SYNTHESIS AND BIOLOGICAL EVALUATION OF METHYLATED DERIVATIVES OF THE COOKED FOOD MUTAGEN METABOLITE 2-AMINO-3,6-DIHYDRO-3-METHYL-7H-IMIDAZO[4,5-f]-QUINOLIN-7-ONE (7-OH-IQ)

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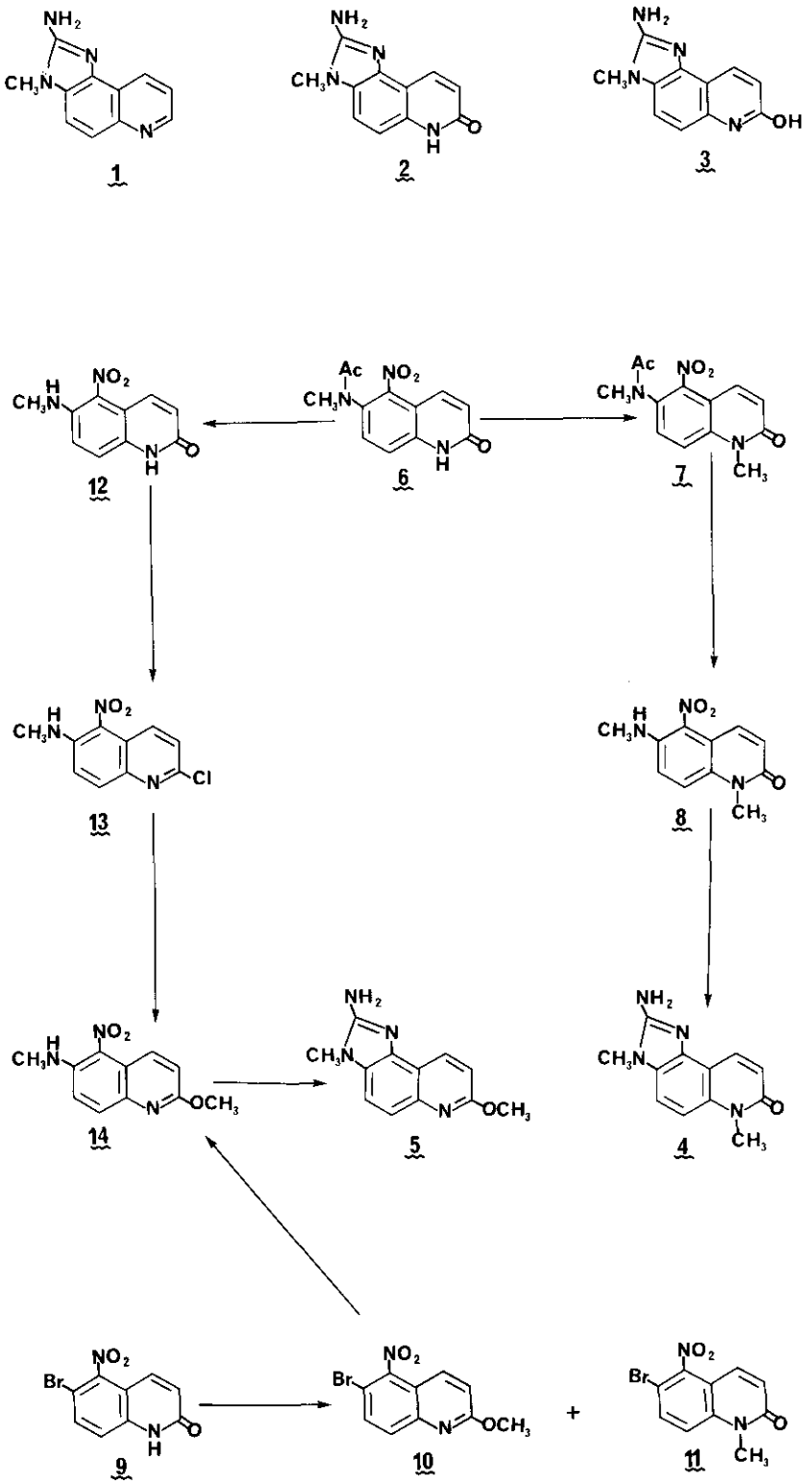
Abstract--The major anaerobic metabolite of the potent cooked food mutacarcinogen IQ is the oxidised product 7-OH-IQ, which is itself a powerful direct-acting mutagen. The O-methyl and N-methyl derivatives of 7-OH-IQ have been prepared to determine whether the tautomeric form of 7-OH-IQ plays any role in its bioactivity. Both N-methyl 7-OH-IQ and O-methyl 7-OH-IQ show comparable mutagenicity when tested directly against the T98 strain of S. typhimurium, indicating that the quinolone structure does not play a major role in the mutagenicity of 7-OH-IQ. Neither 7-OH-IQ nor the methylated derivatives cleaved DNA in the presence of metal cations.

