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Polar effects control the gas-phase reactivity of charged *para*-benzyne analogs

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The gas-phase reactivity of charged *para*-benzynes is entirely unexplored as they and/or their precursors tend to undergo ring-opening upon their generation. We report here a gas-phase reactivity study of two such benzynes, the 2,5-didehydropyridinium and 5,8-didehydro-isoquinolinium cations, generated in a modified dual-linear quadrupole ion trap (DLQIT) mass spectrometer. Both biradicals were found to form diagnostic products with organic molecules, indicating the presence of two radical sites. As opposed to earlier predictions that the singlet–triplet (S–T) splitting controls the radical reactivity of such species, the 2,5-didehydropyridinium cation reacts much faster in spite of its larger S–T splitting. Calculated vertical electron affinities of the radical sites of the *para*-benzynes, a parameter related to the polarity of the transition states of their reactions, appears to be the most important reactivity controlling factor.

1. Introduction

Research on the biological activity of enediyne anticancer antibiotics has been at the forefront of cancer research for many decades because of the ability of these molecules to irreversibly cleave double-stranded DNA [1–4]. The enediynes can intercalate into a double-stranded DNA molecule and undergo Bergman-cyclization to form a σ-type carbon-centered biradical intermediate, usually a *para*-benzyne analog. This highly reactive molecule can abstract a hydrogen atom from each DNA strand, causing irreversible cleavage of the double-stranded DNA and ultimately leading to apoptosis of the cell [1–4]. However, these antibiotics have been found to be extremely cytotoxic [3,5]. In order to be able to rationally design less toxic synthetic drugs, knowledge of the chemical properties of *para*-benzyne analogs is essential.

Because of their short lifetimes, high reactivity, and difficulties in generation, the chemical properties of σ-type carbon-centered aromatic σ,σ-biradicals, such as *para*-benzynes, have proven challenging to study [6–8]. One method of overcoming these challenges is the "distonic ion approach" [9–13], a technique that involves attaching a chemically inert charged group to the reactive intermediate to allow for mass spectrometric manipulation. The charged group is located on a different atom than either of the radical sites. This approach has been successfully utilized to characterize the reactivity of many *meta*-benzyne analogs in Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometers [14–16]. Unfortunately, the generation of charged *para*-benzyne analogs in FT-ICR instruments has been unsuccessful [17]. For example, the attempted generation of the radical sites in **1** and **2** (Scheme 1) via the cleavage of two iodine atoms in 2,5-diiodopyridinium and 5,8-diiodoisoquinolinium cation precursors, respectively, involved two collision-activated dissociation (CAD) events to cleave the C—I bonds in a consecutive manner. However, the monoradical intermediates formed upon loss of one iodine atom were found to undergo ring-opening faster than iodine atom loss upon CAD, eventually forming enediynes (**3** and **4**; Scheme 1) instead of the isomeric *para*-benzynes [17].

In the present study, stable *para*-benzyne analogs **1** and **2** (Scheme 1) were successfully generated in a novel dual-linear quadrupole ion trap mass spectrometer (DLQIT) [18a–c]. Q3 Their reactivity toward allyl iodide, dimethyl disulfide, cyclohexane and tetra-hydrofuran is reported. To better understand the observations made for the *para*-benzyne analogs, their reactivities are compared to those of related monoradicals (Scheme 2).

2. Experimental

2.1. Instrumentation

All reactions were carried out in a differentially pumped dual-LQIT tandem mass spectrometer (DLQIT) [18a,b] equipped with

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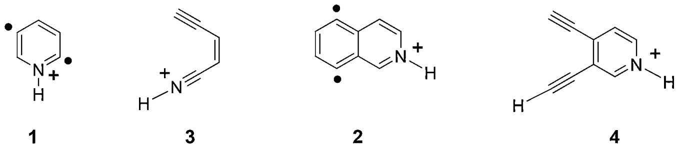


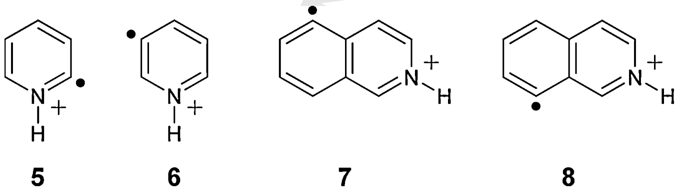
Scheme 1. *para*-Benzynes **1** and **2** and the corresponding enediyne isomers **3** and **4**

a manifold for neutral reagent introduction designed based on a previously described apparatus [18c]. This instrument consists of two differentially pumped Thermo Scientific linear quadrupole ion trap (LQIT) systems that have been connected via an ion transfer octupole encased in a machined manifold. Radical and biradical iodo-precursors were introduced and ionized by protonation via atmospheric pressure chemical ionization (APCI) and the radical sites were formed by ion-source collision-activated dissociation (CAD) of iodine atoms with nitrogen collision gas. The (bi)radicals were transferred into the first linear quadrupole ion trap and allowed to react with each reagent for varying periods of time to determine reaction products and efficiencies, as previously described for FT-ICR instruments [14].

