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Generation and Characterization of a Distonic Biradical Anion Formed from an Enediynone Prodrug in the Gas Phase

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Abstract

A negatively charged biradical intermediate was successfully generated in the gas phase via cyclization of the deprotonated bicyclo[8.3.0]trideca-12-ene-2,7-diyn-1-one precursor. The inherent negative charge of this biradical allows its characterization via collision-activated dissociation and reactions with a variety of neutral substrates in an FT-ICR mass spectrometer. Although the biradical is unreactive toward reagents that usually react rapidly with positively charged biradicals, such as dimethyl disulfide, it reacts with the halogen-containing substrates carbon tetrachloride, carbon tetrabromide and bromotrichloromethane via bromine or chlorine atom abstraction, which supports its biradical structure. The results presented in this study indicate that cyclizations commonly used in solution to form biradical intermediates from enediyne compounds may also occur in the gas phase.

Introduction

The enediyne class of DNA-cleaving agents contains the most potent family of antitumor antibiotics found thus far [1–6]. The DNA-cleaving action of these enediynes results from their unique enediyne core which forms biologically active biradical intermediates upon activation [1–6]. There are two major types of cyclization mechanisms through which reactive biradicals can be formed. In Bergman cyclization [7, 8], an enediyne unit cycloaromatizes to form a *para*-benzyne type biradical. On the other hand, in the Myers cyclization [9–11], an enyne-cumulene intermediate is proposed to yield a 1,5-didehydroindene biradical.

Since the discovery of the enediyne class of DNA-cleaving agents, considerable efforts have been dedicated toward designing simple analogous compounds that form intermediates with similar biological activities as the reactive intermediates of the complex naturally occurring enediynes [5, 12–18]. Synthesis of a simple enediynone, bicyclo[8.3.0]trideca-12-ene-2,7-diyn-1-one (1), that may undergo cyclization to form a biradical, was recently reported [16]. Upon reaction with NaH, the enediynone 1 yielded an intermediate compound whose structure could not be unambiguously determined by using ¹H NMR, ¹H-¹H COSY, HMQC, DEPT, IR, UV-vis, and mass spectrometry. Derivation of the intermediate by using *p*-nitrobenzoyl chloride yielded compound 7 (Scheme 1). Based on this finding, the unknown compound was proposed to have structure 5 [16]. The pathway leading to this compound 2 may undergo isomerization via two possible pathways to eventually form 5. It may cyclize to form biradical 3 that isomerizes to a more stable biradical 4 via intramolecular hydrogen atom transfer. Ring-opening of 4 would form compound 5 (Scheme 1, pathway a). Alternatively, the cumulene enolate 2 might isomerize to form 6 by prototropic transfer.

Compound **6** may cyclize to form compound **5** by a [3,3]-sigmatropic rearrangement (Scheme 1, pathway **b**) [16].

While the chemical properties of highly reactive molecules, such as the biradical discussed above, are very difficult to study in solution, this is possible by using gas-phase experiments. Chemical properties of positively as well as negatively charged phenyl radicals, phenyl carbenes, aromatic σ , σ -biradicals, aromatic Π , Π -biradicals and even triradicals have been successfully examined in mass spectrometers by using the distonic ion approach (*i.e.*, by attaching a charged moiety to a (bi)radical of interest for its mass spectrometric manipulation and analysis) [19–39]. Hence, we decided to examine the reactivity of cumulene enolate 2 by using mass spectrometry. Biradical 3, if formed from 2, may be distinguished from 2 and other cumulenes based on their gas-phase reactivity. The results obtained in these experiments are documented here.

Experimental

All the experiments were carried out in the Finnigan Model 2001 FTMS dual-cell 3 Tesla FT-ICR mass spectrometer equipped with an Odyssey data acquisition system, as described previously [22, 40–42]. The dual-cell is aligned with the magnetic field produced by a 3.0 T superconducting magnet, and differentially pumped by two Edwards diffusion pumps (800 L/s), each backed with a mechanical pump. A nominal base pressure (measured by an ionization gauge located at some distance from the cell) of less than 10⁻⁹ Torr was maintained.

