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Characterization of new metabolites from *in vivo* biotransformation of 2-amino-3-methylimidazo[4,5-*f*]quinoline in mouse by mass spectrometry

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Abstract

In studying the metabolic pathways underlying the mechanism of carcinogenesis of the heterocyclic amine of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), we recently found a new metabolite, which gave a $[M + H]^+$ ion of m/z 217 when subjected to ESI in positive-ion mode. Following ip injection of this metabolite of m/z 217 (designated as m/z 217) to beta-naphthoflavone-treated mice, 57% of the total radioactivity was recovered in a 24-hr mouse urine sample. HPLC separation followed by MS analysis indicates that the urine sample contained m/z 217 ($36 \pm 3\%$ of total recovered radioactivity) and two other peaks that gave rise to the $[M + H]^+$ ions of m/z 393 ($31 \pm 4\%$, designated as m/z 393) and m/z 233 ($14 \pm 1\%$, designated as m/z 233). Beta-glucuronidase treatment of m/z 393 resulted in a radioactive peak corresponding to m/z 217. Electrospray ionization in combination with various mass spectrometry techniques, including multiple-stage mass spectrometry, exact mass measurements, and H-D exchange followed by tandem mass spectrometry was used for structural characterization. The urinary metabolites of m/z 217, 393, and 233 were identified as 1,2-dihydro-2-amino-5-hydroxy-3-methylimidazo[4,5-*f*]quinoline, 1,2-dihydro-2-amino-5-O-glucuronide-3-methylimidazo[4,5-*f*]quinoline, and 1,2-dihydro-2-amino-5,7-dihydroxy-3-methylimidazo[4,5-*f*]quinoline, respectively. Our results demonstrated that m/z 217 is biotransformed *in vivo* to m/z 393 by O-glucuronidation and to m/z 233 by oxidation. The observation of these more polar metabolites relative to IQ suggests that they may arise from a previously undescribed detoxification pathway.

Introduction

Dietary factors play a significant role in the development of colon cancer in humans.[1]. Heterocyclic amines (HCAs)¹ are pyrolysis products of well done red meat.[2]. These compounds are mutagenic in short-term assays.[3] and are carcinogenic at multiple sites in rodents.[4]. 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ), a HCA, is carcinogenic to both rodents and nonhuman primates.[5]. HCA intake from a western diet results in exposure of several micrograms of amine per person per day.[6,7]. The presence of HCAs in urine indicates that they are absorbed from diet.[8].

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¹**Abbreviations:** HCAs, heterocyclic amines; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; P450, cytochrome P450; Demethyl-IQ, 2-amino-imidazo[4,5-*f*]quinoline; BNF, beta-naphthoflavone; ESI, electrospray ionization; MS, mass spectrometry

HCAs are thought to elicit their toxic effects following activation by cytochrome P450 (P450). Binding the N-hydroxyl product to DNA is mediated by N-acetyltransferase catalyzed O-acetylation. This N-acetoxy derivative is hydrolyzed to a nitrenium ion that forms DNA adducts initiating mutagenesis or carcinogenesis.[9]. Mutagenic activation of IQ by human P450 1A2 is consistent with formation of an N-hydroxyl product.[10]. In addition, purified rat and human cytochrome P450 1A2 activates HCAs.[11] and furafylline, a selective 1A2 inhibitor, increases excretion of HCAs upon administration to humans.[8]. Demethylation of aminoimidazole HCAs, like IQ, is a major metabolic pathway in mouse.[12], monkey.[13] and human.[14], but has not been reported in rat.[12]. 2-Amino-imidazo[4,5-f]quinoline (Demethyl-IQ) is mutagenic.[15]. Rat inactivation of HCAs is also thought to involve P450, resulting in ring oxidation at C(5), followed by conjugation with sulfate or glucuronic acid.[16-18]. In monkey, oxidation at C(7) is reported.[13]. Direct conjugation of the exocyclic amine to form N²-glucuronide or sulfamate is another important pathway.[16-19]. These results support an important role of P450s in HCA metabolism in vivo.

