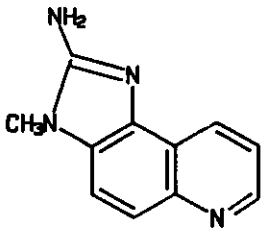
BIOLOGICAL FORMATION AND CHEMICAL SYNTHESIS OF 2-AMINO-3,6-DIHYDRO-3-METHYL-7H-IMIDAZOLO[4,5-f]QUINOLIN-7-ONE, THE MAJOR METABOLITE OF THE DIETARY CARCINOGEN 2-AMINO-3-METHYL-3H-IMIDAZOLO[4,5-f]QUINOLINE (IQ) BY NORMAL INTESTINAL BACTERIA

Mohammad Bashir and David G. I. Kingston*
Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, U.S.A.
Robert J. Carman, Roger L. Van Tassell, and Tracy D. Wilkins
Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, U.S.A.

Abstract—2-Amino-3,6-dihydro-3-methyl-7H-imidazolo[4,5-f]quinolin-7-one (2) has been synthesized from 6-bromo-5-nitroquinoline in seven steps. Biological formation of 2 from IQ (1) involves the addition of water from the medium, followed by oxidation.

The formation of various mutagenic heterocyclic amines on pyrolysis of proteinaceous foods has been reported by many investigators,[1] and several reviews of the subject have appeared.[2] Mutagens of the imidazoquinoline or imidazoquinoxaline class of compounds are the most potent mutagens known in the Ames test, and the parent compound of this group, 2-amino-3-methyl-3H-imidazo[4,5-f]quino-line, or IQ (1) has been shown to be carcinogenic in mice[1a,3] and in rats.[4]



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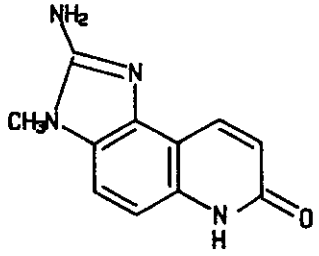

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Because of the significance of IQ as a possible carcinogen in man, a knowledge of its metabolic fate is important to an assessment of its carcinogenic potential. Some studies have been reported on the fate of IQ in rats, and it was shown that the major urinary mutagen is unchanged IQ, with smaller amounts of possible N-acetyl or 3-N-demethylated products.[5] It has also been shown that IQ is metabolized to an active N-hydroxy compound by cytochrome P-448.[6] Recently we reported that IQ is converted to a metabolite identified as 2-amino-3,6-dihydro-3-methyl-7H-imidazolo[4,5-f]-quinolin-7-one (HOIQ) (2) by human fecal flora,[7,8] and we have also shown that this metabolite is a potent direct acting mutagen,[8] in contrast to IQ which requires activation by liver microsomal enzymes.