Bicyclo[8.3.0]trideca-12-ene-2,7-diyn-1-one precursor was synthesized as described previously [16] and introduced into one side of the dual cell via an automatic solids probe at a nominal pressure of $0.8-1.6\times10^{-8}$ Torr. Water was introduced into the instrument via a pulsed valve assembly at a nominal peak pressure of about 1×10^{-5} Torr and subjected to electron ionization (6 eV electron energy, 8 μ A emission current and 50 ms electron beam time) to generate hydroxide ions. The hydroxide ions were allowed to react with the enediynone precursor for about 1 to 2 seconds. Upon deprotonation, a charged molecule with m/z value corresponding to 2 was formed. This anion was transferred into the other cell by grounding the conductance limit plate for about 130 μ s. The quadrupolar axialixation [43] (QA) technique was employed to enhance the ion transfer efficiency. After transfer, the ion of interest was allowed to cool for at least one second via IR emission and by collisions with argon (nominal peak pressure about 1×10^{-5} Torr) pulsed into the cell via a pulsed valve assembly. The ion of interest was isolated by applying a series of SWIFT [44] excitation pulses and then allowed to react with several neutral reagents. The reaction sequence is shown in Figure 1.

For comparison purposes, reactions of the phenoxide ion with the same neutral reagents were also examined. Phenol was introduced into one side of the dual cell via a variable leak valve at a nominal pressure of $1.9-2.9\times10^{-8}$ Torr. Dimethyl disulfide was introduced into the instrument via a batch inlet at a nominal pressure of about 4.0×10^{-8} Torr and subjected to electron ionization (2 eV electron energy, 8 μ A emission current and 1.5-3.5 s electron beam time) to generate methyl sulfide ion. This ion was allowed to react with phenol for about 7 seconds to yield the phenoxide ion. This anion was transferred into the other cell by grounding the conductance limit plate for about $100~\mu s$. After transfer, the phenoxide ion was allowed to cool for at least one second via IR emission and by collisions with argon. The phenoxide ion was isolated by applying a series of SWIFT excitation pulses before reactions with the neutral reagents.

All the neutral reagents were commercially available and used as received. Dimethyl disulfide, 1,4-cyclohexadiene, bromoform, carbon tetrachloride and bromotrichloromethane were introduced into the cell via the batch inlet at a nominal pressure of $1.5 - 3.9 \times 10^{-8}$ Torr. Iodine, thiophenol, iodoform, carbon tetrabromide and boron trifluoride were introduced into the cell via the variable leak valve at a nominal pressure of $0.6 - 2.9 \times 10^{-8}$ Torr. The ion of interest was allowed to react with these neutral reagents for variable periods of time ranging from 0.1 to 600 seconds. Under these conditions, the reactions follow pseudo-first-order kinetics. The second-order reaction rate constant k_{exp} was calculated from a semilogarithmic plot of the relative abundance of the reactant ion versus time and the concentration of each neutral reagent. In order to determine this concentration, pressure readings of the ion gauge were corrected for its sensitivity [45] toward each neutral reagent and for the pressure gradient between the cell and the ion gauge [46]. The correction factor for each reagent was obtained by measuring the rate of a highly exothermic reaction, i.e., electron transfer from the substrate to ionized argon, which was assumed to occur at collision rate. The reaction efficiencies (the fraction of collisions that lead to reaction products) are given as k_{exp}/k_{coll}. k_{coll} was calculated using a parameterized trajectory theory [47]. The accuracy of reaction rate constant measurements is estimated to be $\pm 50\%$ and the precision is greater than $\pm 10\%$.

Then enthalpy changes for selected reactions were calculated with the Gaussian 03 electronic structure program suite [48] at the B3LYP/cc-pVTZ or UB3LYP/cc-pVTZ// UB3LYP/cc-pVTZ level of theory, as specified in text.

Results and Discussion

Collision-activated Dissociation and Reactions with Common Radical Probes

Transformation of 2 to 6 (Scheme 1) requires migration of a hydrogen atom. In high vacuum, as in the experiments discussed here, this reaction would have to occur unimolecularly. This is not possible for 2 due to the C≡C bond that separates the hydrogen atom donor and acceptor sites. The transition of 3 to 4 is also unlikely due to the large distance between the hydrogen atom donor and acceptor sites and the orientation of the orbitals involved. Molecular orbital calculations (UB3LYP/cc-pVTZ//UB3LYP/cc-pVTZ level of theory) on the model compound 8 (Figure 2) suggest that the energy barrier for the hydrogen transfer to convert 3 to 4 (Scheme 1) is 48.5 kcal/mol. Hence, it is highly unlikely that these rearrangements can occur in the gas-phase experiments discussed here. Therefore, the two most probable structures for the ion generated from 1 in the gas phase are 2 and 3.