Pathways involved in HCA metabolism are important risk factors contributing to human cancer and enzymatic demethylation is an important pathway present in human and mouse, but not in rat.[12,14]. The enzymes involved are genetically variant and/or inducible and can modify tumorigenicity. Because mouse is an attractive model for assessing these parameters, we have evaluated mouse IQ metabolism demonstrated that new IQ metabolites (seen as protonated cations at m/z 217, 393 and 233 by electrospray ionization) are present in beta-naphthoflavone (BNF)-treated mice.[20], but are not detected in rat.[12]. Herein, we report the characterization of these new urinary metabolites by use of mass spectrometry. The identification of these compounds affords unveiling a previously unknown pathway involving conversion of IQ to m/z 217, which is further transformed by O-glucuronidation to m/z 393 and oxidation to m/z 233.

Experimental Procedures

Materials

[2-¹⁴C]-IQ (10 mCi/mmol, >98% radiochemical purity) and IQ were purchased from Toronto Research Chemicals (Toronto, ON, Canada). All chemicals were purchased from Sigma-Aldrich Corporation (St. Louis, MO) unless otherwise stated. Ultima-Flo AP was purchased from PerkinElmer LAS, Inc., Shelton, CT. Metabolites seen as protonated cations of m/z 217, 393, and 233 by mass spectrometry (designated as m/z 217, 393, and 233, respectively, hereon) were prepared from mouse urine as previously described.[20].

Animals and dosing

Six-week old C57Bl/6 female mice were purchased from Jackson Laboratory, Bar Harbor, ME. Mice were fed AIN-76A from Bio-Serv, Frenchtown, NJ for 8 weeks before use. Experiments were performed according to the National Institutes of Health standards for care and use of experimental animals and the St. Louis VA Medical Center Animal Care and Use Committee. Animals were group-housed, maintained on a 12-h light/dark cycle, and had access to food and water *ad libitum*. To induce hepatic phase I and II enzymes, animals were treated with 40 mg/kg beta-naphthoflavone (BNF) (0.2 ml per 20 gm injected ip; 13% DMSO : 87% corn oil) for 3 consecutive days. To assess m/z 217 metabolism in vivo, mice were administered 60 ug of purified material (0.5 ml per 20 gm injected ip; 50% DMSO : saline), placed in metabolic cages, and a 24-hour urine was collected.

Processing urine and analysis

The 24-hour urine samples were treated with 4 volumes of methanol: acetone (1:1), mixed, allowed to stand for 60 min at 4°C, and centrifuged to precipitate protein and debris. The

supernatants were transferred to a silicized glass tubes, evaporated, and dissolved in a small volume of methanol. Urine was analyzed by a Beckman HPLC with System Gold software using a 5 μ m, 4.6 $\times$ 150 mm C-18 Ultrasphere column attached to a guard column. The mobile phase contained 20 mM ammonium acetate (pH 5.0) in 2% acetonitrile, 0-5 min; 2-5% acetonitrile, 5-23 min; 5-40% acetonitrile, 23-28 min; 40-2% acetonitrile, 28-33 min at a flow rate of 1 ml/min (solvent system 1). Radioactivity in HPLC eluents was continuously measured using a FLO-ONE (PerkinElmer LAS, Inc., Shelton, CT) radioactive flow detector. Data are expressed as percent of total radioactivity after HPLC.