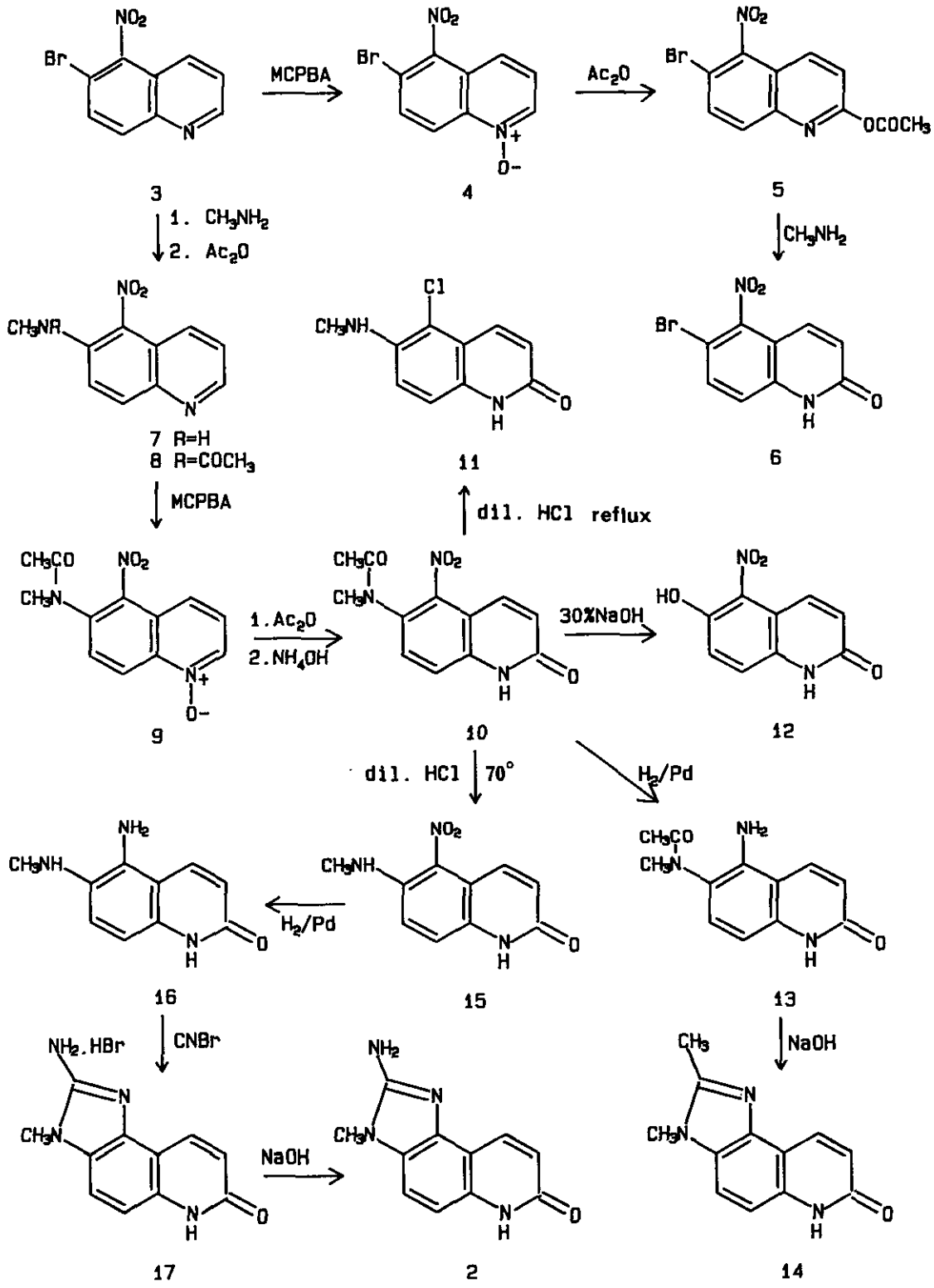
Since HOIQ is potentially a greater cancer risk than IQ itself, due to its direct acting nature, it thus became important to prepare it by synthesis, both as a confirmation of structure and to provide adequate material for biological testing. In addition, the mechanism of the unusual formation of an oxidized compound under anaerobic conditions is of interest. In this manuscript, we report the synthesis of HOIQ and a study of its metabolic formation.

RESULTS AND DISCUSSION

The first synthetic route to HOIQ that we attempted was a simple modification of the published route to IQ.[9] Oxidation of 6-bromo-5-nitroquinoline **3**[9] with m-chloroperbenzoic acid yielded the N-oxide **4**. Rearrangement of **4** in the presence of acetic anhydride gave the acetate **5**, but reactions of this compound with methylamine, under conditions which were successful when applied to **3**,[9] failed to effect a displacement of bromide. We thus sougnt an alternate route to avoid this problem.

Reaction of 6-bromo-5-nitroquinoline **3** with methylamine as previously described[9] gave the aminoquinoline **7**, which was acetylated to the acetamidoquinoline **8**. Treatment of **8** with m-chloroperbenzoic acid gave the N-oxide **9** in 70% yield. Rearrangement of **9** in the presence of acetic anhydride and sodium acetate, according to a literature procedure,[10] gave substantial amounts of desoxy material (**8**) in addition to the desired rearrangement product. The desoxy product was presumably formed by attack of acetate ion on the N-acetoxyquinolinium ion to give methyl acetate and carbon dioxide, and the undesired reaction was avoided by the use of acetic anhydride alone for the rearrangement. The 2(1H)-quinolinone **10** was obtained in 50% yield after a work-up which included a mild basic hydrolysis.