To probe the structure of the ion, collision-activated dissociation (CAD) was performed in the FT-ICR mass spectrometer since characteristic fragment ions often provide useful information on the connectivity of atoms in an ion. In FT-ICR mass spectrometers, dissociation experiments are often carried out by using the sustained off-resonance irradiation collision-activated dissociation [49] (SORI-CAD) technique which activates the ions slowly so that often only the cleavage of the weakest bond is usually observed. Upon SORI-CAD, the ion of interest yields two major fragment ions corresponding to loss of ethylene and loss of two hydrogen atoms (Figure 3). These findings are in best agreement with the biradical structure 3 since radical or charge site induced cleavages are the most common fragmentation pathways observed in mass spectrometers [50]. As shown in Scheme 2, radical site induced cleavage of two C—C bonds in 3 can lead to the loss of an ethylene molecule. Additionally, radical induced cleavage of one C—C bond and two C—H bonds can result in the loss of a hydrogen molecule from 3. Neither reaction is likely for 2 and 4.

Validation of the existence of two radical sites in an ion can be accomplished by ion-molecule reactions with certain reagents. Dimethyl disulfide and a variety of hydrogen atom

donors, such as 1,4-cyclohexadiene and thiophenol, are often used since positively charged radicals react with dimethyl disulfide by thiomethyl radical abstraction and with hydrogen donors by hydrogen atom abstraction [23, 24, 26, 30, 31]. Two sequential atom or group abstractions have been reported for biradicals [30,31]. Therefore, the ion of interest was allowed to react with dimethyl disulfide and selected hydrogen atom donors. The results obtained are shown in Table 1 and discussed below.

The ion of interest was found to be unreactive toward dimethyldisulfide, 1,4-cyclohexadiene and 1,4-dioxane. Upon reaction with thiophenol, proton transfer was observed. Proton transfer probably occurs at the charged oxygen atom (the proton affinity of phenoxide ion is 350.0 kcal/mol [51] while the proton affinity of thiophenoxide ion is 340.4 kal/mol [51]). Exothermic proton transfer usually dominates over radical reactions because it is barrierless and hence fast. These results are consistent with previous studies [29] on the reactivity of a negatively charged *meta*-benzyne analog, 3,5-didehydrobenzoate, which does not react with either dimethyl disulfide or 1,4-cyclohexadiene.

A comparison of the reactivity of a positively charged phenyl radical 9 (N-(3dehydrophenyl)pyridinium ion) to that of an analogous, negatively charged phenyl radical 10 (3-dehydrobenzoate ion) (Figure 2) demonstrated that the polarity of the charged group has a substantial influence on the reactivity of radicals in the gas phase [26]. Positively charged radicals react readily with electron-rich, nucleophilic substrates, such as tert-butyl isocyanide and dimethyl disulfide, characterized by relatively low ionization energies. However, the negatively charged radical was found to react substantially slower or not at all with these electron-rich substrates. In contrast, the negatively charged radical favors reactions with electron-deficient substrates, such as halogens and halogenated hydrocarbons, characterized by high electron affinities. For example, thiomethyl radical abstraction from dimethyl disulfide occurs six times faster for N-(3-dehydrophenyl)pyridinium ion than for 3dehydrobenzoate ion while bromine atom abstraction from carbon tetrabromide occurs 70 times faster for 3-dehydrobenzoate ion than for N-(3-dehydrophenyl)pyridinium ion. The reactivity difference was attributed to the different ability of each reacting system to stabilize the transition state by polarization [26]. For a negatively charged singlet biradical, such as the ones discussed here, a large S-T gap is expected to further reduce its reactivity towards all substrates [30, 31, 34]. Thus, it is not surprising that no radical reactions were observed upon reaction of the ion of interest with the four electron-rich reagents listed in Table 1. In order to characterize the negatively charged ion of interest, examination of its reactions with electron-deficient substrates is necessary. These reactions are discussed in the following sections.