Mass spectral analysis

Low-energy CAD tandem mass spectrometry experiments were conducted both on a Finnigan (San Jose, CA) LTQ linear ion-trap (IT) mass spectrometer (MS) with Xcalibur operating system and a Finnigan TSQ-7000 triple-stage quadrupole (TSQ) mass spectrometer with ICIS operating system. High-resolution accurate mass measurements were performed on a Finnigan LTQ-FT instrument with internal calibration. Methanol was continuously infused onto the ESI source with a syringe pump at a flow rate of 5 μ L/min and the methanolic sample solution was loop injected. The skimmer was at ground potential, and the electrospray needle was at 4.5 kV. The heated capillary temperature was set at 250°C. To obtain collisionally activated dissociation (CAD) tandem mass spectra with a TSQ instrument, the collision energy was set at 22 eV. The precursor ions were selected in the first quadrupole (Q1), collided with Ar (2.3 mTorr) in the rf-only second quadrupole (Q2) and analyzed in the third quadrupole (Q3). For source CAD product-ion spectra, the skimmer voltage was set at 25 eV to yield product ions that were selected for further MS/MS. For the MSⁿ experiments carried out with LTQ, the automatic gain control of the ion trap was set to 5×10^4 , with a maximum injection time of 100 ms. Helium was used as the buffer and collision gas at a pressure of 1×10^{-3} mbar (0.75 mTorr). The relative collision energy ranging from 10-30% with an activation time of 30 ms and with an activation q value at 0.25 was used to leave a minimal residual abundance of precursor ion (around 20%) for the MSⁿ spectra. The mass resolution of the ion-trap and tandem quadrupole instruments was tuned to 0.6 Da at half peak height, while the resolution of the LTQ-FT was set at 200,000. The tandem quadrupole product ion spectra were acquired at the scan rate of one scan per 3 sec. Mass spectra were accumulated in the profile mode, typically for 3-5 min for MSⁿ spectra (n = 2, 3, 4).

Results and Discussions

in vivo Metabolic conversion of *m/z* 217 to 393 and 233 in mouse

To determine whether *m/z* 217 is further metabolized, purified material (60 μ g) from mouse urine was i.p. injected into BNF-treated mice. Results from HPLC analysis (Figure 1) indicated that 57% of the total radioactivity injected was recovered in the 24-hour urine, which contained peaks of *m/z* 217 ($36 \pm 3\%$ of total radioactivity recovered by HPLC), *m/z* 393 ($31 \pm 4\%$), and *m/z* 233 ($14 \pm 1\%$), along with a new peak eluting at 2.3 min ($12 \pm 2\%$). *E. coli* beta-glucuronidase (Type VII-A) treatment of *m/z* 393 completely converted it to *m/z* 217 (not shown). However, *m/z* 233 was not hydrolyzed by beta-glucuronidase. The results indicate the majority of *m/z* 217 (64%) has been further metabolized to *m/z* 233 and 393, which accounted for about 80% of the radioactivity, while an unidentified peak eluted at 2.3 min is accountable for the remaining 20%.

Structural characterization of 1,2-dihydro-2-amino-5-hydroxy-3-methylimidazo[4,5-f]quinoline (*m/z* 217)

The ESI/MS spectra obtained with either an ion trap or a tandem quadrupole instruments in the positive-ion mode exhibited a predominant $[M + H]^+$ ion at *m/z* 217 (data not shown). High-resolution mass measurement of the ion with an LTQ-FT mass spectrometer gave an accurate

m/z value of 217.1084 (Table 1), corresponding to an elemental composition of $C_{11}H_{13}ON_4$ (Calculated mass: 217.1089), which indicated that the number of unsaturation of the compound is eight. ESI/MS spectrum after H-D exchange (data not shown) showed a mass shift of 5 Da (from m/z 217 to m/z 222), suggesting that the molecule contains four exchangeable hydrogen atoms. These results are in accord with the assigned structure of 1,2-dihydro-2-amino-5-hydroxy-3-methylimidazo-[4,5-f]-quinoline, which contains five exchangeable atoms, two on the amino group, one on imine and one on the hydroxyl residue attached to C(5). The tandem mass spectrometric approaches toward to the structural assignment are described below.

As shown in Figure 2, the product-ion spectra of the $[M + H]^+$ ion at m/z 217 obtained with a tandem quadrupole (Panel a) and a linear ion-trap (Panel b) instruments contained abundant ions at m/z 200, 186, and 161, arising from the charge remote fragmentation (CRF) processes that lose NH_3 , CH_3NH_2 , and $NH=C=N-CH_3$ residues, respectively (Scheme 1). The losses are confirmed by high-resolution mass measurements (Table 1) and are consistent with the observation of the ions at m/z 32 and 57, representing the protonated ions of CH_3NH_2 (i.e., $CH_3NH_3^+$), and $NH=C=N-CH_3$ (i.e., $^+NH_2=C=N-CH_3$), respectively, in Figure 2a. However, these latter two ions are absent in the MS^2 spectrum of m/z 217 obtained with a LIT instrument (Figure 2b) because of the low mass cut-off nature of the instrument. The neutral losses of NH_3 , CH_3NH_2 , and $NH=C=N-CH_3$ residues are further supported by the observation of the analogous ions at m/z 202 ($222 - ND_3$), 189 ($222 - CH_3ND_2$), and 165 ($222 - ND=C=N-CH_3$) in the LIT MS^2 spectrum of the deuterated ion at m/z 222 (Figure 2c), arising from H-D exchange.

