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SYNTHESIS OF 2-AMINO-3,8-DIMETHYLIMIDAZO[4,5-*f*]QUINOXALINE (Me-IQx), A POTENT MUTAGEN ISOLATED FROM FRIED BEEF

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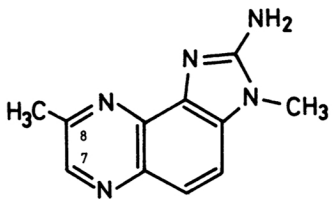
A potent mutagen, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (Me-IQx), isolated from fried beef and its 3,7-dimethyl derivative were synthesized from 6-amino-3-methylquinoxaline and 6-amino-2-methylquinoxaline, respectively. These compounds showed strong mutagenic activity towards *Salmonella typhimurium* TA98 in the presence of S9 Mix.

We previously reported isolation of a new mutagenic compound from fried beef and its structural determination as 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (Me-IQx, structure 1)[1]. It is an aza-derivative of the mutagenic compounds 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (Me-IQ), which were previously isolated from broiled sardines[2-5]. For the final assignment of the structure of Me-IQx and for further studies on its biological properties, it was necessary to synthesize chemically Me-IQx and its isomer having a methyl group in the 7-position in place of the 8-position. In this communication, we report a chemical synthesis of the mutagen Me-IQx and its isomer.



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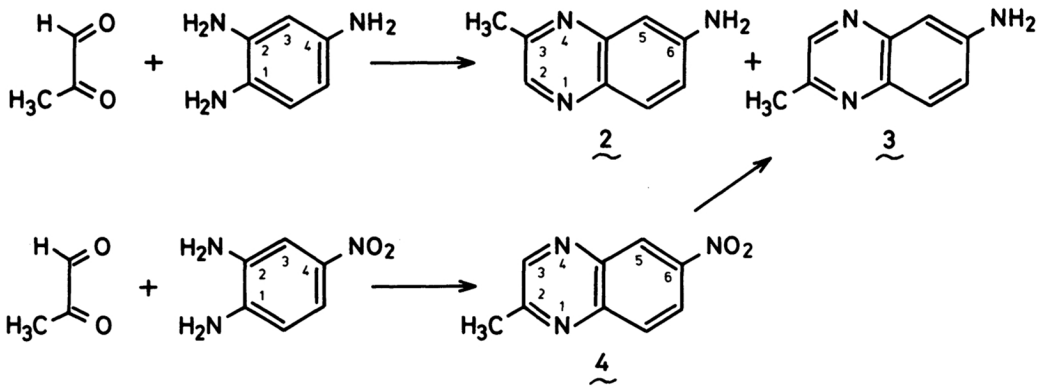
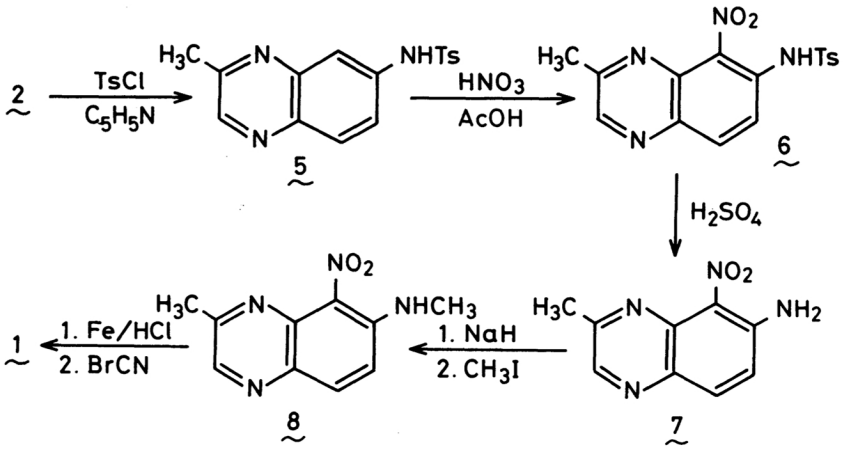
Me-IQx (1) was synthesized from 6-amino-2-methylquinoxaline (2) as shown in scheme 2 by a similar procedure to that used for the synthesis of IQ[3] and Me-IQ[5]. The starting material for this synthesis, compound 2, was prepared by condensation of 1,2,4-triaminobenzene with methylglyoxal by the method of Klicnar and Kosek[6]. Contrary to their finding[6] that only 6-amino-3-methylquinoxaline (2) was formed as a reaction product, in our experiment compound 2 and its isomer 3 were produced in about equal proportions. These products were separated by high pressure liquid chromatography on a TSK-GEL LS-410 column. Structural assignments of these compounds were



