 1,2-dihydroxybenzene[16] and 3,4-diiodocyclobutene by iodide ion substitution on 3,4-dichlorobutene.[17] All other reagents were commercially available and used without further purification.



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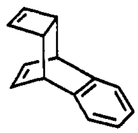

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Results and Discussion

Reference Ions. As described previously,[8] reference ions $\mathbf{a}^{\bullet+}$ and $\mathbf{b}^{\bullet+}$ were prepared by ionization of the corresponding molecules, and $\mathbf{c}^{\bullet+}$ and $\mathbf{d}^{\bullet+}$ by the Alder-Rickert decomposition of ionized **1** and **2**, respectively. Both the $^{+}$NR^{+} and the $^{+}$NR^{-} spectra of $\mathbf{a}^{\bullet+}$–$\mathbf{d}^{\bullet+}$ (Table I) show significant differences characteristic of their structures,[8,18] with at least one peak having the largest (or smallest) relative abundance of all the isomers. The $^{+}$NR^{+} spectra of $\mathbf{a}^{\bullet+}$ and $\mathbf{b}^{\bullet+}$ are independent of electron ionization energy, evidence that **a** and **b** are ionized without appreciable isomerization.[8] The Table I data show that the reference $\mathbf{c}^{\bullet+}$ and $\mathbf{d}^{\bullet+}$ ions contain <25% and <10% respectively of $\mathbf{a}^{\bullet+}$ plus $\mathbf{b}^{\bullet+}$, while the dissociation of $C_4H_4^{\bullet+}$ from **d**-*1,2-d*$_2$ demonstrates that even the degenerate isomerization to **d**-*2,3-d*$_2^{\bullet+}$ is incomplete.[5e] Further, collisionally exciting the $C_4H_4^{\bullet+}$ ions from **2** with helium (first field-free region) under multiple collision conditions allowing 30% ion transmittance[19] did not change the $^{+}$NR spectra outside experimental error, while 20% transmittance conditions gave $^{+}$NR spectra indicating <5% of isomers other than $\mathbf{d}^{\bullet+}$; this is consistent with the previous estimate[8] that the $\mathbf{d}^{\bullet+} \rightarrow \mathbf{a}^{\bullet+}$ rearrangement requires 218 kJ mol^{-1}. The remaining discussion assumes that the reference $^{+}$NR^{+} and $^{+}$NR^{-} spectra represent those of $\mathbf{a}^{\bullet+}$–$\mathbf{d}^{\bullet+}$ of satisfactory purity.

Ionization of C_6H_6 Compounds. The loss of C_2H_2 producing $C_4H_4^{\bullet+}$ is a major pathway in the unimolecular decomposition of seven ionized C_6H_6 isomers.[9] The Table I data are consistent with the previous qualitative isomeric characterizations;[1-6] the $C_4H_4^{\bullet+}$ products from three C_6H_6 compounds are the linear vinylacetylene ($\mathbf{a}^{\bullet+}$) and cyclic methylenecyclopropene ($\mathbf{c}^{\bullet+}$) ions (the initial NR study reported 30% $\mathbf{a}^{\bullet+}$ and 70% $\mathbf{c}^{\bullet+}$ from benzene).[8] Supporting the later photodissociation results[7b] over the initial ones,[7a] no $\mathbf{b}^{\bullet+}$ (or $\mathbf{d}^{\bullet+}$) is observed. The $^{+}$NR^{+} and $^{+}$NR^{-} spectral data show that the same proportions of $\mathbf{a}^{\bullet+}$ and $\mathbf{c}^{\bullet+}$, within experimental error, are produced by electron ionization (EI) of the three C_6H_6 isomers. This is consistent with previous suggestions[1-6] that the $C_6H_6^{\bullet+}$ ion, prior to dissociation, rearranges to the most stable benzene isomer.