Attempted conversion of **10** to the desired desacetyl product **15** produced some mild surprises. Hydrolysis with 30% NaOH resulted in displacement of the N-methylacetamido group by hydroxide ion to give the 6-hydroxy-5-nitro-2(1H)-quinolinone **12**. Reduction prior to hydolysis did not help, since the aminoquinolinone **13** simply underwent cyclization on treatment with base to give the 2,3-


NO2
Br
N
MCPBA
N+
O-
Ac2O
OCOCH3
3
4
5
1. CH3NH2
2. Ac2O
CH3NH2
CH3NH
Cl
O
H
7 R=H
8 R=COCH3
11
6
MCPBA
dil. HCl reflux
CH3CO
CH3N
1.Ac2O
2.NH4OH
30%NaOH
HO
9
10
12
dil. HCl
70°
H2/Pd
NH2
16
15
13
H2/Pd
NH2.HBr
CNBr
NaOH
CH3
17
2
14

dimethylimidazoquinolin-2-one **14**. Vigorous hydrolysis in the presence of hydrochloric acid also yielded an unexpected product in the shape of 5-chloro-6-(methylamino)-2(1H)-quinolinone **11**. Similar displacements of nitro groups by chloride ion have been reported when 4-nitroquinoline-N-oxides are heated with hydrochloric acid.[11]

Conversion of the nitroquinolinone **10** to the desired diaminoquinolinone **16** was finally achieved by mild acid hydrolysis to the aminonitroquinolinone **15**, followed by catalytic reduction to **16**. Completion of the synthesis was then brought about by the method previously used to make IQ.[9]

The isolated product **2** had identical chemical properties (^{1}H nmr, tlc, ms) to the metabolite obtained from IQ,[7] and it also showed the same mutagenicity in the Ames assay.[8]

The biochemical formation of compound **2** under anaerobic conditions is surprising, since formally it is an oxidation product of IQ (**1**). The hydroxylation of aromatic rings by microorganisms is an important step in the metabolism of various physiologically important compounds, and it occurs by three different pathways. Two of these pathways involve oxidation by dioxygen, with catalysis by monooxygenases or by dioxygenases, and these are the normal pathways for the aerobic metabolism of aromatic compounds.[12] A third pathway involves the addition of water followed by hydride transfer to an electron acceptor, and this is the pathway that has been demonstrated in the oxidation of nicotinic acid under anaerobic conditions.[13]

Incubation of IQ in a culture of the anaerobic bacterium Eubacterium moniliforme VPI 13480, which metabolizes IQ,[8] with added $H_2{}^{18}O$ (20% wt/vol), yielded a sample of HOIQ (**2**) which contained about 12% ^{18}O as determined by mass spectrometry. Since a control experiment indicated that oxygen exchange of the amide with water does not occur under the experimental conditions, this result shows that the formation of HOIQ from IQ must involve an initial hydration of the 1,2-bond of the quinoline ring, followed by oxidation of the resulting dihydroquinoline. The electron acceptor for this oxidation step is at present unknown.

In conclusion, we have synthesized the unusual metabolite HOIQ, thus confirming its structure, and have determined that it arises by a hydration-dehydrogenation sequence on the food mutagen IQ. Studies on the biological activity of HOIQ are in progress, and will be reported elsewhere.[8]

EXPERIMENTAL

Melting points (uncorrected) were determined in open capillaries. Proton spectra were obtained in the indicated solvent on an IBM WP-270 nuclear magnetic resonance (nmr) spectrometer; chemical shifts are reported in ppm downfield from internal tetramethylsilane. Splitting patterns are designated as s(singlet), d(doublet), t(triplet), q(quartet), and m(multiplet). Coupling constants are given in Hertz. Infrared spectra (ir) were recorded on a Perkin-Elmer instrument. Ultraviolet (uv) spectra were obtained from solutions in ethanol on a Hitachi-Perkin-Elmer spectrophotometer.

