A Study of the Reaction Between Quinone and 2R4F Cigarette Smoke Condensate*

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SUMMARY

A study using atomic emission detection (AED) as an approach to explore the fate of quinone added into 2R4F cigarette smoke condensate (CSC) have been performed. Both natural isotope quinone and ^{13}C labeled quinone were used in the study. When coupled with a gas chromatographic separation (GC/AED), the AED provided informative new data on ^{13}C isotope enriched products generated following reactions between 2R4F CSC and the quinone. Two ^{13}C containing species were detected by GC/AED. Matching chromatographic separation using gas chromatography/mass selective detection (GC/MSD) allowed for a structural assignment of a relatively minor CSC $^{13}\text{C}_6$ quinone reaction product as nitrohydroquinone ($^{13}\text{C}_6\text{NO}_2\text{HQ}$). The chemical mechanism accounting for the formation of $^{13}\text{C}_6\text{NO}_2\text{HQ}$ in the CSC was envisioned to be a reaction product between HONO and $^{13}\text{C}_6$ Quinone ($^{13}\text{C}_6\text{Q}$) to form $^{13}\text{C}_6\text{NO}_2\text{Q}$, followed by reduction of $^{13}\text{C}_6\text{NO}_2\text{Q}$ to $^{13}\text{C}_6\text{NO}_2\text{HQ}$. The amount of $^{13}\text{C}_6\text{NO}_2\text{HQ}$ accounted for ~6% of the added $^{13}\text{C}_6\text{Q}$. Identical trends in reaction chemistries were found for experiments with $^{12}\text{C}_6\text{Q}$. The major reaction product detected upon addition of $^{13}\text{C}_6\text{Q}$ to the 2R4F CSC sample was $^{13}\text{C}_6\text{HQ}$. $^{13}\text{C}_6\text{HQ}$ accounted for, on average, ~47% of the initial $^{13}\text{C}_6\text{Q}$ concentration. Identical trends in reaction chemistries were found for experiments with $^{12}\text{C}_6\text{Q}$. No additional ^{13}C containing species were detected. A ^{13}C AED compound independent calibration (CIC) approach under the operating conditions was not possible. This work further expands the knowledge regarding possible reactions of quinone and hydroquinone in CSC. [Beitr. Tabakforsch. Int. 13 (2009) 359–367]

ZUSAMMENFASSUNG

Das Schicksal von Chinon, das zum Zigarettenrauchkondensat (CSC) einer 2R4F Zigarette hinzugefügt wurde, wurde mittels Atomemissionsdetektion (AED) untersucht. Sowohl das natürliche Isotop des Chinons als auch ^{13}C -markiertes Chinon wurden eingesetzt. Gekoppelt mit gaschromatographischer Trennung (GC/AED) lieferte die AED informative Daten über die mit dem ^{13}C -Isotop angereicherten Produkte, die aus den Reaktionen zwischen dem CSC der 2R4F Zigarette und dem Chinon hervorgegangen waren. Mittels GC/AED wurden zwei Spezies, die ^{13}C enthielten, gefunden. Mit massenspektrometrischer Detektion (GC/MSD) konnte der kleinere Peak als Nitrohydrochinon ($^{13}\text{C}_6\text{NO}_2\text{HQ}$) identifiziert werden. Es wird angenommen, dass es sich bei dem chemischen Mechanismus, der zur Bildung des $^{13}\text{C}_6\text{NO}_2\text{HQ}$ im CSC führte, um eine Nitrierung des $^{13}\text{C}_6$ Quinon ($^{13}\text{C}_6\text{Q}$) mit salpetriger Säure (HONO) handelt, wobei $^{13}\text{C}_6\text{NO}_2\text{Q}$ gebildet wurde mit nachfolgender Reduktion zu $^{13}\text{C}_6\text{NO}_2\text{HQ}$. Der Anteil des $^{13}\text{C}_6\text{NO}_2\text{HQ}$ betrug ungefähr 6% des zugefügten $^{13}\text{C}_6\text{Q}$. Identische Trends in der Reaktionschemie wurden bei Experimenten mit $^{12}\text{C}_6\text{Q}$ gefunden. Das Hauptreaktionsprodukt, dass nach Zugabe von $^{13}\text{C}_6\text{Q}$ zum 2R4F CSC gefunden wurde, war $^{13}\text{C}_6\text{HQ}$. Der Anteil des $^{13}\text{C}_6\text{HQ}$ betrug ungefähr 47% der ursprünglichen $^{13}\text{C}_6\text{Q}$ Konzentration. Identische Trends bei der Reaktionschemie wurden bei Experimenten mit $^{12}\text{C}_6\text{Q}$ gefunden. Es wurden keine zusätzlichen ^{13}C -haltigen Spezies gefunden. Ein Nachweis des ^{13}C mittels AED-CIC (compound independent calibration) war unter den vorherrschenden Bedingungen nicht möglich. Diese Arbeit vertieft das Wissen über mögliche Reaktionen von Chinon und Hydrochinon im CSC. [Beitr. Tabakforsch. Int. 13 (2009) 359–367]