Reactions with an Electron-deficient Substrate - Boron Trifluoride

Boron trifluoride generally reacts readily with electron-rich molecules, such as negatively charged ions. Thus, reactions of the ion of interest and the phenoxide ion (for comparison purposes) with boron trifluoride were examined in the FT-ICR mass spectrometer. The results are summarized in Table 2. Upon reaction with boron trifluoride, addition and addition followed by elimination of a hydrogen fluoride molecule were observed for the ion of interest (Figure 4). Phenoxide ion reacts with boron trifluoride via addition at a similar efficiency (48% for the ion of interest vs. 46% for phenoxide ion), which suggests that the oxygen atom in the ion of interest adds to boron trifluoride and the radical sites (if present) play no role. Furthermore, for both anions, addition is followed by the same secondary reaction, formation of boron tetrafluoride ion, which suggests that the adducts have a similar structure and that the radical sites in the unknown ion (if present) have not participated in the reaction. A proposed mechanism for the addition of the ion to boron trifluoride and the

following secondary reaction, formation of boron tetrafluoride ion, is shown in Scheme 3 for the biradical candidate.

Besides the formation of a stable adduct, addition followed by the loss of a hydrogen fluoride molecule was observed for the ion of interest. This reaction does not occur for phenoxide. The proposed mechanism for this slow addition/elimination reaction of the biradical candidate likely involves an initial addition of the Π electrons of the ion to the porbital on boron followed by elimination of HF via a six-membered transition state, as shown in Scheme 4. The reaction was calculated to be exothermic by 3.2 kcal/mol (B3LYP/cc-pVTZ level of theory). Elimination of HF via this mechanism is not possible for phenoxide. The HF loss product undergoes a fast addition to another boron trifluoride molecule to form a secondary addition product (Scheme 4), which supports the proposal that the first boron trifluoride molecule has added to a site other than the oxygen atom, which is still available to undergo the observed secondary addition.

Reactions with boron trifluoride reveal that the ion of interest is a nucleophile although its radical or nonradical character remains unclear at this point. Similar reactivity, addition of boron trifluoride to a negatively charged oxygen atom in a related biradical, 3,5-didehydrobenzoate anion, has been reported earlier [29]. No reactions involving the biradical moiety were observed, just as here.

Reactions with Halogen-containing Substrates

Previous studies have demonstrated that halogen atom abstraction from halogens and halogenated hydrocarbons is characteristic for negatively charged (bi)radicals [26,29]. This section reports results obtained upon examination of reactions with different halogen-containing reagents (Table 3). Upon reaction of the ion of interest with iodine, charge transfer was observed. This indicates that the electron affinity of iodine (2.52 eV [52]) is higher than that of the phenoxy radical analog 11 shown in Figure 2 (electron affinity of phenoxy radical is 2.25 eV [52]), as expected. Proton abstraction was observed in reactions with bromoform and iodoform. Hence, the proton affinity of the ion of interest must be higher than that of tribromomethyl anion and triiodomethyl anion (349.7 kcal/mol [50] and <349.7 kcal/mol, respectively), as expected (proton affinity of phenoxide ion is 350.0 kcal/mol [50]). Proton transfer was also observed between the phenoxide ion and bromoform. These findings indicate that the proton transfer reactivity of the ion of interest is associated with the charged oxide moiety rather than a (bi)radical site.

In the reactions with tetrachloride, tetrabromide, and bromotrichloromethane, product ions corresponding to abstraction of a chlorine or bromine atom by the ion of interest and elimination of a hydrogen atom ($[M+Cl-H]^-$ or $[M+Br-H]^-$) were observed. A mass spectrum measured for the reaction of the ion of interest with bromotrichloromethane is shown in Figure 5. These results indicate that the structure of the ion of interest is likely to be that of biradical 3 since it is highly unlikely for the uncycloaromatized molecules, 2 or 6, to react with tetrabromide or tetrachloride by a halogen atom abstraction. To confirm that the reactions observed for these *per*-halogenated substrates arise from the (bi)radical sites of the ion of interest, reactions of phenoxide ion with the same substrates were examined. No reactions were observed. Therefore, we conclude that the observed products $[M+Br-H]^-$ and $[M+Cl-H]^-$ in the reactions of the unknown ion are radical reaction products, and hence, the structure of the ion of interest is that of 3.