Of the $C_4H_4^{\bullet+}$ isomers formed from benzene, one, probably $\mathbf{a}^{\bullet+}$, reacts with benzene, while the other(s), probably $\mathbf{c}^{\bullet+}$, does not.[4] In agreement with this (Table I), the fraction of $\mathbf{a}^{\bullet+}$ from ionized benzene is halved as the benzene pressure in the ion source is increased from 5×10^{-6} to 1×10^{-4} Torr.

Other Cyclic/Unsaturated Hydrocarbons. The $C_4H_4^{\bullet+}$ ions from the C_6H_8 isomers 1,3,5-hexatriene and 1,3- and 1,4-cyclohexadiene and the C_8H_8 cyclooctatetraene also produce two stable isomers, $\mathbf{a}^{\bullet+}$ and $\mathbf{c}^{\bullet+}$. For their proportions all seven C_6H_6, C_6H_8, and C_8H_8 precursors (Table I) give values of ~32% $\mathbf{a}^{\bullet+}$ and ~68% $\mathbf{c}^{\bullet+}$. For cyclooctatetraene this is not surprising; in its mass spectrum[9] $C_6H_6^{\bullet+}$ formed by C_2H_2 loss is the most abundant fragment ion, so that its subsequent dissociation should produce these $C_4H_4^{\bullet+}$ isomers. Although the mass spectra of the three C_6H_8 isomers of Table I show a $C_6H_6^{\bullet+}$ peak that is unimportant compared to the major $C_6H_5^+$ and $C_6H_7^+$ fragment ions,[9] Franklin[1a] postulated that $C_4H_4^{\bullet+}$ from these $C_6H_8^{\bullet+}$ isomers was formed through $C_6H_6^{\bullet+}$ (and for two $C_6H_8^{\bullet+}$ precursors the data are consistent with the benzene$^{\bullet+}$ structure); the Table I data fully confirm his conclusions. Further, the CAD spectra of $C_6H_5^+$ and $C_6H_7^+$ from a wide variety of hydrocarbons are the same with insignificant $C_4H_4^{\bullet+}$ or $C_6H_6^{\bullet+}$ ions,[5b] while the CAD spectrum of the $C_6H_8^{\bullet+}$ ions from 1,3-cyclohexadiene shows that most (~88%) of its $C_4H_4^{\bullet+}$ ions are formed from $C_6H_6^{\bullet+}$.[20] Further, reionization of the neutrals formed by CAD of these $C_6H_8^{\bullet+}$ ions shows C_2H_2 is the dominant neutral (C_2 or larger) lost, with C_2H_4 loss only 14% of the C_2H_2 value. C_2H_2 is also the dominant neutral from CAD of $C_6H_7^+$ and $C_6H_6^{\bullet+}$, with C_2H_4 loss ~10% and ~5%, respectively, of the C_2H_2 value. The formation of $C_6H_5^+$ (phenyl$^+$), $C_6H_6^{\bullet+}$ (benzene$^{\bullet+}$), and $C_6H_7^+$ (benzene-H$^+$) from 1,3-cyclohexadiene$^{\bullet+}$ requires 443, 74, and 170 kJ mol^{-1}, respectively;[10] the far greater abundance of $C_6H_5^+$ and $C_6H_7^+$ versus $C_6H_6^{\bullet+}$ reflects the high entropy penalty for H_2 loss and the low abundance of $C_6H_8^{\bullet+}$ ions formed with internal energies of 75–175 kJ mol^{-1} as indicated by the photoelectron spectra.[21] On the other hand, formation of $C_6H_5^+$ and $C_6H_6^{\bullet+}$ from $C_6H_7^+$ requires 273 and 340 kJ mol^{-1}, respectively, consistent with the dominance of $C_6H_5^+$ in the CAD spectrum of $C_6H_7^+$.[5b] As originally proposed by Franklin,[1a] the $C_6H_7^+ \rightarrow C_6H_6^{\bullet+}$ pathway should be favored at high energies over H_2 loss; such benzene$^{\bullet+}$ ions of energies well above the threshold (461 kJ mol^{-1})[10] for $\mathbf{c}^{\bullet+}$ formation could then yield the observed $C_4H_4^{\bullet+}$ isomers. The quantitative agreement between the $[\mathbf{a}^{\bullet+}]/[\mathbf{c}^{\bullet+}]$ values for the different molecular sources indicates that the energy distributions of the dissociating $C_6H_6^{\bullet+}$ precursor ions are similar and/or that a common intermediate results in little effect of energy on the branching ratio.