The cooked-food mutagen 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ, **1**) has been shown to be a carcinogen in rats[1] and may thus play a significant role in the etiology of colon cancer. In the presence of human feces and also in the human intestine it is metabolized to the oxidized derivative 2-amino-3,6-dihydro-3-methyl-7H-imidazo[4,5-f]quinolin-7-one (7-OH-IQ, **2**).[2,3] Significantly, although IQ requires metabolic activation for its mutagenicity, 7-OH-IQ is a powerful direct-acting mutagen.[4] The possibility thus exists that the carcinogenicity of IQ is mediated in part through its conversion to 7-OH-IQ in the human colon, followed by cancer induction by 7-OH-IQ. For this reason, it is essential to understand the origin of the direct acting mutagenicity of 7-OH-IQ. Direct acting mutagens are normally presumed to be alkylating agents,[5] following the initial proposal that active carcinogen metabolites act as electrophilic reagents.[6] We thus initially elected to test the hypothesis that 7-OH-IQ (**2**) functions as an alkylating agent. The structure of 7-OH-IQ is predominantly the quinolin-2-one form **2** rather


NH2
CH3N
N
1
2
3
Ac
NO2
H
O
OH
CH3
12
6
7
13
8
Cl
OCH3
14
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than the tautomeric 2-hydroxyquinoline form **3**; the evidence for this is based on the spectroscopic properties of the isolated metabolite[2a] and on the structure of quinolin-2-one itself.[7] It is thus possible that the α,β-unsaturated amide function of 7-OH-IQ acts as a weakly electrophilic group by reacting as a Michael acceptor, and is thus the cause of its direct mutagenicity. The simpler α,β-unsaturated amide acrylamide has been shown to alkylate both 2'-deoxynucleosides and calf thymus DNA at pH 7.0 and 37°C,[8] and it has also been shown to induce a dose-dependent cytotoxic effect in C3H/10T 1/2 and NIH/3T3 mouse fibroblast cells.[9] It is not, however, a direct acting mutagen against five TA strains of Salmonella typhimurium.[10]

If 7-OH-IQ is mutagenic by virtue of its ability to act as a Michael acceptor, then its N-methyl derivative **4** should show comparable mutagenicity to the parent compound while its O-methyl derivative **5**, which lacks the a,β-unsaturated amide function, should not show comparable mutagenicity. We thus prepared compounds **4** and **5** for mutagenicity testing.

RESULTS AND DISCUSSION

The synthesis of N-methyl 7-OH-IQ **4** was achieved in a straightforward manner from the known intermediate 6-(N-acetyl-N-methylamino)-5-nitroquinolin-2-one (**6**).[2b] Alkylation of **6** with methyl iodide in methanolic potassium carbonate yielded the N-methyl derivative **7** exclusively. Mild hydrolysis of **7** followed by reduction and reaction with bromocyanogen yielded **4** in an overall yield of 60% from **6**.

Preparation of O-methyl 7-OH-IQ **5** proved more difficult. Initial experiments aimed at preparing **5** by a direct alkylation procedure showed that the N-methyl derivative always predominated, even when alkylation was carried out in aprotic solvents. The best yield of O-methyl product was obtained by alkylation of 6-bromo-5-nitro-2(1H)-quinolinone (**9**) to give a 1:6 ratio of O-methyl (**10**) to N-methyl (**11**) derivatives. Treatment of the O-methyl derivative **10** with methylamine yielded the same amino nitro quinoline **14** as obtained by the route described below.

The most efficient preparation of O-methyl 7-OH-IQ **5** was found to be by displacement of a 2-chloroquinoline derivative with sodium methoxide. The known 6-methylamino-5-nitro-2(1H)-quinolinone (**12**)[2b] was treated with phosphorus oxychloride to give the 2-chloroquinoline **13**. Reaction of **13** with sodium methoxide gave the 2-methoxyquinoline **14**, identical with the compound prepared by the method described above. Reduction of **14** and treatment with bromocyanogen gave O-methyl 7-OH-IQ (**5**).