A reaction mechanism for bromotrichloromethane is proposed in Scheme 5. This mechanism involves an initial bromine atom abstraction by the biradical from bromotrichloromethane. The same reaction has been observed earlier for a related negatively charged, carbon-centered σ -radical, 3-dehydrobenzoate, as well as for a related carbon-

centered σ , σ -biradical, 3,5-didehydrobenzoate, upon interaction with bromotrichloromethane [26,29]. After bromine atom abstraction, a fast electron transfer from the so-generated monoradical to the trichloromethyl radical may occur within the collision complex, which leads to the formation of trichloromethyl anion (Scheme 5, pathway **a**) (as expected based on the similar electron affinities of trichloromethyl radical and phenoxy radical: 2.16 eV [52] and 2.25 eV [52], respectively). Alternatively, the trichloromethyl radical may abstract a hydrogen atom accompanied by ring opening to yield an *ortho*-benzyne analog (Scheme 5, pathway **b**) that corresponds to [M + Br – H]⁻. This reaction was calculated to be exothermic by 28.9 kcal/mol (at the B3LYP/cc-pVTZ level of theory). Some of the product ions fragment via loss of bromide ion. Proposed mechanism of this dissociation is shown in Scheme 6.

Dissociation of $[M + Cl - H]^-$ to yield chloride ion was observed in the reaction with carbon tetrachloride. A product corresponding to the loss of a hydrogen chloride molecule from $[M + Cl - H]^-$ was also observed. Its formation is proposed to occur by proton transfer to a leaving chloride ion (Scheme 7). The observation of this reaction only for carbon tetrachloride can be attributed to the higher proton affinity of chloride ion (333.4 kcal/mol [51]) than bromide ion (323.5 kcal/mol [51]).

It is notable that biradical 3 reacts more efficiently with bromotrichloromethane (reaction efficiency 60%) than with either tetrabromide (reaction efficiency 51%) or with tetrachloride (reaction efficiency 45%). Based on the homolytic C—Br bond dissociation energies [53] (55.3 kcal/mol for bromotrichloromethane and 56.2 kcal/mol for carbon tetrabromide), the exothermicity of bromine atom abstraction from bromotrichloromethane and carbon tetrabromide should be very close. This less than 1 kcal/mol difference in reaction exothermicity may not account for the nearly 10% difference in reaction efficiencies observed for bromotrichloromethane and carbon tetrabromide. Dipole moments of these reagents may be responsible for the observed reactivity difference. Compared to carbon tetrabromide and carbon tetrachloride (with no dipole moment), bromotrichloromethane with a dipole moment of 0.1 Debye (calculated at the B3LYP/6-31+G (d) level of theory [26]) will result in a greater solvation energy for this reagent. If the barrier for atom abstraction is the same for all reagents, the energy difference between the separated reactants and transition state will be greater for bromotrichloromethane than the other two reagents. Hence, reaction of biradical 3 with bromotrichloromethane is predicted to be faster than with carbon tetrabromide and carbon tetrachloride. In addition, the fact that C—Cl bond dissociation energy (~73 kcal/mol [53]) is greater than that of the C—Br bond (~55 kcal/mol [53]) accounts for slow chlorine atom abstraction from carbon tetrachloride and no chlorine atom abstraction from bromotrichloromethane.

Conclusions

A negatively charged biradical intermediate was successfully generated in the gas phase via cyclization of the deprotonated bicyclo[8.3.0]trideca-12-ene-2,7-diyn-1-one precursor. The inherent negative charge of this biradical allows its characterization via collision-activated dissociation and reactions with a variety of neutral substrates in an FT-ICR mass spectrometer. Although neither hydrogen atom abstraction from common hydrogen atom donors nor thiomethyl radical abstraction from dimethyl disulfide was observed, the biradicals' reactions with halogen-containing substrates, carbon tetrachloride, carbon tetrabromide and bromotrichloromethane, support the biradical structure. Reaction products involving bromine atom or chlorine atom abstraction were observed. The lack of radical reactivity toward nucleophilic substrates can be attributed to the polarity of the biradical. As a result, the biradical ion favors reactions with electrophilic reagents. Addition to boron trifluoride reveals the nucleophilic character of the biradical intermediate.