Further resonance excitation dissociation on the ion of m/z 200 ($217 \rightarrow 200$, Figure 3a) in an ion trap yielded the ions at m/z 185 ($200 - CH_3\cdot$), 183 ($200 - NH_3$), 173 ($200 - HCN$), 171 ($200 - CH_2=NH$), and 159 ($200 - CH_3CN$), arising from losses of $CH_3\cdot$, NH_3 , HCN , $CH_2=NH$, and CH_3CN residues, respectively. These losses were also confirmed by accurate mass measurement on the individual ions with FT-ICR (Table 1) and were further supported by the LIT MS^3 spectrum of the analogous ion of m/z 202 ($222 \rightarrow 202$, Figure 3b), arising from H-D exchange. The spectrum contains the analogous ions with the expected mass shifts seen at m/z 187 (loss of $CH_3\cdot$), 183 (loss of ND_2H), 174 (loss of DCN), 172 (loss of $CH_2=ND$), and 161 (loss of CH_3CN), consistent with the ion structures proposed in Scheme 1.

The MS^3 on the ion at m/z 186 ($217 \rightarrow 186$, Figure 3c) gave rise to the ions at m/z 159, 158, 145 and 144, arising from losses of HCN , CO , $CH_2=C=NH$ and H_2N-CN , respectively (Scheme 1). The formation of these ions were also supported by the MS^3 spectrum of the analogous ion at m/z 189 arising from H-D exchange ($222 \rightarrow 189$, Figure 3d), which contains ions at m/z 161 ($(189 - DCN)$; $(189 - CO)$), 147 ($189 - CH_2=C=ND$), and 145 ($189 - D_2N-CN$). The ions at m/z 145 and 144 also gave rise to m/z 117 and 116 by further loss of a CO residue (data not shown). The observation of the ions at m/z 158 ($186 - CO$), 117 ($145 - CO$), and 116 ($144 - CO$) resulting from further loss of a CO residue further confirms that the compound contains a hydroxyl group, probably at C(5).¹⁶⁻¹⁸ This structural assignment is also consistent with the observation of a 1,2-dihydro-2-amino-5-O-glucuronide-3-methylimidazo[4,5-f]quinoline metabolite at m/z 393, which gave rise to compound of m/z 217 after beta-glucuronidase treatment.

The MS^3 spectrum of the ion of m/z 161 ($217 \rightarrow 161$, Figure 3e) is dominated by the ion at m/z 144, arising from loss of a NH_3 residue, along with the ions at m/z 143 and 133, arising from losses of a H_2O and CO , respectively, and the ion at m/z 116 arising from further loss of a CO residue from m/z 144. The formation of these ions is again, supported by the MS^3 on the H-D exchanged analogous ion of m/z 165 ($222 \rightarrow 165$, Figure 3f), which yielded ions at m/z 145 ($165 - ND_3$), 146 ($165 - HDO$), 137 ($165 - CO$), and 117 ($165 - ND_3 - CO$). The ion at m/z 116 is the most prominent seen in Figure 2a and in the MS^4 spectrum of m/z 144 ($217 \rightarrow 161 \rightarrow 144$)

(data not shown). This prevalent formation of the ion of m/z 116 (Figure 2a) from m/z 144 via loss of a CO residue suggested that the hydroxyl group is most likely located at C(5), and the ion of m/z 116 may represent a stable highly conjugated 1,5-pyridine cation (Scheme 1). Collectively, the above structural information confirms that the compound is a 1,2-dihydro-2-amino-5-hydroxy-3-methylimidazo-[4,5f]-quinoline.