Mass spectra were obtained on a VG 7070 instrument operating in the electron ionization (ei) or chemical ionization (ci) mode. Flash chromatography was carried out on E. Merck silica gel (230-400 mesh) according to the Still procedure.[14] Thin layer chromatography was carried out on E. Merck precoated silica gel plates, 0.2mm. Microanalyses were performed by Micanal (Tucson, Az.)

6-Bromo-5-nitroquinoline-N-oxide(4).

A solution of 3 (1 g, 3.96 mmol) in $CHCl_3$ (5 ml) was cooled to 0°C and to this solution MCPBA (0.72 g, 4.18 mmol) in 10 ml of $CHCl_3$ was added dropwise at the same temperature. Stirring was continued for 3 h, and the reaction mixture was kept overnight at 0°C. The resulting solution was washed with saturated $NaHCO_3$ and then with water. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. Purification by flash chromatography ($CHCl_3$) afforded 4 (0.33 g, 31%) which crystallized from EtOH, mp 204-206°C: ^1H nmr ($CDCl_3$) 7.29-7.51(2H,m), 7.94(1H,d,J=9.3), 8.59(1H,d,J=5.6), 8.77(1H,d,J=9.3); eims *m/z* (relative intensity) 270,268($M^{+\cdot}$,100,98), 254,252(30,30), 240,238(36,34), 224,222(42,42), 196,194(25,27), 143(80), 127(90), 115(70); uv λ_{max} 345 nm(ε 5913), 236(30107); ir($CHCl_3$), 1550, 1360, 1270, 1260, 1160 cm^{-1}.

2-Acetoxy-6-bromo-5-nitroquinoline (5).

Compound 4 (64 mg, 0.238 mmol) was dissolved in 5 ml of acetic anhydride and refluxed for 3 h in an N_2 atmosphere. Solvent was then removed *in vacuo*. The residue was basified with NH_4OH and extracted with $CHCl_3$. The organic layers were combined, dried (Na_2SO_4) and evaporated. Preparative tlc ($CHCl_3$: EtOAc, 1:1) yielded 5 (22 mg, 30%) as brown needles from EtOH, mp 83-85°C: ^1H nmr ($CDCl_3$) 2.43(3H,s), 7.40(1H,d,J=8.9), 7.92(1H,d,J=8.9), 8.01(1H.d.J=9.5), 8.12(1H.d.J=9.5); eims *m/z* (relative intensity) 312,310($M^{+\cdot}$, 50,44), 270/268 (100,100), 224,222(20,22), 196,194(22,25), 115(50); uv λ_{max} 335nm (ε 2713); 325(3488), 310(2713), 275(3100), 232(37209); ir($CHCl_3$), 1780, 1540, 1370, 1170 cm^{-1}.

6-Bromo-5-nitro-2(1H)-quinoline(6).

A solution of 5 (22 mg, 0.07 mmol) in EtOH (1.5ml) was refluxed with 40% aq methylamine (0.1 ml) for 1 h. Solvent was removed and the residue was purified by preparative tlc ($CHCl_3$: EtOAc, 1:1) to give 6 (18 mg, 94%), mp >290°C(MeOH): ^1H nmr(DMSO) 6.71(1H,d,J=9.9), 7.44(1H,d,J=8.9), 7.66(1H,d,J=9.9), 7.93(1H,d,J=8.9); eims *m/z* (relative intensity) 270,268 ($M^{+\cdot}$,50,50), 224,222(22,20), 212,210(30,36), 196,194(35,40), 167(20), 115(100); uv λ_{max} 345 nm (ε5376), 275(2150), 238(35483); ir (KBr) 3000, 2900, 1620, 1510, 1460, 1420, 1340, 1220 cm^{-1}.

6-Methylamino-5-nitroquinoline (7).