The structures of the alkylated 7-OH-IQ derivatives follow from their methods of preparation and their spectroscopic properties. In particular, the N-methyl derivatives consistently showed an infrared absorption for an amide carbonyl group in the 1640-1670 cm^{-1} range, while the O-methyl derivatives showed only absorption for the aromatic ring in the 1600-1630 cm^{-1} range. In their ^1H nmr spectra the signal for the O-methyl protons was consistently downfield of the signal for

the N-methyl protons of the isomeric compound by about 0.3 ppm, and the vicinal coupling constant of the 3,4-protons was slightly higher for the N-methyl derivative.

The two methylated 7-OH-IQ derivatives **4** and **5** were tested for mutagenicity against S. typhimurium strain TA98 by the methods described previously.[4] Testing was carried out without metabolic activation by arochlor-induced S9 fractions so that only direct-acting mutagenicity was determined. The results are shown below in Table 1.

Table 1

Mutagenicity of IQ Analogs against S. typhimurium TA98

Compound	Revertants/plate		Average net revertants
	Control	Compound	
IQ **1**	28, 30, 33	25, 27, 31	0
7-OH-IQ **2**	28, 30, 33	445, 472, 524	455
N-Me 7-OH-IQ **4**	28, 30, 33	362, 382, 424	362
O-Me 7-OH-IQ **5**	28, 30, 33	280, 291, 313	272

The data of Table 1 indicate that there is no major difference in direct-acting mutagenicity between the N-methyl and O-methyl analogs **4** and **5**. Both compounds show significant direct-acting mutagenic effects on S. typhimurium TA98, although both compounds are slightly less active in this respect than 7-OH-IQ itself. It thus appears that the mutagenicity of these compounds is not related to their ability to act as Michael acceptors.

A second possible explanation of the mutagenicity of 7-OH-IQ is that it could chelate metal cations and thus facilitate metal-catalyzed oxygen-mediated DNA strand scission, analogous to that observed with a metal chelator such as 1,10-phenanthroline.[11] However, a test of this hypothesis using ΦX174 DNA and the method described by Hecht[12] failed to reveal any DNA-cleaving activity for 7-OH-IQ. The precise mechanism of action of 7-OH-IQ as a direct-acting mutagen thus remains to be determined.

EXPERIMENTAL

General experimental procedures were as previously described.[2b] Uv spectra were measured in ethanol.

6-(N-Acetyl-N-methylamino)-1-methyl-5-nitroquinolin-2-one **(7)**.

6-(N-Acetyl-N-methylamino)-5-nitroquinolin-2-one **(6)**[2b] (250 mg, 0.957 mmol) in MeOH (20 ml) was treated with K_2CO_3 (200 mg). After stirring for 15 min CH_3I (1.5 ml) was added and the suspension stirred overnight. The solvent was then removed and the residue fractionated between

H_2O and $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated, and the crude product was purified by flash chromatography ($CHCl_3$, then $CHCl_3$:MeOH, 98.2), to yield **7** (250 mg, 95%), mp 223-226°C (EtOH): ^1H Nmr ($CDCl_3$) 1.86 (3H,s), 3.19 (3H,s), 3.76 (3H, s), 6.89 (1H, d, J=9.9 Hz), 7.51-7.58 (3H, m); eims, m/z (relative intensity) 275 (M^+, 4), 232 (55), 229 (100), 187 (45), 172 (15), 159 (16), 130 (20), 84 (35); uv λ_{max} 240 nm (6,100), 238 (44,400); ir ($CHCl_3$) 1680, 1545, 1440, 1220 cm^{-1}. Anal. Calcd for $C_{13}H_{13}N_3O_4$: C, 56.72; H, 4.72, N, 15.27. Found: C, 56.69; H, 4.70; N, 15.19.

1-Methyl-6-Methylamino-5-nitroquinolin-2-one (8).

Compound **7** (200 mg, 0.727 mmol) was dissolved in 5% HCl (20 ml) and heated to 70°C for 20 h in an N_2 atmosphere. The solution was then basified with 10% NH_4OH and extracted with $CHCl_3$, and the $CHCl_3$ fractions were combined, dried over Na_2SO_4, and evaporated. Flash chromatography ($CHCl_3$ then $CHCl_3$:MeOH, 98.2) yielded **8** (165 mg, 97%) which was recrystallized from EtOH, mp 237-240°C: ^1H Nmr ($CDCl_3$) 3.04 (3H, d, J=5 Hz), 3.73 (3H, s), 6.82 (1H, d, J=10 Hz), 7.07 (1H, bs), 7.13 (1H, d, J=9.6 Hz), 7.55 (1H, d, J=9.6 Hz), 8.26 (1H, d, J=10 Hz); eims m/z, (relative intensity) 233 (M^+, 100), 216 (5), 203 (10), 187 (40), 172 (25), 158 (30), 143 (30), 130 (45), 117 (25), 103 (32), 89 (30); uv λ_{max} 460 nm (2,400), 244 (36,000); ir ($CHCl_3$) 3450, 2950, 1660, 1615, 1510, 1470, 1350 cm^{-1}. Anal. Calcd for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.72; N, 18.02. Found: C, 56.65; H, 4.66; N, 17.96.