RESUME

Le devenir de la quinone apportée au condensat de la fumée (CSC) de la cigarette 2R4F a été examiné au moyen de détection par émission atomique (AED). L'isotope naturel et la quinone marquée au ^{13}C ont été utilisés dans cette étude. Couplé avec une chromatographie à phase gazeuse (GC/AED) l'AED a fournit des données nouvelles sur des produits enrichis par l'isotope ^{13}C et qui ont été générés par la réaction entre le CSC de la cigarette 2R4F et la quinone. Deux espèces moléculaires ont été détectées par GC/AED. La détection sélective de masse (GC/MSD) a permis l'identification du spectre de nitro-hydroquinone ($^{13}\text{C}_6\text{NO}_2\text{HQ}$). Il est supposé que $^{13}\text{C}_6\text{NO}_2\text{HQ}$ ait été généré par la réaction entre HONO et la $^{13}\text{C}_6$ Quinone ($^{13}\text{C}_6\text{Q}$) et la formation de $^{13}\text{C}_6\text{NO}_2\text{Q}$, et réduction consécutive de $^{13}\text{C}_6\text{NO}_2\text{Q}$ en $^{13}\text{C}_6\text{NO}_2\text{HQ}$. La quantité de $^{13}\text{C}_6\text{NO}_2\text{HQ}$ s'élève à environ 6% de la $^{13}\text{C}_6\text{Q}$ apportée. Les réactions chimiques ont été comparables dans les études avec la substance $^{12}\text{C}_6\text{Q}$. La molécule $^{13}\text{C}_6\text{HQ}$ a été le principal produit de réaction après apport de $^{13}\text{C}_6\text{Q}$ au CSC de la cigarette 2R4F, représentant en moyen ~47% du $^{13}\text{C}_6\text{Q}$ initiale. Les réactions chimiques ont été comparables dans les études avec la substance $^{12}\text{C}_6\text{Q}$. Aucune espèce moléculaire supplémentaire ne pouvait être détectée. Dans les conditions opératoires une détection du ^{13}C par CIC (compound independent calibration) n'a pas été possible. L'objectif de cette revue est d'approfondir le savoir actuel sur les réactions possibles de la quinone et hydroquinone dans le CSC. [Beitr. Tabakforsch. Int. 23 (2009) 359–367]

INTRODUCTION

For a number of years, cigarette smoke has been recognized as a complex mixture of organic and inorganic species (1). The chemical complexity of cigarette smoke is strongly dependent on the heating conditions inside the lit cigarette (2). Recently, special emphasis has been placed on investigations related to the redox chemistry and free radical content of cigarette smoke (3,4). In addition, research efforts have been placed toward understanding the role of selected hydroxybenzenes, benzoquinone (quinone), Q, and hydroquinone, HQ, in the chemistry and toxicity of cigarette smoke, as well as in their chemistries and toxicities (5–19). A portion of the understanding and focus has centered about the free radical nature of the hydroquinone-semiquinone-quinone reaction and the potential of this reaction to influence the nature and possibly the toxicity of cigarette smoke. In addition, benzoquinone has been shown to react with dienes in mainstream vapor phase smoke to yield Diels-Alder reaction products (20). In fact, the reactions between benzoquinone and dienes culminated in the Nobel Prize in Chemistry for Professor OTTO DIELS and his student KURT ALDER in 1950 (18, 19). The initial investigations concerning these reactions took place in 1928.