Characterization of 1,2-dihydro-2-amino-5,7-dihydroxy-3-methylimidazo[4,5-f]quinoline (m/z 233)

Similar to m/z 217, the product-ion spectra of the $[M + H]^+$ ion at m/z 233 obtained with a tandem quadrupole (Figure 4a) and a linear ion-trap (Figure 4b) contained major ions at m/z 216, 202, and 177, arising from losses of NH_3 , CH_3NH_2 , and $NH=C=N-CH_3$, respectively, along with the ions at m/z 32 and 57 corresponding to the protonated ion species of CH_3NH_2 and $NH=C=N-CH_3$, respectively (seen in Figure 4a). The ions at m/z 216, 202, and 177 are 16 Da heavier than the ions of m/z 200, 186, and 161, respectively, seen in Figure 2a and 2b, and the profiles of these two spectra (Figure 4a and 4b) are also similar to those of Figure 2a and Figure 2b, respectively. The results suggest that the two compounds undergo the similar fragmentation processes upon subjection to CAD and the structures of m/z 217 and 233 are similar. The losses of the NH_3 , CH_3NH_2 , and $NH=C=N-CH_3$ residues are further supported by the MS^2 spectrum of the H-D exchanged $[M + D]^+$ ion at m/z 239 (Figure 4c), which contained the ions at m/z 219 ($239 - ND_3$), 206 ($239 - CH_3ND_2$), and 182 ($239 - ND=C=N-CH_3$). These results indicate that the compound of m/z 233 represents a 1,2-dihydro-2-amino-5,7-dihydroxy-3-methylimidazo-[4,5f]-quinoline, which is 16 Da heavier than the compound of 1,2-dihydro-2-amino-5-hydroxy-3-methylimidazo-[4,5f]-quinoline (m/z 217) and contains an additional hydroxyl group at C(7). The presence of the additional hydroxyl group is also supported by the observation of the $[M + D]^+$ ion at m/z 239 after H-D exchange, indicating the presence of one additional exchangeable hydrogen atom (as compared to m/z 217).

The ions at m/z 175, 160, 147 and 132 seen in Figure 4a obtained with a triple stage quadrupole instrument are analogous to the ions of m/z 159, 144, 131 and 116 seen in Figure 2a. These ions of m/z 175, 160, 147 and 132 arise from further dissociation of the ion at m/z 216, as evidenced by the MS^3 spectrum of the ion at m/z 216 ($233 \rightarrow 216$, Figure 5a). The results are consistent with the notion that the ions of m/z 159, 144, 131 and 116 seen in Figure 2a arose from further dissociation of the ion of m/z 200 (Figure 3a). The profiles of the two spectra (i.e., Figure 3a and 5a) are also similar, indicating that the structures of the two compounds are similar and consistent with the suggestion that the compound of m/z 233 contains an additional hydroxyl group. The proposed structure is also supported by the MS^3 spectrum of the ion of m/z 219 ($239 \rightarrow 219$, Figure 5b), an analogous ion (to m/z 216) arising from H-D exchange. The spectrum contained the analogous ions at m/z 178, 162, 150, and 132 that match the mass shift(s) expected for the structure of m/z 233 (Scheme 2).

The charge site of the protonated ion of m/z 233 is probably located at N(6).[21], and the additional hydroxyl group is possibly located at C(7). The electron-donating group of the hydroxyl substituent that is ortho to the N(6) stabilizes the charge site (Scheme 2) and the N(6) charge site is also the remotest from the sites where the CRF processes yielding the ions of m/z 177, 175, and 160 take place, resulting in their favorable formation. In contrast, the analogous ions at m/z 161, 159 and 144 seen for m/z 217 (Figure 2a) may arise from the CRF processes in which the charge site of the precursor ions is situated on the tertiary amine at N(3) or at N(1) (for the m/z 186 precursor ion that yields ions of m/z 159 and 144) (Scheme 1), which are less remote from the sites of fragmentation. As a result, the ions of m/z 161, 159 and 144 are less abundant.[22,23].