2-Amino-3,6-dihydro-3,6-dimethyl-7H-imidazo[4,5-f]quinolin-7-one (N-Me-7-OH-IQ, **4**).

Compound **8** (60 mg, 0.257 mmol) was dissolved in EtOH (15 ml) and Raney Ni (1/2 spatula) was added. The reaction mixture was hydrogenated for 20 min at room temperature with stirring. The catalyst was filtered off, and the filtrate was evaporated to 8 ml and treated with BrCN (45 mg, 0.42 mmol), with stirring for 14 h at room temperature. The crude hydrobromide product was filtered off, basified with 10% NH_4OH, and again filtered off. The residue was washed with water and finally with EtOH. Recrystallization from EtOH afforded **4** (39 mg, 67%), mp > 330°C: ^1H Nmr (DMSO) 3.53 (3H, s), 3.60 (3H, s), 6.50 (1H, d, J=9.5 Hz), 6.70 (2H, s), 6.98 (1H, d, J=8.7 Hz), 7.41 (1H, d, J=8.7 Hz), 8.15 (1H, d, J=9.5 Hz); eims m/z (relative intensity) 228 (M^+, 100), 213 (10), 199 (20), 185 (12), 129 (5), 114 (5), 83 (8); uv λ_{max} 365 nm (4,100), 327 (11,000), 320 (12,800), 270 (16,500), 222 (45,800); ir (KBr) 3400, 3150, 1638, 1600, 1540, 1440, 1330, 1235, 1200 cm^{-1}. Calcd for $C_{12}H_{12}N_4O$: M^+ 228.1011. Found: M 228.1010.

6-Bromo-2-methoxy-5-nitroquinoline (**10**) and 6-bromo-1-methyl-5-nitro-2(1H)-quinolinone (**11**).

A large excess of CH_3I was added to 6-bromo-5-nitro-2(1H)-quinolinone (**9**)[2b] (0.9 g, 3.383 mmol) and K_2CO_3 (0.92 g) in 100 ml of DMF, and the mixture was stirred for 18 h at room temperature.

Solvent was then removed in vacuo and the residue was dissolved in $CHCl_3$ and washed with water. The organic layer was dried (Na_2SO_4), evaporated, and purified by flash chromatography (CH_2Cl_2). Early fractions afforded **10** (122 mg, 13%), mp 123-125°C (EtOH): ^{1}H Nmr ($CDCl_3$) 4.07 (3H, s), 7.04 (1H, d, J=9.2 Hz), 7.76-7.86 (3H, m); eims m/z (relative intensity) 284, 282 (M^+, 98, 100), 255, 253 (25, 30), 238, 236 (25, 30), 208, 206 (18, 18), 192 (30), 177 (25), 157 (55), 127 (50), 114 (100); uv, λ_{max} 332 nm (2,100), 328 (2,100), 222 (64,500); ir ($CHCl_3$) 1600, 1530, 1480, 1380, 1300 cm^{-1}. Later fractions of the column yielded **11** (0.77 g, 82%) which was crystallized from EtOH, mp 175-178°C: ^{1}H Nmr ($CDCl_3$) 3.72 (3H, s), 6.84 (1H, d, J=9.7 Hz), 7.36 (1H, d, J=8.9 Hz), 7.45 (1H, d, J=8.9 Hz), 7.77 (1H, d, J=9.7 Hz); eims m/z (relative intensity 284, 282 (M^+, 38, 40), 238, 236 (16, 18), 226, 224 (10, 10), 210, 208 (12, 14), 198 (10), 167 (10), 157 (20), 145 (18), 114 (40), 102 (35), 87 (48), uv λ_{max} 343 nm (5,700), 240, (45,500); ir ($CHCl_3$) 1660, 1580, 1535, 1360, 1230 cm^{-1}.

6-Methylamino-5-nitro-2 chloroquinone (**13**).