Atomic emission detection (AED) coupled with gas chromatographic separation (GC/AED) provides for an ideal means of determining the fate of a molecule(s) present in a reactive/complex mixture, particularly if the molecule(s) of interest is composed of certain elements, such as

phosphorus, sulfur, or nitrogen (21–30). The AED approach is based on the generation of atomic species from degradation of organic molecules in a microwave plasma. In a recent study (28), the fate of deuterium (D) labeled compounds was determined in mainstream and sidestream tobacco smoke. More specifically, the fates of nicotine- d_4 and pyridine- d_5 were monitored after their addition to the tobacco rod of full flavor cigarettes. GC/AED approaches have also been defined for monitoring the fate of ^{13}C labeled compounds in the presence of ^{12}C species. Linking the chromatographic separation from GC/AED with chromatographic separation using a gas chromatography/mass spectrometric detection (GC/MSD) provided for confident structural assignments of the $^{13}\text{C}_6\text{Q}$ Diels-Alder reaction products in mainstream smoke (20).

In the early 1970's KLUS and KUHN determined nitrophenols in cigarette smoke condensate (31) demonstrating a dependency on the nitrate content of the tobacco blend and nitrophenol yields. However, the issue of possible artifact formation was not addressed and no one has repeated the investigations. Thus, based in part on the information surrounding the characteristics of mainstream cigarette smoke as portrayed by hydroxybenzenes, the chemical complexity of mainstream cigarette smoke, possible artifact formation, as well as smoke chemistry disclosed (32, 33), the dynamic reactivity of mainstream cigarette smoke would seem to have been confirmed and well documented. During the course of our GC/AED investigations into $^{13}\text{C}_6\text{Q}$ and $^{13}\text{C}_6\text{HQ}$ reactions with mainstream smoke, a ^{13}C response was noted in the cigarette smoke condensate from 2R4F. Preliminary indications from GC/MSD data suggested that this new ^{13}C species could possibly contain nitrogen. Under the same separation conditions, this new ^{13}C species was also found to have a notably different retention time that the Diels-Alder $^{13}\text{C}_6\text{Q}$ reaction products previously detected (20) in 2R4F mainstream smoke. In this present report is described yet another reaction between well known cigarette smoke components. Data collected via GC/AED and GC/MSD provide information on an additional reaction pathway(s) in the complex chemistry of the hydroquinone-semiquinone-quinone shuttle as it relates to mainstream cigarette smoke.

EXPERIMENTAL SECTION

Chemicals

Hydroquinone $^{13}\text{C}_6$ and quinone $^{13}\text{C}_6$ were obtained from IsoSciences, King of Prussia, PA. Solutions of known concentration of hydroquinone $^{13}\text{C}_6$ and quinone $^{13}\text{C}_6$ were prepared in acetone. $^{12}\text{C}_6$ nitrohydroquinone ($^{12}\text{C}_6\text{NO}_2\text{HQ}$) was obtained from Frinton Laboratories, Vineland, NJ. Acetone, cyclohexanol-1- ^{13}C , diethylmalonate-1,3- $^{13}\text{C}_2$, ethyl acetoacetate-3,4- $^{13}\text{C}_2$, toluene- ^{13}C (methyl), benzaldehyde- $^{13}\text{C}_6$, benzyl alcohol- $^{13}\text{C}_6$, nitrobenzene- $^{13}\text{C}_6$, and acetone was obtained from Aldrich Chemical Company, St. Louis, MO. Catechol- $^{13}\text{C}_6$, 4-hydroxybenzaldehyde- $^{13}\text{C}_6$ and phenol- $^{13}\text{C}_6$ were obtained from Cambridge Isotope Laboratories, Andover, MA. All reagents were used as received.