6-Bromo-5-nitroquinoline (10 g, 39.52 mmol) was heated under reflux in 120 ml of ethanol, and to this refluxing solution 40% aq methylamine (20 g, 258 mmol) was added dropwise over 1 h. After refluxing for another 3 h tlc showed the absence of starting material. The solution was then poured onto ice water and extracted with $CHCl_3$. The organic fractions were combined, dried (Na_2SO_4) and evaporated. Crystallization of the residue from acetone gave yellow needles of 7 (6.4 g, 80%), mp 190°C (lit. 194°C); ^1H nmr ($CDCl_3$) 3.20(3H,d,J=5), 7.33(1H,d,J=9.7), 7.50(1H,dd,J_1=8.9,J_2=4.2), 8.09(1H,d,J=9.7), 8.69(1H,dd,J_1=4.2, J_2=1.4), 9.15(1H, broad s), 9.18(1H,d,J=8.9); eims m/z (relative intensity) 203($M^{+\cdot}$,100), 186(12), 173(10), 156(40), 128(60), 115(15), 101(30), 77(20); uv λ_{max} 430 (ε 23571), 315(20000), 245(66428); ir($CHCl_3$) 3600, 3050, 1620, 1540, 1220, 940 cm^{-1}.

6-(N-Acetyl-N-methylamino)-5-nitroquinoline (8).

Compound 7 (5 g, 24.63 mmol) was dissolved in acetic anhydride (50 ml) and pyridine (10 ml) and refluxed for 8 h in an N_2 atmosphere. Solvent was removed in vacuo and the residue was dissolved in $CHCl_3$ and washed with water. The organic layer was dried (Na_2SO_4) and concentrated to a gum. Purification by flash chromatography ($CHCl_3$) afforded 8 (5.25 g, 87%) which crystallized from EtOAc, mp 84-85°C: ^1H nmr ($CDCl_3$) 1.90(3H.s), 3.29(3H.s) 7.27-7.69(2H,m), 8.16(1H,d,J=8.4), 8.38(1H,d,J=9), 9.10(1H,d,J=4.2); cims m/z (relative intensity) 246($M+1^+$,90), 238(10), 216(20 204(45), 198(95), 170(30), 145(10); uv λ_{max} 315nm(ε3750), 307(3000), 218(39500); ir($CHCl_3$) 3050, 1700, 1560, 1400, 1350, 1200 cm^{-1}. Anal. Calcd for $C_{12}H_{11}N_3O_3$: C, 58.77; H_3 4.48; N, 17.14. Found: C, 58.49; H, 4.15; N, 16.88.

6-(N-Acetyl-N-methylamino)-5-nitroquinoline-N-oxide (9).

A solution of m-chloroperbenzoic acid (4.95 g, 28.77 mmol) in 90ml of $CHCl_3$ was added dropwise over 2 h, to a stirred solution of 8 (4 g, 16.32 mmol) in 20 ml of $CHCl_3$ at 0°C in an N_2 atmosphere. The reaction mixture was stirred for an additional 6 h at the same temperature and then kept overnight at 0°C. The solution was then treated with a saturated solution of $NaHCO_3$ and finally with water. The organic layer was dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography ($CHCl_3$, $CHCl_3$:MeOH 95:5) gave 9 (2.98 g, 70%) as a yellow solid, which was recrystallized from EtOH, mp 181-182°C: ^1H nmr ($CDCl_3$) 1.88(3H,s), 3.25(3H,s), 7.50(1H,m), 7.64(2H,m), 8.60(1H,d,J=5.6), 9.0(1H,d,J=9.4); cims m/z (relative intensity) 262($M+1^+$,90), 246(60), 220(53), 215(100), 199(85), 186(13), 170(20); uv λ_{max}, 350 nm(ε7000), 230(27666), 218(28000); ir($CHCl_3$) 3050, 1690, 1550, 1360, 1270cm^{-1}. Anal. calcd. for $C_{12}H_{11}N_3O_4 \cdot \frac{1}{2}H_2O$: C,53.33; H,4.44; N,15.55. Found: C,52.92; H,4.01; N,15.19.

6-(N-Acetyl-N-methylamino)-5-nitroquinolin-2-one (10).