The ions at m/z 160 and 74 are among the most prominent seen in Figure 4a, and these two ions were also seen in Figure 4b obtained with an ion-trap instrument. The prominence of the ion at m/z 160 is consistent with the notion that the ion may arise from direct cleavage, probably across the N(1)-C(1b) and N(3)-C(3a) bonds of the C-ring, leading to formation of a stable highly conjugated ion of protonated 5,6-dehydro-2,8-dihydroxy-quinoline via CRF process that eliminates a N-methyl-amino-diaziridine residue (Scheme 2). This fragmentation process is in agreement with the observation of the ion of m/z 74, probably representing a protonated N-methyl-amino-diaziridine cation. The observation of these two ions is also in agreement with the presence of the hydroxyl groups at C(7) for the compound of m/z 233. In contrast, the similar CRF process leading to formation of the analogous ions of m/z 144 and 74 for the compound of m/z 217 is less favorable and the ions of m/z 144 and 74 are of low abundance (Figure 2a, and 2b).

The observation of the ion of m/z 184 in Figure 4a is also consistent with the presence of the hydroxyl group at C(7). This ion is formed from m/z 202 by loss of H_2O , probably involving in the participation of the proton at N(6) and the hydroxyl group at C(7) (Scheme 2). The H_2O loss is supported by the observation of the ion at m/z 184 ($202 - H_2O$) in the MS^3 spectrum of m/z 202 ($233 \rightarrow 202$, Figure 5c), and of the ion at m/z 186 ($206 - D_2O$) in the MS^3 spectrum of the ion at m/z 206 ($239 \rightarrow 206$, Figure 5d), arising from H-D exchange. Further losses of a H_2O residue were also seen for the ions of m/z 216 ($233 \rightarrow 216$, Figure 5a) and 177 ($233 \rightarrow 177$, Figure 5e), which gave rise to ions at m/z 198, and 159, respectively. These losses of water residue are also supported by the observation of the ions of m/z 199 ($219 - D_2O$) in the MS^3 spectrum of m/z 219 ($239 \rightarrow 219$, Figure 5b) and the ion at m/z 162 ($182 - D_2O$) in the MS^3 spectrum of the ion of m/z 182 ($239 \rightarrow 182$, Figure 5f), arising from loss of D_2O . The ions from further loss of a water residue is also seen at m/z 159 (from m/z 177) and 129 (from m/z 147) in Figure 4a (Scheme 2). By contrast, the ions expected at m/z 168, 143, and 113 arising from water loss (analogous to 184, 159, and 129 seen for m/z 233) are absent in the production spectrum of 1,2-dihydro-2-amino-5-hydroxy-3-methylimidazo-[4,5-f]-quinoline (Figure 2a). The above results support that the ion at m/z 233, indeed represents a 1,2-dihydro-2-amino-5,7-dihydroxy-3-methylimidazo[4,5-f]quinoline.

Characterization of 1,2-dihydro-2-amino-5-O-glucuronide-3-methylimidazo[4,5-f]quinoline (m/z 393)

The tandem quadrupole mass spectrum of the $[M + H]^+$ ion of m/z 393 is dominated by the ion at m/z 217, arising from loss of glucuronic acid (loss as [glucuronic acid - H_2O]) (Figure 6), along with ions at m/z 200, 186, 161, arising from further dissociation of the ion at m/z 217. The presence of glucuronic acid in the molecule is consistent with the observation of the ion of m/z 217 arising from m/z 393 by loss of a glucuronic acid upon beta-glucuronidase treatment. Further CAD on the ion of m/z 217 following source CAD of the ion of m/z 393 gives rise to the spectrum that is identical to that shown in Figure 2a (data not shown). The results clearly demonstrated that the compound of m/z 393 represents a 1,2-dihydro-2-amino-5-O-glucuronide-3-methylimidazo[4,5-f]quinoline.

Conclusions

The mass spectrometric approaches described above are readily applicable for structural identification of metabolites of HCA of 2-amino-3-methylimidazo[4,5-f]quinoline, leading to insight into the metabolic pathways underlying the mechanism of carcinogenesis. The identification of the structures of 1,2-dihydro-2-amino-5-hydroxy-3-methylimidazo[4,5-f]quinoline for metabolite of m/z 217 and of 1,2-dihydro-2-amino-5,7-dihydroxy-3-methylimidazo[4,5-f]quinoline for metabolite of m/z 233 is consistent with the previous findings that the oxidation site of IQ in rat and monkey is located at C(5), while N-demethylated

and C-7 hydroxylated compounds are metabolites of aminoimidazole HCAs reported for monkey and human.[22,23]. The identification of these new metabolites that are relatively more polar than IQ also suggests that they may arise from a previously undescribed detoxification pathway.[20].