Table 1. Gas chromatography/atomic emission detection (GC/AED) operating conditions

System configuration	Agilent 6890 GC equipped with a G2350 AED (Wilmington, DL, USA)
Column	DB-1701 30 meters, 0.25 mm I. D., 0.25 µm film thickness
Injection port temperature	280 °C
Injection	Splitless
Column flow	Constant, 1mL/min He
Column oven initial temperature	50 °C
Column oven initial time	0 min
Column oven initial ramp 1 rate	2.5 °C/min
Column oven 1 final temperature	90 °C
Column oven 1 final time	0 min
Column oven ramp 2 rate	10 °C/min
Column oven 2 final temperature	280 °C
Column oven 2 final time	0 min
Elements	Carbon12, 177.491 nm Carbon13, 176.934 nm
AED vent times	On @ 2.5 min, Off @ 5.00 min
Hydrogen pressure	8.3 psi
Oxygen pressure	35.5 psi
Purge vent flow	32 mL/min
Cavity vent flow	61 mL/min
Cavity pressure	1.5 psi
AED transfer line temperature	280 °C
AED cavity temperature	290 °C

Table 2. Gas chromatography/mass spectrometry (GC/MS) operating conditions

System configuration	Agilent 6890 GC equipped with a 5973 MSD (Wilmington, DL, USA)
Column	DB-1701 30 meters, 0.25 mm I. D., 0.25 µm film thickness
Injection port temperature	280 °C
Injection	Splitless
Column flow	Constant, 0.6 mL/min He
Column oven initial temperature	50 °C
Column oven initial time	0 min
Column oven initial ramp 1 rate	2 °C/min
Column oven 1 final temperature	80 °C
Column oven 1 final time	0 min
Column oven ramp 2 rate	9.8 °C/min
Column oven 2 final temperature	280 °C
Column oven 2 final time	0 min
Mass spectrometer transfer line temp	280 °C
Mass spectral databases	NBS, Wiley
Mass spectrometer configuration	Electron Impact, 70 eV

Instrumentation

Gas chromatography (GC)/atomic emission detection (AED) analysis conditions appear in Table 1. Gas chromatography (GC)/mass selective detection (MSD) analysis conditions appear in Table 2.

2R4F cigarette smoke condensate (CSC) sample collection and preparation: 2R4F CSC collection and dose solution collection and preparation were performed following a literature method (34).

CSC sample preparation for GC/AED and GC/MSD analyses: To 1 mL of cigarette smoke condensate solution (CSC) was added 1 mL $^{13}\text{C}_6\text{Q}$ in acetone at 7010 µg/mL. The solution was shaken gently, diluted 1:10 with acetone, and then placed in a GC vial. The solutions were subsequently monitored by GC/AED and GC/MSD, *vide infra*.

A calibration curve for $^{13}\text{C}_6\text{HQ}$ was prepared using GC/AED responses for known concentrations of $^{13}\text{C}_6\text{HQ}$. The slope and R^2 values for the calibration curve were 16.599 and 0.9992, respectively.

Compound independent calibration (CIC) and sample preparation: Acetone solutions of each ^{13}C compound were prepared as to yield a well defined relatively representative concentration of mmoles of ^{13}C . The GC/AED conditions, Table 1, were employed for data generation. ^{13}C AED areas were then plotted against mmoles ^{13}C .

A brief description of the AED CIC approach seems indicated here. Under optimized AED operating conditions, the CIC is often very useful. Fundamentally, the CIC theory is based on the complete quantitative conversion of the labeled analytes of interest to a common simple molecule, like ^{13}CO in our case. Thus, plots of concentration now depend on the AED response to an amount of a common

Figure 1. $^{13}\text{C}_6$ GC/AED response from 1 mL 2R4F CSC (Reference 35) + 1 mL $^{13}\text{C}_6\text{Q}$ @ 7010 $\mu\text{g/mL}$

molecule, ^{13}CO , whose origin is directly related to the concentration of the precursor molecule but under optimized AED conditions is not a function of the molecular structure of the precursor molecule.

RESULTS AND DISCUSSION

Figure 1 presents the GC/ ^{13}C AED responses when $^{13}\text{C}_6\text{Q}$ was added into 2R4F CSC. The detection of the presence of ^{13}C isotope signals serves to clearly illustrate the uniqueness of the AED. More specifically, the advantage/uniqueness of the AED rests with its ability to selectively detect the presence of specific unique atomic isotopes in the presence of additional atomic species. In the case under study herein, the advantage of the AED is its ability to detect relatively low concentration of the ^{13}C isotope in the presence of relatively abundant concentrations of ^{12}C isotopes. Such detection allows for the trace of the isotope through a chemical reaction of series of chemical reactions. Thus, several observations were made from the data appearing in Figure 1 because of the uniqueness of the AED:

- ▶ The AED readily detected the presence of both $^{13}\text{C}_6\text{Q}$ ~ 15 min and $^{13}\text{C}_6\text{HQ}$ ~ 27 min).
- ▶ The AED response for $^{13}\text{C}_6\text{Q}$, ~ 15 min, was relatively low.
- ▶ The AED response for $^{13}\text{C}_6\text{HQ}$, ~ 27 min, was relatively substantial.
- ▶ Addition of $^{13}\text{C}_6\text{Q}$ to the CSC samples resulted in essentially complete elimination of any $^{13}\text{C}_6\text{Q}$ response and appearance of a ^{13}C AED signal attributable to $^{13}\text{C}_6\text{HQ}$.
- ▶ These results were in concert to previous findings (20).
- ▶ A ^{13}C containing product not attributable to $^{13}\text{C}_6\text{Q}$ or $^{13}\text{C}_6\text{HQ}$ was observed at ~ 30 min in the 2R4F CSC sample.
- ▶ Two additional responses were observed at 24.5 and 25.5 min. These responses were due to relatively high concentrations of ^{12}C nicotine and ^{12}C triacetin in the 2R4F CSC. Relatively high concentrations of ^{12}C

isotopes will produce signals within a ^{13}C isotope scan due to the close proximity of the absorbance wavelengths employed in the AED methodology, ^{12}C , 177.491 nm; ^{13}C , 176.934 nm..

- ▶ Responses due to comparable similar quinone-like ^{12}C species were not detected in the 2R4F CSC without the addition of Q or HQ.

Close examination of the AED chromatogram in Figure 1 revealed the presence of a ^{13}C containing species with a retention time of ~ 30 min. Since the separation conditions and retention times from the AED were duplicated on the GC/MSD the mass spectrum of the component at ~ 30 min could readily be obtained. For the component with retention time ~ 30 min, the mass spectrum revealed a relatively simple ion profile with an intense base peak at $m/z = 155$ when $^{12}\text{C}_6\text{Q}$ was employed, Figure 2A. When $^{13}\text{C}_6\text{Q}$ was employed the mass spectrum base peak m/z shifted by 6 units from $m/z = 155$ to $m/z = 161$, Figure 2B. Accompanying the shift in m/z of 6 units were additional shifts in m/z values linked to the presence of ^{13}C atoms within the structure, Figure 2. Thus, this yet unidentified component at retention time ~ 30 min did contain 6 ^{13}C atoms. This strongly suggested that the 6 membered ring structure of the added $^{13}\text{C}_6\text{Q}$ remained intact for this particular structure. Positive and negative ion GC/MSD experiments were performed on the $^{12}\text{C}_6\text{Q}$ and $^{13}\text{C}_6\text{Q}$ solutions. The results of these experiments further indicated that $m/z = 155$ was indeed the molecular ion (36). With a proposed intact 6 membered ring structure, the presence of a nitrogen atom, and a molecular weight of 155, four reasonable empirical formulas are/were possible: 1) $\text{C}_6\text{H}_5\text{NO}_4$, 2) $\text{C}_6\text{H}_9\text{N}_3\text{O}_2$, 3) $\text{C}_6\text{H}_7\text{N}_2\text{O}_3$, and 4) $\text{C}_6\text{H}_{11}\text{N}_4\text{O}$. Taking the simplest approach and assuming the addition of a species containing one nitrogen to the $^{13}\text{C}_6\text{Q}$ structure, empirical formula number 1 seemed to be the best candidate. Such an approach yielded Figure 3, nitrohydroquinone, $^{12}\text{C}_6\text{NO}_2\text{HQ}$.

Analysis of the mass spectrum of an authentic standard of $^{12}\text{C}_6\text{NO}_2\text{HQ}$ yielded a retention time consistent with the unknown at retention time ~ 30 min. The mass spectrum of $^{12}\text{C}_6\text{NO}_2\text{HQ}$ can be found in Figure 3A. The similarity observed from overlaying the mass spectrum of the unknown at retention time ~ 30 min with that of authentic $^{12}\text{C}_6\text{NO}_2\text{HQ}$, Figure 3B, provided substantial support for structural assignment of the unknown as $^{12}\text{C}_6\text{NO}_2\text{HQ}$. Thus, having essentially identical GC retention times and strikingly similar mass spectra, convincingly defined the unknown in the reaction between 2R4F CSC with $^{12}\text{C}_6\text{Q}$ as $^{12}\text{C}_6\text{NO}_2\text{HQ}$.