Finally, the results presented in this study indicate that cyclizations commonly used in solution to form biradical intermediates from enediyne precursors may also occur in the gas phase. By attaching a charged moiety to the biradical intermediates, reactions with a variety of substrates can be studied in mass spectrometers to characterize the biradicals and to provide insightful information on their intrinsic reactivity.

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Figure 1.
Reaction sequence in the FT-ICR mass spectrometer

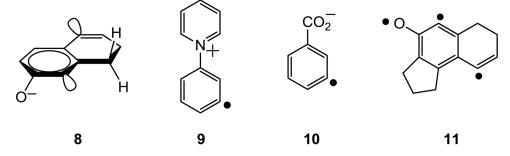


Figure 2. Structures of related compounds

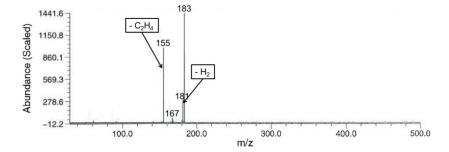


Figure 3. A SORI-CAD mass spectrum measured for the isolated ion (m/z 183) of interest

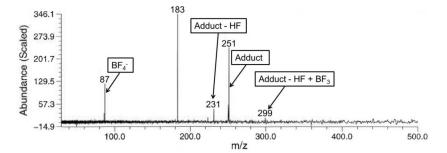


Figure 4. A mass spectrum measured for the reaction of the ion of interest (m/z 183) with boron trifuoride

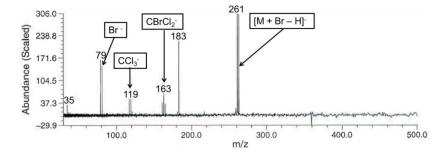


Figure 5. A mass spectrum measured for the reaction of the ion of interest (m/z 183) with bromotrichloromethane

Scheme 1.

Scheme 2.

$$F_{B}-F$$

$$F_{B$$

Scheme 3.

Scheme 4.

Scheme 5.

Scheme 6.

Scheme 7.

Table 1

Products observed for the reactions of the ion of interest with neutral reagents

	or C=C=
CH₃SSCH₃	No products observed
	No products observed
	No products observed
ян — sн	C ₆ H ₅ S ⁻ (100%)

Table 2

Efficiencies a and products b,c for the reactions of the ion of interest and phenoxide ion with boron trifluoride

	BF ₃
or or c=c=	Adduct 91% BF_4^- (2°) Adduct - HF 9% + BF_3 (2°) Efficiency = 48%
-0	Adduct 100% BF ₄ ⁻ (2°) Efficiency = 46%

^aReaction efficiency (Eff.) = $k_{reaction}/k_{collision} \times 100\%$.

 $^{^{\}mbox{\it b}}_{\mbox{\footnotesize Product}}$ branching ratios are given after each primary reaction product.

 $^{^{\}textit{C}}\textsc{Secondary}$ products are given underneath the primary product that produced them.

Table 3

Efficiencies^a and products^{b,c} for reactions of the unknown ion with halogenated substrates (efficiencies were only measured for radical reactions)

	or C=C
I_2	$I_3^ I_3^ (2^0)$ I_2^- Efficiency not measured
CHBr ₃	Br ⁻ CBr ₃ ⁻ Efficiency not measured
CHI ₃	I ⁻ CI ₃ ⁻ Efficiency not measured
CCl ₄	[M + Cl - H] ⁻ 71% Cl ⁻ 16% CCl ₃ ⁻ 13% [M + Cl - H - HCl] ⁻ (trace) Efficiency = 45%
CBr ₄	CBr ₃ - 72% [M + Br - H] ⁻ 17% Br- 11% Efficiency = 51%
CBrCl ₃	[M + Br - H] ⁻ 58% Br ⁻ 21% CCl ₃ ⁻ 21% CBrCl ₂ ⁻ (2°) Cl ⁻ (trace) m/z 259 ^d (trace) Efficiency = 60%

^aReaction efficiency (Eff.) = $k_{reaction}/k_{collision} \times 100\%$.

 $[\]ensuremath{^b}\xspace$ Product branching ratios are given after each primary reaction product.

 $^{^{\}mbox{\it C}} Secondary products are given underneath the primary product that produced them.$

 $^{^{}d}_{\hbox{Unidentified products.}}$