2,4,6-Cycloheptatrien-1-one. Although photodissociation studies of this ionized molecule indicated that at least three structurally distinct $C_4H_4^{\bullet+}$ ions are produced,[7a] the Table I data show that only the $\mathbf{a}^{\bullet+}$ and $\mathbf{c}^{\bullet+}$ isomers are formed, and in proportions similar to those from the hydrocarbon precursors. The $C_6H_6^{\bullet+}$ ion is the most abundant peak in the EI mass spectrum of this compound, again suggesting that the same $C_4H_4^{\bullet+}$ isomeric mixture is produced from dissociation of an intermediate benzene ion.

3-Butyn-1-ol. The $^{+}$NR$^{+,-}$ spectral data (Table I) of $C_4H_4^{\bullet+}$ formed from ionized 3-butyn-1-ol through loss of H_2O show these products to represent the vinylacetylene isomer $\mathbf{a}^{\bullet+}$. This is consistent with the expected mechanism[22] and further supports the purity of the $\mathbf{a}^{\bullet+}$ reference ions inferred above.

1,4-Dibromo-2-butyne. Chemical debromination of this compound yields butatriene.[23] The $^{+}$NR$^{+,-}$ data show that unimolecular debromination of the corresponding ions also yields butatriene ions $\mathbf{b}^{\bullet+}$, but in addition 44% $\mathbf{d}^{\bullet+}$ ions are formed (Table I). Although $\mathbf{b}^{\bullet+}$ can be formed by simple 1,4-elimination of Br_2, this apparently is accompanied by ring closure and hydrogen

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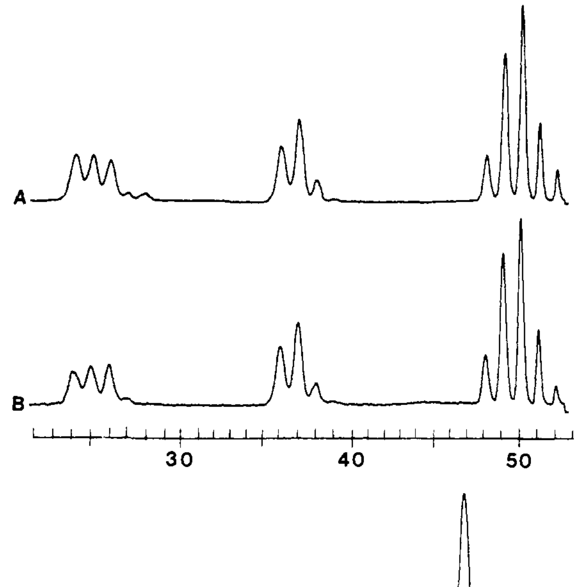
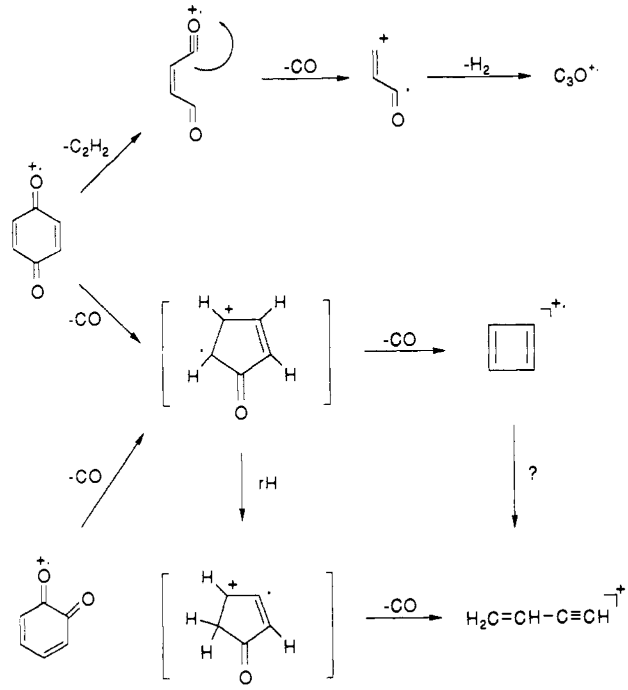