The question then arises as to the reaction mechanism(s) that could yield the $^{12}\text{C}_6\text{NO}_2\text{HQ}$ in the CSC. It seemed safe to conclude that nitrous acid would be the "nitrating" reagent in this case (37). However, when nitrous acid was added to $^{12}\text{C}_6\text{HQ}$ hydroquinone in air-saturated aqueous solution, the $^{12}\text{C}_6\text{HQ}$ was converted quantitatively to $^{12}\text{C}_6\text{Q}$ (38). Thus, under smoking conditions (air and water), nitration of $^{12}\text{C}_6\text{HQ}$ to yield $^{12}\text{C}_6\text{NO}_2\text{HQ}$ does not seem to be a rational reaction mechanism to account for the appearance of the $^{12}\text{C}_6\text{NO}_2\text{HQ}$. Hence, this leaves the possible nitration of quinone or semiquinone. However, nitroquinones, are known to be unstable species (39). These proposed reactions are supported by the observation

Figure 2. Mass spectrum of ^{12}C containing species having a retention time of ~ 30 min from the reaction between 2R4F CSC and $^{12}\text{C}_6\text{Q}$ (A) and mass spectrum of ^{13}C containing species having a retention time of ~ 30 min from the reaction between 2R4F CSC and $^{13}\text{C}_6\text{Q}$ (B)

that nitric oxide oxidizes phenols to form their quinone derivatives (40).

Proposing that a nitroquinone is involved in the mechanism to form $^{12}\text{C}_6\text{NO}_2\text{HQ}$ requires that the nitroquinone be classified as a relatively short-lived reaction intermediate. Such a classification does seem appropriate here based in part on the previous observations regarding the relatively fast rate of conversion of quinone to hydroquinone by cigarette smoke (20). Therefore, a plausible reaction mechanism that would account for the presence of $^{12}\text{C}_6\text{NO}_2\text{HQ}$ appears in Figure 4.

An additional, very important aspect concerning the presence of the $^{12}\text{C}_6\text{NO}_2\text{HQ}$ still remains to be discussed: 1) What was the yield of the $^{12}\text{C}_6\text{NO}_2\text{HQ}$? That is, what degree of conversion of $^{12}\text{C}_6\text{Q}$ to $^{12}\text{C}_6\text{NO}_2\text{HQ}$ was realized upon addition of a known amount of $^{12}\text{C}_6\text{Q}$ to the 2R4F CSC? Compound independent calibration (CIC) with AED has been shown feasible for a number of isotopes (28, 29, 30). CIC allows for the calculation of component concentrations based on the optimized AED response obtained from a known concentration of a known species, regardless of initial molecular structure. For example, in

the reactions under study here, new ^{13}C containing molecules were generated from a $^{13}\text{C}_6\text{Q}$ precursor. Under optimized AED conditions, all of these new ^{13}C containing molecules would have been quantitatively converted to ^{13}CO , thereby eliminating any precursor molecular structural impact on the AED response other than the ^{13}C content within the molecule. Optimizations of cavity and vent flows are often necessary to obtain adequate conditions for CIC. These optimizations can be rather involved requiring optimization of a number of parameters that are not necessarily independent from one another. However, initial investigations into CIC for ^{13}C species under the present operating conditions revealed no such trends, Figure 5. Should AED CIC for ^{13}C species have proven valid, calculation of the yield of $^{13}\text{C}_6\text{NO}_2\text{HQ}$ would have been straight forward (28, 29, 30, 41). Consequently, calculations of the yields of $^{12}\text{C}_6\text{NO}_2\text{HQ}$ were performed by building a $^{12}\text{C}_6\text{NO}_2\text{HQ}$ calibration curve (slope = 1318.9, intercept = -65363, $R^2 = 0.9995$) from mass spectral extracted ion ($m/z = 155$) area values versus $^{12}\text{C}_6\text{NO}_2\text{HQ}$ concentration. Extracted ion ($m/z = 155$) values attributed to $^{12}\text{C}_6\text{NO}_2\text{HQ}$ from the reaction of 2R4F CSC sample with $^{12}\text{C}_6\text{Q}$ were also