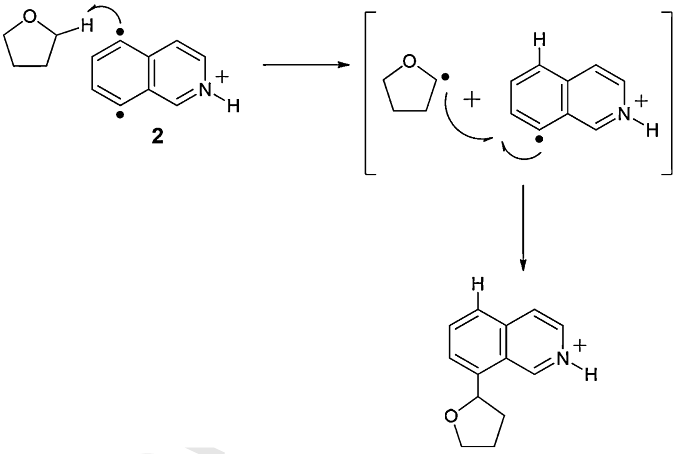
3. Results and discussion

The reactivity of the *para*-benzynes, **1** and **2**, was expected to be similar to that of related monoradicals but lower due to the stabilizing coupling of the (singlet) biradical electrons [19,20]. When the ions having m/z values of **1** and **2** were allowed to react with each of the neutral reagents, fast proton transfer was observed, indicative of the presence of the enediynes **3** and **4**, like in the earlier study [17]. The only exception was cyclohexane, which has a proton affinity too low to accept a proton from either protonated enediyne [17]. However, a second, slowly reacting isomer was also observed in each case (with the exception of cyclohexane for which only one isomer was detected). The second isomer yields reaction products similar to those of the related monoradicals. Hence, the second, slowly reacting isomers are likely to be the desired *para*-benzynes **1** and **2**. Their reactivity provides support for this hypothesis as it is analogous to that of related monoradicals, as discussed below.

Monoradicals **5**, **6**, **7**, and **8** show major reaction products, with branching ratios at or near 100%, that are diagnostic [21] for the presence of a radical site, such as a H atom abstraction product for cyclohexane and tetrahydrofuran, I atom abstraction product for allyl iodide and SCH$_3$ abstraction product for dimethyl disulfide (see Tables 1 and 2). The same primary reactions were observed for the *para*-benzynes. Furthermore, similar secondary reactions, identified by isolating the primary products and allowing them to react further, were observed for the *para*-benzynes with all four neutral reagents (Tables 1 and 2). This second set of abstractions reveals the presence of the second radical site, hence unambiguously demonstrating that the reacting ions are biradicals. Both biradicals show lower reactivity than the corresponding monoradicals, as expected, due to the stabilizing coupling of the nonbonding electrons in these (ground) singlet state biradicals Many additional reaction products were also observed (Tables 1 and 2). For example, a product resulting from the addition of the entire neutral reagent molecule to the biradical was observed for all reagents with **2** and all except cyclohexane and dimethyl disulfide with **1**. In addition, a product resulting from abstraction of HSCH$_3$ from dimethyl disulfide was observed for both biradicals. In general, the formation of these products is an additional indication that these molecules contain a second radical site. For example, addition of tetrahydrofuran to the *para*-benzyne analogs likely occurs by initial hydrogen atom abstraction by one of the two radical sites in **1** and **2**, followed by radical-radical recombination of the remaining radical site and the newly formed reagent radical (Scheme 3). Similarly, the product resulting from abstraction of HSCH$_3$ from dimethyl disulfide is likely initiated by SCH$_3$ abstraction by the more reactive radical site followed by H atom abstraction from the eliminated SCH$_3$ radical by the unquenched radical site in **1** and **2** to form CH$_2$S.



Scheme 2. The monoradicals studied.



Scheme 3. Proposed mechanism for the addition of tetrahydrofuran to **2**.

A significantly larger amount of the enediyne isomer was observed for **1** than **2**. For **2**, ca. 2–7% of the ion population corresponds to the enediyne **4** whereas for **1**, ca. 96–98% of the ion population corresponds to the enediyne, **3**. Previous calculations have shown that during the generation of these *para*-benzyne analogs, ring opening occurs upon CAD of the monoradical precursors formed via cleavage of the first C—I bond from the diiodoprecursors [17]. Hence, the cause for the large difference in the amounts of the enediyne isomers is likely the difference in the ring-opening barriers of the relevant monoradicals [17]. Indeed, the calculated ring opening barrier for the monoradical iodoprecursor of **1**, 53.5 kcal mol^{-1}, is lower than that for **2**, 56.8 kcal mol^{-1} (both calculated at the (U)B3LYP/6-311G(d,p)//(U)B3LYP/6-311G(d,p) level of theory). Furthermore, loss of an iodine atom from the iodoprecursor to generate the *para*-benzyne requires more energy for **1** than **2** (65.0 and 63.5 kcal mol^{-1}, respectively) [17].

As for differences in reaction efficiencies, **1** reacts at least two orders of magnitude faster than **2** with each neutral reagent studied. The closest reaction efficiencies were found for cyclohexane, in which case **1** reacts with an efficiency of 0.1% and **2** with 0.002%. The magnitude of the S–T splitting has been used in the past to explain differences in reaction efficiencies for radical reactions of related (ground) singlet state biradicals [19,20,22,23]. Stronger coupling between the biradical nonbonding electrons has been suggested to lead to lower (radical) reactivity [19]. However, **1** and **2** do not follow the expected trend. Biradical **1** with a S–T splitting of −4.6 kcal mol^{-1} (calculated at the RHF-BCCD(T)/cc-pVTZ//UB3LYP/cc-pVTZ (singlet state) or RHF-UCCSD(T)/cc-pVTZ//UB3LYP/cc-pVTZ (triplet state) level of theory) reacts much faster than **2** with a S–T splitting of −3.7 kcal mol^{-1}. However, the reactivity difference for **1** and **2** can be rationalized based on the electrophilicity of the radical sites, quantified here by the (calculated) vertical electron affinity (EA$_v$) of the

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