A solution of 9 (2 g, 7.66 mmol) in 50 ml of acetic anhydride was refluxed for 30 h in an N_2 atmosphere. Solvent was then removed in vacuo and the residue was basified with NH_4OH and extracted with $CHCl_3$. The organic phases were combined, dried (Na_2SO_4) and evaporated. Purification by flash chromatography ($CHCl_3$ ---→ $CHCl_3$: MeOH, 90:10) afforded 10 (1g, 50%), mp 259-260°C (EtOH): ^1H nmr ($COCl_3$) 1.86(3H,s), 3.21(3H,s), 6.90(1H,d,J=9.8), 7.46(1H,d,J=8.8), 7.64(1H,d,J=8.8), 7.74(1H,d,J=9.8), 12.05(1H,bd.s); cims, m/z (relative intensity) 262($M+1^+$,100), 233(30), 220(25), 215(70), 199(12), 186(25), 175(15); uv λ_{max} 335 nm(ε6190), 270(3809), 235(40952); ir($CHCl_3$) 3675, 3050, 1690, 1550, 1440, 1240, 1050cm^{-1}. Anal. Calcd. for $C_{12}H_{11}N_3O_4$: C,55.17; H,4.2; N,16.09. Found: C,54.89; H,4.16; N,16.13.

3,6-Dihydro-2,3-dimethyl-7H-imidazo[4,5-f]quinolin-7-one (14).

A mixture of 10 (50 mg, 0.19 mmol) and 10% Pd/C (10 mg) in 5 ml of acetic acid was hydrogenated at room temperature. After 3 h tne catalyst was filtered off and the filtrate was evaporated. The residue was basified with NH_4OH and extracted with $CHCl_3$, and the $CHCl_3$ fractions were dried (Na_2SO_4) and evaporated to afford crude product. Purification by preparative tlc ($CHCl_3$:MeOH, 95:5) afforded the unstable amine 13 (13 mg). Compound 13 (13 mg) was refluxed with 20% NaOH (1ml) for 2 h. After this time water was removed from the reaction mixture and the product was directly purified by preparative tlc to give 14 (4.4 mg, 36.7%): ^1H nmr (CD_3OD) 2.54(3H,s), 3.72(3H,s), 6.60(1H,d,J=9.5), 7.15(1H,d,J=8.8), 7.57(1H,d,J=8.8), 8.40(1H,d,J=9.5); cims, m/z (relative intensity) 214($M+1^+$,100), 199(10), 171(6).

5-Chloro-6-methylaminoquinolin-2-one (11).

A solution of 10 (10 mg, 0.038 mmol) in 5% HCl (1.5ml) was refluxed for 15 h in an N_2 atmosphere. The solution was basified with NH_4OH, extracted with $CHCl_3$ and the organic phases were combined, dried (Na_2SO_4) and concentrated to dryness in vacuo. Purification by preparative tlc ($CHCl_3$: EtOAc, 1:1) afforded 11 (6 mg, 75%): ^1H nmr(CD_3OD) 2.79(3H,s), 6.55(1H,d,J=9.8), 6.97(1H,d,J=9.0), 7.14(1H,d,J=9.0), 8.09(1H,d,J=9.8); eims m/z (relative intensity) 210,208 ($M^{+\cdot}$ 32,100), 195,193(8.25), 173(5), 166(7).

6-Hydroxy-5-nitro-2(1H)-quinolinone (12).

Compound 10 (80 mg, 0.305 mmol) in 2.5ml of 30% NaOH was refluxed for 4 h in an N_2 atmosphere. Water was removed in vacuo and the residue was purified by flash chromatography (EtOAc-→EtOAc:MeOH, 70:30) to yield 12 (60 mg, 95%; ^1H nmr (CD_3OD), 6.36(1H,d,J=9.3), 6.70(1H,d,J=9.0), 7.10(1H,d,J=9.3), 7.76(1H,d,J=9.0).

6-Methylamino-5-nitro-2(1H)-quinolinone (15).