Figure 1. Spectra of m/z 52 from (A, C) 1,4-benzoquinone and (B, D) 1,2-benzoquinone: (A, B) $^{+}$NR^{+}, Hg(90% T)/He(30% T); (C, D) $^{+}$NR^{-}, benzene (30% T). The small m/z 52 in spectrum D is due to $^{13}CC_3H_3^{-}$. (A) $[50^{+}]/I_0 = 1.0 \times 10^{-3}$; (C) $[49^{-}]/I_0 = 1.9 \times 10^{-5}$.

transfer to form the surprising cyclobutadiene ion product $\mathbf{d}^{\bullet+}$. Note that the $\mathbf{b}^{\bullet+}$:$\mathbf{d}^{\bullet+}$ relative abundances (56:44) reflect their ΔH_f values (1232:1238 kJ mol^{-1}).[8,10]

Benzoquinones. In an early study[24] Beynon et al. postulated that the loss of two CO molecules from ionized anthraquinone (the dibenzo analogue of 1,4-benzoquinone) yielded ionized dibenzocyclobutadiene. Supporting this mechanism, the $^{+}$NR$^{+,-}$ spectra (Table I and Figure 1) indicate that the cyclobutadiene ion $\mathbf{d}^{\bullet+}$ is the main $C_4H_4^{\bullet+}$ product from both isomeric benzoquinone ions. The mass spectrum of 1,2-benzoquinone shows $C_4H_4^{\bullet+}$ as the most abundant product; however, $C_3H_2O^{\bullet+}$ ($M^{\bullet+} - CO - C_2H_2$) is five times as abundant as $C_4H_4^{\bullet+}$ in the mass spectrum of the 1,4-isomer.[9] The $^{+}$NR$^{+,-}$ spectra of the 1,2- and 1,4-isomers (Figure 1) are virtually identical, except for a reionized *undissociated* m/z 52 in the 1,4-$^{+}$NR^{-} spectrum, yet none of the $C_4H_4^{\bullet-}$ anions $\mathbf{a}^{\bullet-}$–$\mathbf{d}^{\bullet-}$ should be stable.[8,18] High-resolution measurements show that the precursor $C_4H_4^{\bullet+}$ ions from the 1,4-isomer contained ~20% C_3O^{+} (Scheme I; those from the 1,2-isomer contained <5%); anions with terminal oxygen atoms are often stable.[25] Correcting the $^{+}$NR$^{+,-}$ spectra for the C_3O^{+} contributions gives the data of Table I, showing that 1,2- and 1,4-benzoquinone ions both yield ~86% $\mathbf{d}^{\bullet+}$ and ~14% $\mathbf{a}^{\bullet+}$. This suggests (Scheme I) that the initial CO loss from both isomeric ions produces a common cyclopentadienone intermediate.[24,26] A second CO loss from this intermediate directly yields $\mathbf{d}^{\bullet+}$, while $\mathbf{a}^{\bullet+}$ could be formed by hydrogen rearrangement followed by CO loss or by rearrangement of excited (>~218 kJ mol^{-1})[8] $\mathbf{d}^{\bullet+}$ to the slightly more stable $\mathbf{a}^{\bullet+}$ product (Scheme I).