Figure 3. Mass spectrum of $^{12}\text{C}_6$ -nitroquinone, mass spectrum of authentic $^{12}\text{C}_6$ -nitrohydroquinone (A) and unknown $^{12}\text{C}_6$ Q reaction product (B) at retention time ~30 min

measured. These measured values were then used to calculate the $^{12}\text{C}_6\text{NO}_2\text{HQ}$ yield of approximately 320 $\mu\text{g/mL}$. Thus, with an initial concentration of $^{12}\text{C}_6\text{Q}$ of 3510 $\mu\text{g/mL}$, a relatively low conversion percentage of 6.35% was calculated for $^{12}\text{C}_6\text{NO}_2\text{HQ}$.

Figure 1 has indicated the significant depletion of AED signal for $^{13}\text{C}_6\text{Q}$ coupled with a significant increase in AED response for $^{13}\text{C}_6\text{HQ}$ for the 2R4F CSC sample, *vide supra*. Using the AED calibration curve values, *vide supra*, a calculation of $^{13}\text{C}_6\text{HQ}$ concentration revealed a $^{13}\text{C}_6\text{HQ}$ yield of approximately 1279 $\mu\text{g/mL}$. With a starting $^{13}\text{C}_6\text{Q}$ concentration of 2715.32 $\mu\text{g/mL}$, the yield of $^{13}\text{C}_6\text{HQ}$ represented an approximate conversion percentage of 47.13%. Thus, the percent conversion to $^{12}\text{C}_6\text{NO}_2\text{HQ}$ of 6.35% coupled with the percent conversion to $^{13}\text{C}_6\text{HQ}$ of

47.13% accounts for ~53% of the starting $^{12}\text{C}_6\text{Q}$ concentration. Obviously, other as yet undetermined reactions were occurring between the 2R4F CSC and $^{12}\text{C}_6\text{Q}$.

CONCLUSION

When coupled with a gas chromatographic separation (GC), an atomic emission detector (AED) provided informative new data on ^{13}C isotope enriched products closely linked on a structural basis to the depletion of $^{13}\text{C}_6$ quinone ($^{13}\text{C}_6\text{Q}$) when added to cigarette smoke condensate (CSC). Matching chromatographic separation using gas chromatography/mass selective detection (GC/MSD)

Figure 4. Proposed mechanism for the production of nitrohydroquinone from the reaction of quinone with nitrous acid in cigarette smoke

Figure 5. ^{13}C AED response as a function of compound structure

allowed for a confident structural assignment of a relatively minor CSC $^{13}\text{C}_6$ quinone reaction product as nitrohydroquinone ($^{13}\text{C}_6\text{NO}_2\text{HQ}$). A chemical mechanism accounting for the formation of $^{13}\text{C}_6\text{NO}_2\text{HQ}$ was postulated to be a reaction product between nitrous acid, HONO, and $^{13}\text{C}_6$ quinone ($^{13}\text{C}_6\text{Q}$) to form $^{13}\text{C}_6\text{NO}_2\text{Q}$, quickly followed by reduction of $^{13}\text{C}_6\text{NO}_2\text{Q}$ to $^{13}\text{C}_6\text{NO}_2\text{HQ}$. The amount of $^{13}\text{C}_6\text{NO}_2\text{HQ}$ accounted for ~6% of the added $^{13}\text{C}_6\text{Q}$. Identical trends in reaction chemistries were found for experiments with $^{12}\text{C}_6\text{Q}$. On the other hand, the major reaction product detected upon addition of $^{13}\text{C}_6\text{Q}$ to the 2R4F CSC sample was $^{13}\text{C}_6\text{HQ}$. $^{13}\text{C}_6\text{HQ}$ accounted for, on average, ~47% of the initial $^{13}\text{C}_6\text{Q}$ concentration. No additional ^{13}C containing species were detected. A ^{13}C AED compound independent calibration (CIC) approach under the operating conditions was not possible. This body of work further expands the knowledge regarding possible reactions of quinone and hydroquinone in CSC.

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