Compound **10** (0.7 g, 2.68 mmol) was dissolved in 5% HCl (30 ml) and heated to 70°C for 24 h in an N_2 atmosphere. After this time a red precipitate was filtered off, washed with water and basified with NH_4OH. The basic solution was extracted with a large excess of $CHCl_3$. The $CHCl_3$ fractions were combined, washed with water, dried (Na_2SO_4) and concentrated, to give **15** (406 mg, 70%) as a red solid. Recrystallization from acetic acid give red crystals, mp 310-313°C: ^1H nmr (DMSO) 2.92(3H,d,J=4.9), 6.60(1H,d,J=10), 7.30(1H,d,J=9.4), 7.5(1H,d,J=9.4), 7.73(1H,br s), 8.25(1H,d,J=10), 11.89(1H,a); eims, m/z (relative intensity) 219($M^{+\cdot}$,100), 202(15), 185(10), 173(30), 128(15), 116(50), 89(15); uv λ_{max} 470nm (ε5555), 340(2777), 248(27777); ir(KBr) 3500, 2900, 1740, 1700, 1670, 1550, 1400, 1365, 1340cm^{-1}.

5-Amino-6-methylamino-2(1H)-quinolinone (16).

A stirred heterogeneous mixture of **15** (0.32 g, 1.46 mmol) and 10% Pd/C (100 mg) in 100 ml of acetic acid was hydrogenated at room temperature for 10 h. The catalyst was removed by filtration, the filtrate was evaporated, and the residue was basified with NH_4OH and extracted with EtOAc. Organic phases were combined, dried (Na_2SO_4), and evaporated in vacuo. Purification by flash chromatography ($CHCl_3$---→$CHCl_3$: MeOH, 90:10) yielded **16** (135mg, 49%). ^1H nmr (DMSO) 2.70(3H,d,J=5), 4.09(1H,q,J=5), 5.23(2H,s), 6.21(1H,d,J=9.7), 6.48(1H,d,J=8.5), 6.90(1H,J=8.5), 8.08(1H,d,J=9.7), 11.12(1H,s); eims, m/z (relative intensity) 189($M^{+\cdot}$, 90), 174(98), 147(35), 129(7), 119(10), 104(12), 92(8).

2-Amino-3,6-dihydro-2-3-methyl-7H-imidazo[4,5-f]quinolin-7-one.(HOIQ, 2).

Cyanogen bromide (126 mg, 1.18 mmol) and **16** (113 mg, 0.5 mmol) were dissolved in 8 ml of MeOH and stirred overnight at room temperature. Next day the hydrobromide **17** was filtered off, basified with 1N NaOH solution, and filtered. The residue was washed with water and finally with EtOH. Crystallization of the residue from EtOH yielded **2** (60 mg, 46.8%) as light green iridescent crystals, mp>360°C: ^1H nmr (DMSO) 3.50(3H,s), 6.50(1H,d,J=9.5), 6.60(2H,s), 6.51(1H,d,J=8.5), 7.30(1H,d,J=8.5), 8.10(1H,d,J=9.5), 11.48(1H,s); eims, m/z (relative intensity) 214($M^{+\cdot}$, 100), 199(30), 186(15), 171(20); uv λ_{max} 360 nm (ε3333), 330(9444), 315(1111), 270(15555), 225(30555); ir (KBr) 3300, 3100, 1630, 1600, 1580, 1550, 1440, 1380, 1340, 1240, 1220 cm^{-1}.
The isolated product was identical (^1H nmr, tlc, co-tlc) with the metabolite isolated from feces.[7]

Metabolism of IQ in $H_2{}^{18}O$.

Brain heart infusion (BHI) broths (Difco) were prepared using [^{18}O] water (20 atom % ^{18}O; MSD isotopes, Montreal, Canada) and supplemented with [^{14}C] IQ (5 μg/ml). Each tube was inoculated

with 100µl of an actively growing BHI culture of Eubacterium moniliforme VPI 13480, a known metabolizer of IQ[16]. The tubes were incubated anaerobically for 18 h at 37°C after which HOIQ and the residue of the labelled substrate were extracted with blue cotton.[7] HOIQ was purified as previously described,[7] and analyzed by mass spectrometry. HOIQ prepared from $H_2{}^{16}O$ had peaks in the molecular ion region at m/z 214 (M^+, 100), 215(13.6), 216(2.0), while HOIQ prepared from $H_2{}^{18}O$ as described had peaks at m/z 214 (M^+, 100), 215(16.8), and 216(16.7). A control experiment in which HOIQ was incubated with $H_2{}^{18}O$ in BHI broth under the same conditions yielded recovered HOIQ which contained no significant amount of ^{18}O.

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