Scheme I



3,4-Diiodocyclobutene. Heating this compound to 120 °C has been reported to produce a mixture of C_4H_4 isomers.[27] Not only did this instability make the $^{+}$NR measurements difficult, but the m/z 52 ions formed by ionizing 3,4-diiodocyclobutene contain ~20% of isobaric $^{13}CC_3H_3^{\bullet+}$, for which the NR cross section is five times that of $C_4H_4^{\bullet+}$. However, only $^{13}CC_3H_3^{\bullet-}$ (not $C_4H_4^{\bullet-}$) is stable, allowing its $^{+}$NR^{-} recovery m/z 52 peak to be used to subtract the spectrum of the $^{13}CC_3H_3^{\bullet+}$ ions. The $[48^{-}]/[51^{-}]$ ratio of the resulting $^{+}$NR^{-} spectral data (Table I), although of much lower precision, do indicate that cyclobutadiene ions $\mathbf{d}^{\bullet+}$ are a major product from unimolecular dissociation of ionized 3,4-diiodocyclobutene.

Quantitative Determination of Isomeric Ion Composition. Evaluation of the reliability for this determination utilizing CAD mass spectra[12] has been hampered by the lack of comparative analytical results or standard isomeric mixtures of known composition. In the present studies the same $C_4H_4^{\bullet+}$ isomeric mixtures have been analyzed using both $^{+}$NR^{+} and $^{+}$NR^{-} spectra, which are relatively independent methods. For the nine precursors that apparently produce the same $[\mathbf{a}^{\bullet+}]/[\mathbf{c}^{\bullet+}]$ ratio (Table I, entries 5–6, 8–14), the average of the $^{+}$NR^{+} values for $[\mathbf{c}^{\bullet+}]$ is 69.3 ± 2.1% and of the $^{+}$NR^{-} values is 65.5 ± 5.2%; for the latter the larger standard deviation reflects the much lower $^{+}$NR^{-} cross sections. The absolute deviations from the average for the individual $^{+}$NR^{+} and $^{+}$NR^{-} measurements for each of 13 precursors (Table I, entries 5, 7–18) exhibit an average of 1.8 ± 2.6%, and the largest difference of the 13 individual values from the $^{+}$NR^{+}/$^{+}$NR^{-} average is 5.5%. The overall accuracy of the isomeric composition determination must additionally include compensating errors of the $^{+}$NR^{+} and $^{+}$NR^{-} methods and errors in reference spectra determination; however, it appears that isomeric composition accuracies of <10% absolute are achievable with the $^{+}$NR methodology.

Conclusions

Despite their high heat of formation values, the four $C_4H_4^{\bullet+}$ isomers appear to be relatively stable toward both isomerization and dissociation. Each has been formed as the major $C_4H_4^{\bullet+}$

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product in one or more unimolecular dissociation reactions, of which by far the most common pathway apparently involves the formation of excited benzene ions whose dissociation yields $\sim$68% $\mathbf{c}^{\bullet+}$ and $\sim$32% $\mathbf{a}^{\bullet+}$. The surprisingly high correspondence between the $^+NR^+$ and $^+NR^-$ values for the relative quantities of all four isomers produced from 13 precursors indicates a high accuracy for such ^+NR isomeric analyses.

Acknowledgment. The authors thank C. Wesdemiotis for advice and experimental results,[20] D. E. Drinkwater, M. Marchetti, R. F. Porter, and F. Turecek for helpful discussions, S. R. Kass for a preprint of ref 18, the National Science Foundation (Grants CHE-8712039 and CHE-9014883) for generous financial support, and the National Institutes of Health (GM16609) for instrumentation.

