

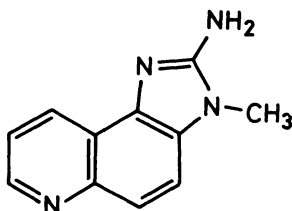
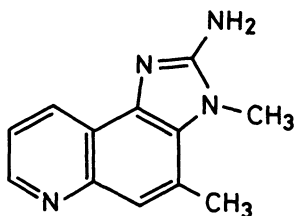
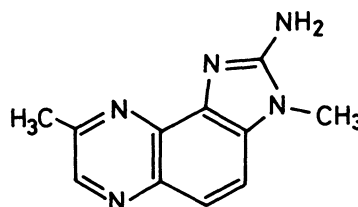
STRUCTURE OF A POTENT MUTAGEN ISOLATED FROM FRIED BEEF

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A new potent mutagen was isolated from fried beef. The molecular formula was determined as $C_{11}H_{11}N_5$ by exact mass measurement. The chemical structure of this mutagen was determined to be 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (Me-IQx) by the analyses of its UV, mass and 1H -NMR spectra, and chemical synthesis.

We previously isolated two potent mutagens from broiled fish and showed that they were 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ, structure 1) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (Me-IQ, structure 2)¹⁾⁻⁴⁾. The mutagen IQ has also been isolated from heated beef extract⁵⁾ and fried beef⁶⁾, suggesting that it is present in a variety of cooked foods. In addition to these two mutagens isolated from cooked foods, we have found another potent mutagen in fried beef. In this communication we report studies showing that this new mutagen is 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (Me-IQx, structure 3).

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The mutagen Me-IQx was purified from fried beef by acid-base partition, LH-20 chromatography and high pressure liquid chromatography (HPLC). Up to the stage of LH-20 chromatography, Me-IQx was copurified with IQ, and then Me-IQx was purified by HPLC on a μ Bondapak C₁₈ column.

The UV spectrum of Me-IQx (λ_{\max} in methanol: 214, 274, 340 nm) is similar to that of IQ, suggesting that the structures of Me-IQx and IQ are similar. The mass spectrum of Me-IQx showed a molecular ion at m/e 213 (M^+) and fragment ions at m/e 212 (M^+-H), m/e 197 (M^+-H-CH_3), m/e 185 ($M^+-H-HCN$), and m/e 144 ($M^+-H-HCN-CH_3CN$). Elemental analysis by exact mass measurement (calculated mass, 213.1014; observed mass, 213.1029) showed that Me-IQx was C₁₁H₁₁N₅. From these results, it seemed likely that Me-IQx was an aza-derivative of IQ with two methyl groups.

The 270 MHz ¹H-NMR spectrum of Me-IQx in CDCl₃ obtained using about 50 μ g of the material revealed the presence of 11 protons (Fig. 1). The chemical shifts and the coupling constants of N-CH₃, H-4 and H-5 protons of Me-IQx were nearly equal to those of IQ, indicating that like IQ, Me-IQx has an N-methylimidizobenzene ring. Furthermore NOE was observed only for H-4 (ca. 10 %) when the N-methyl protons were irradiated, and the presence of an amino group was confirmed by the saturation transfer technique. Among the possible structures of Me-IQx with an imidazoquinazoline or imidazoquinoxaline nucleus, 2-amino-3,8-dimethyl-

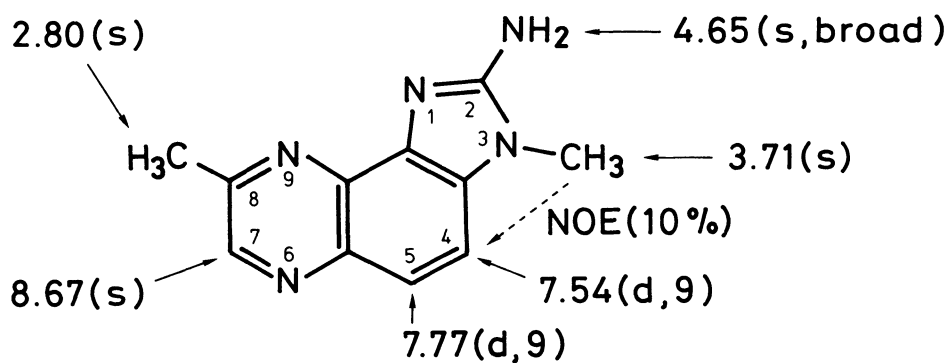


Fig. 1. 270-MHz ¹H-NMR data for Me-IQx in CDCl₃. Chemical shifts (ppm) and coupling constants (Hz) are shown by arrows.

imidazo[4,5-f]quinoxaline (3) and its 3,7-dimethyl derivative (4) were the most probable, judging by comparison of chemical shift of the aromatic proton (8.67 ppm) of Me-IQx with those of model compounds such as 2-methylquinoxaline and quinazoline. Therefore compounds 3 and 4 were chemically synthesized from 6-amino-3-methyl-5-nitroquinoxaline and 6-amino-2-methyl-5-nitroquinoxaline, respectively, by the similar procedure as described previously^{2,3}). Detailed data on the synthesis of 3 and 4 will be published elsewhere. The UV, mass and NMR spectra and R_f values on thin-layer chromatography of Me-IQx from fried beef were identical with those of compound 3 but different from those of compound 4. Thus the structure of Me-IQx was unambiguously determined to be 3.

It is interesting that all three mutagenic compounds, IQ, Me-IQ and Me-IQx, isolated from broiled fish and fried beef have a 2-amino-3-methylimidazobenzene ring. Me-IQx showed strong mutagenic activity towards *S. typhimurium* TA98, when tested by Ames' method with the modification of preincubation⁷). Me-IQx required metabolic activation by microsomal enzymes (S-9) for mutagenicity. The specific mutagenicity of Me-IQx was 100,000 revertants/ μ g, which is a little weaker than those of IQ and Me-IQ^{2,3}). We are now synthesizing Me-IQx for use in carcinogenic tests and for tests on its other biological activities.

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