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Figure 1.

A representative HPLC profile of urinary excretion of ^{14}C - m/z 217 from BNF-treated mice. Mice were administered an ip dose of m/z 217 and a 24-hour urine was collected. The peaks were identified by retention times relative to standards and by their susceptibility to specific treatments (see text for detail).

Figure 2. The MS² product-ion spectrum of the [M + H]⁺ ion of 1,2-dihydro-2-amino-5-hydroxy-3-methylimidazo-[4,5f]-quinoline at m/z 217, a new HCA metabolite of IQ isolated from mouse urine, obtained with a triple-stage quadrupole (a), with a linear ion-trap (b) instrument, and (c) the LIT MS² spectrum of the analogous [M + D]⁺ ion at m/z 222 prepared by H-D exchange.

Figure 3.

The LIT MS³ product-ion spectra of the ions of m/z 200 ($217 \rightarrow 200$) (a), 186 ($217 \rightarrow 186$) (c), 161 ($217 \rightarrow 161$) (e), from the $[M + H]^+$ ion at m/z 217, and of the analogous ions of m/z 202 ($222 \rightarrow 202$) (b), 189 ($222 \rightarrow 189$) (d), 165 ($222 \rightarrow 165$) (f), from the $[M + D]^+$ ion at m/z 222, prepared by H-D exchange. The observed mass shifts of the fragment ions seen in panels b, d, and f confirm the fragmentation processes proposed in Scheme 1.

Figure 4. The MS² product-ion spectra of the [M + H]⁺ ion of 1,2-dihydro-2-amino-5,7-dihydroxy-3-methylimidazo-[4,5f]-quinoline at m/z 233, another IQ metabolite isolated from mouse urine, obtained with a triple-stage quadrupole (a), with a linear ion-trap (b) instrument, and (c) the LIT MS² spectrum of the analogous [M + D]⁺ ion at m/z 239 prepared by H-D exchange.

Figure 5.

The LIT MS³ product-ion spectra of the ions of m/z 216 ($233 \rightarrow 216$) (a), 202 ($233 \rightarrow 202$) (c), 177 ($233 \rightarrow 177$) (e), from the $[M + H]^+$ ion at m/z 233, and of the analogous ions of m/z 219 ($239 \rightarrow 219$) (b), 206 ($239 \rightarrow 206$) (d), 182 ($239 \rightarrow 182$) (f), from the $[M + D]^+$ ion at m/z 239, prepared by H-D exchange. The observed mass shifts of the fragment ions seen in panels b, d, and f confirm the fragmentation processes proposed in Scheme 2.

Figure 6. The tandem quadrupole product-ion spectrum of the $[M + H]^+$ ion of 1,2-dihydro-2-amino-5-O-glucuronide-3-methylimidazo[4,5-*f*]quinoline at m/z 393.

Scheme 1.

The fragmentation processes proposed for 1-hydro-2-amino-3-methyl-5-hydroxyimidazo [4,5-f]quinoline

Scheme 2.

The fragmentation processes proposed for 1-hydro-2-amino-3-methyl-5,7-dihydroxy-imidazo [4,5-f]quinoline

*The observed mass is off by 1Da (from expected), probably due to hydrogen rearrangement

Table 1

High Resolution Mass Measurements of Major Fragment ions from m/z 217

Nominal Mass (m/z)	Measured Mass (Da)	Calculated Mass (Da)	Elementary Composition
217	217.1084	217.1089	C ₁₁ H ₁₃ O ₁ N ₄
200	200.0818	200.0824	C ₁₁ H ₁₀ O ₁ N ₃
186	186.0662	186.0667	C ₁₀ H ₈ O ₁ N ₃
171	171.0553	171.0558	C ₁₀ H ₇ O ₁ N ₂
161	161.0709	161.0715	C ₉ H ₉ O ₁ N ₂
183	183.0553	183.0558	C ₁₁ H ₇ O ₁ N ₂
159	159.0553	159.0558	C ₉ H ₇ O ₁ N ₂