6-Methylamino-5-nitro-2(1H)-quinolinone (**12**)[2b] (200 mg, 0.913 mmol) was added to $POCl_3$ (5 ml) and heated to 100°C for 1 h. The reaction mixture was then poured into ice water and extracted with CH_2Cl_2, and the organic fractions were combined, washed with water, dried (Na_2SO_4) and evaporated. Purification by flash chromatography (CH_2Cl_2) afforded **13** (166 mg, 77%) as a yellow solid, mp 140-142°C (CH_2Cl_2): ^{1}H Nmr ($CDCl_3$) 3.19 (3H, d, J=5 Hz), 7.35 (1H, d, J=9.7 Hz), 7.47 (1H, d, J=8.9 Hz), 8.00 (1H, d, J=9.7 Hz), 9.00 (1H, bs) 9.12 (1H, d, J=8.9 Hz); eims m/z (relative intensity) 239,237 (M^+, 45, 100), 222,220 (8, 18), 204 (10), 191 (35), 176 (20), 164 (30), 155 (18), 127 (40), 97 (42), 83 (40), 71 (50); uv λ_{max} 432 nm (2,900), 320 (2,500), 260 (12,200); ir ($CHCl_3$) 3420, 2960, 1620, 1570, 1500, 1400, 1340, 1200, 1170 cm^{-1}. Anal. Calcd for $C_{10}H_8N_3O_2Cl$: C, 50.42; H, 3.36; N, 17.64. Found: C, 50.50; H, 3.16; N, 17.45.

2-Methoxy-6-methylamino-5-nitroquinoline (**14**).

Method a. Compound **13** (130 mg, 0.548 mmol) was added to a solution of sodium methoxide prepared by dissolving 2.8 g of Na in 80 ml of anhydrous methyl alcohol, and the reaction mixture was refluxed for 2 h, after which time tlc showed the absence of starting material. The solvent was removed, the residue fractionated between water and $CHCl_3$, and the chloroform fractions combined, dried (Na_2SO_4) and evaporated. Crystallization of the residue from EtOH gave **14** as needles (78 mg, 61%) mp 163-165°C: ^{1}H Nmr ($CDCl_3$) 3.14 (3H, d, J=5 Hz), 4.00 (3H, s), 6.99 (1H, d, J=9.5 Hz), 7.23 (1H, d, J=9.3 Hz), 7.90 (1H, d, J=9.3 Hz), 8.72 (1H, bs), 8.99 (1H, d, J=9.5 Hz); eims m/z (relative intensity) 233 (M^+, 100), 216 (15), 203 (10), 187 (25), 172 (15), 158 (22), 144 (30), 129 (25), 116 (12), 102 (15), 89 (10); uv λ_{max} 445 nm (10,300), 325 (8,400), 255 (38,300), 235 (43,000); ir($CHCl_3$) 3000, 1635, 1515, 1485, 1400, 1380, 1280 cm^{-1}. Anal. Calcd for

$C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.72; N, 18.02. *Found:* C, 56.54; H, 4.59; N, 17.86.

Method b. 6-Bromo-2-methoxy-5-nitroquinoline (**10**) (44 mg, 0.154 mmol) was treated with 33% methylamine in EtOH (5 ml) and the solution was refluxed for 24 h. Solvent was then removed in vacuo and the residue was purified by flash chromatography ($CHCl_3$) to afford **14** (30 mg, 83%), identical with that prepared by method a.

2-Amino-7-methoxy-3-methyl-3H-imidazo[4,5-f]quinoline (O-Me-7-OH-IQ, **5**).

A stirred heterogeneous mixture of **14** (33 mg, 0.141 mmol) and Raney Ni (1/4 spatula) in 10 ml of EtOH was hydrogenated for 15 min at room temperature. The catalyst was removed by filtration and the filtrate was concentrated to 5 ml and treated with BrCN (30 mg, 0.28 mmol) for 14 h. The hydrobromide was then filtered off, basified with 10% NH_4OH and again filtered off. The residue was washed with water and with MeOH and crystallized from MeOH to yield **5** (17 mg, 52%), mp 293-296°C: ^1H Nmr (DMSO) 3.58 (3H, s), 3.93 (3H, s), 6.51 (2H, s), 6.90 (1h, d, J=8.8 Hz), 7.31 (1H, d, J=8.7 Hz), 7.55 (1H, d, J=8.7 Hz), 8.41 (1H, d, J=8.8 Hz); eims *m/z* (relative intensity) 228 (M^+, 100), 199 (28), 185 (20), 167 (10), 157 (10), 129 (12), 98 (50), 84 (72); uv λ_{max} 345 nm (4,600), 310 (5,700), 300 (6,900), 260 (48,300), 219 (44,800); ir ($CHCl_3$) 2950, 1615, 1580, 1580, 1460, 1430, 1410, 1350 cm^{-1}. Calcd for $C_{12}H_{12}N_4O$: M^+ 228.1011. Found: M 228.1011.

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