Decay and Fate of the Hydroxyl Radical Adduct of α-Phenyl-*N*-*tert*-butylnitrone in Aqueous Media†

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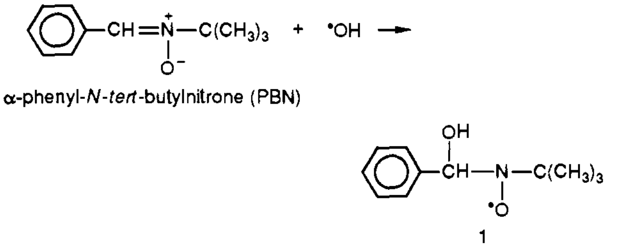
Abstract: The lifetime of α-(hydroxylbenzyl)-*tert*-butylaminoxyl, the hydroxyl radical adduct of α-phenyl-*N*-*tert*-butylnitrone (PBN) produced by spin trapping, is measured with an EPR spectrometer at various pH values in phosphate buffer. The decay of the EPR signal of the hydroxyl adduct of PBN is first order with respect to the concentration of the aminoxyl and the decay rate constant is strongly dependent on the pH of the solution. The rate constant increases as a function of increasing pH, indicating that the decay mechanism involves the dissociation of an acidic proton. Clear evidence is obtained that the hydroxyl adduct of PBN spontaneously transforms to *tert*-butylhydroaminoxyl.

As a first line of attack hydroxyl radicals add to the unsaturated functions of available substrates. The rates of addition are extremely fast and approach those of diffusion-controlled reactions. Rate constants for addition to targets have been measured, and little variation is found as a function of structure of the unsaturated site.[1] Nitrone spin traps[2] offer no particular exception to this generalization since the rate constants estimated for addition of hydroxyl radicals fall within the range of values frequently encountered for organic molecules, namely 10^7–10^9 mol L^{-1} s^{-1}.[3–6] Thus in evaluating the analytical usefulness of a spin trap for the detection of hydroxyl radicals, the actual rate constant of the spin trapping reaction is not of great importance since it can be assumed to be fast. Of more relevance is the persistance of the spin adduct and the uniqueness of the EPR spectrum.

One of the most often used nitrone spin traps, α-phenyl-*N*-*tert*-butylnitrone (PBN), produces relatively stable spin adducts of a variety of free radicals. The hydroxyl radical produced in various reactions is readily trapped by PBN:[7–9]



α-phenyl-*N*-*tert*-butylnitrone (PBN)

1

α-(hydroxylbenzyl)-*tert*-butylaminoxyl

However, the persistence of the hydroxyl adduct (**1**) may depend on the property of the solubilizing media. In spite of the large number of publications describing experiments utilizing PBN as a spin trap, detailed studies on factors influencing the stability of the hydroxyl adduct have not been published. The fate of the adduct is also unknown. This study is concerned with the decay rates of the hydroxyl spin adduct of PBN produced by the photolysis of hydrogen peroxide in aqueous media as a function of pH and the structure of the primary degradation product obtained in the decay reaction.

Experimental Section

PBN used in this study was synthesized in these laboratories and purified by sublimation before use.[10] Hydrogen peroxide was obtained from Sigma Chemical Co. as a 30% aqueous solution. Distilled water treated with the Millipore MilliQ system was used. D_2O was purchased from KOR Isotopes. The pH of the solution was adjusted by using HCl or KOH in potassium phosphate buffer (0.05–0.1 mol L^{-1}). The solution pH was adjusted after mixing the reagents because hydrogen peroxide usually lowers the pH considerably. Iron-depleted potassium phosphate buffer was prepared by passing 0.05 M buffer through a Chelex 100 column.

A typical experiment involves introducing a solution containing 1% hydrogen peroxide and 4×10^{-3} mol L^{-1} PBN into a flat quartz EPR sample cell. The sample solution was loaded with an injector (Fisher

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(10) PBN decomposes slowly and forms benzaldehyde in water. When unpurified PBN was used the reproducibility of the rate constants was poor.

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