Scheme 1



Scheme 2

made by comparison of their properties with those of an authentic sample of compound 3, prepared separately by reduction of 2-methyl-6-nitroquinoxaline (4) which was obtained by condensation of 1,2-diamino-4-nitrobenzene with methylglyoxal (Scheme 1). In the latter condensation reaction, the more basic 2-amino-group reacts with the aldehyde group in methylglyoxal by nucleophilic addition to give a single product 4 and the structure of its reduction product 3 was established previously by unequivocal synthesis from 2,4-dinitrophenylalanine[6].

Compound 2, thus obtained, was first converted to the tosyl derivative 5 and nitrated with HNO_3-AcOH mixture to afford compound 6, which was hydrolyzed with H_2SO_4 to yield 6-amino-3-methyl-5-nitroquinoxaline (7). Compound 7 was then treated with NaH and methylated with CH_3I to afford compound 8. Then compound 8 was reduced to 5-amino derivative with Fe-HCl mixture and the neutralized reaction mixture was treated with BrCN to afford compound 1. UV (λ_{max}^{MeOH}, ε):214 (24,300), 274 (41,100), 240 (3,900); NMR (δ_{CDCl_3}, J): 8.65 ppm (1H, s, 7-H), 7.75 (1H, d, 9Hz, 5-H), 7.51 (1H, d, 9Hz, 4-H), 4.88 (2H, s, broad, $-NH_2$), 3.69 (3H, s, $N-CH_3$), 2.80 (3H, s, $C-CH_3$).

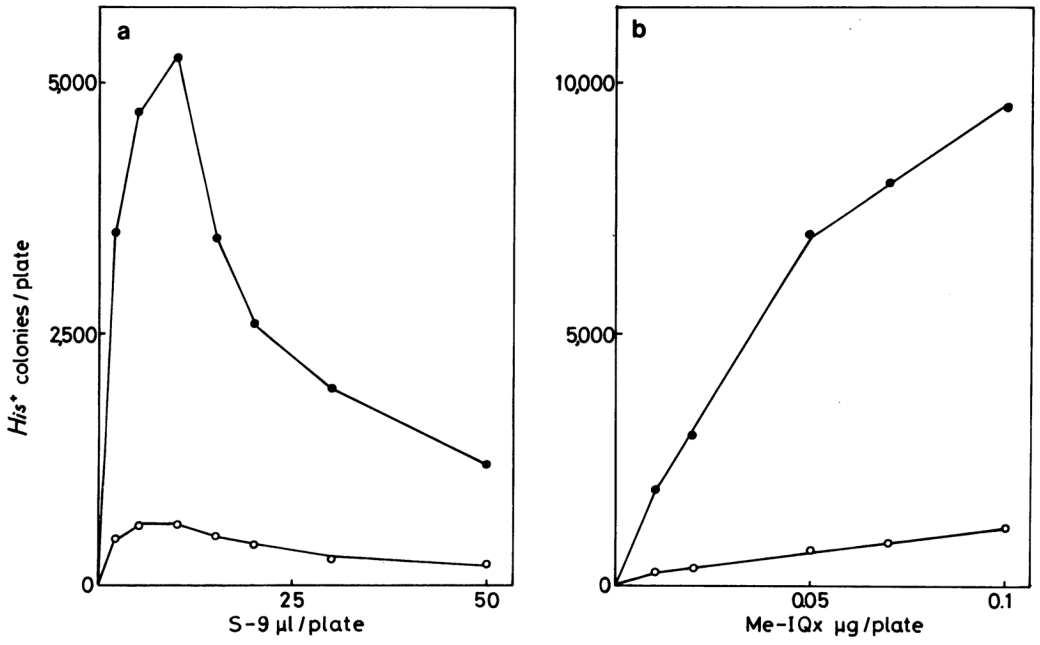


Fig. 1. Mutagenicity of Me-IQx to *S. typhimurium*.
a: The mutagenicity of 0.05 µg of Me-IQx was tested with various amounts of S9.
●—● TA98; o—o TA100.
b: Dose-response curve for mutagenicity obtained with S9 Mix containing 10 µl S9.
●—● TA98; o—o TA100.

In the same fashion, 2-amino-3,7-dimethylimidazo[4,5-*f*]quinoxaline (9), an isomer of Me-IQx, was synthesized from 6-amino-2-methylquinoxaline (3). UV (λ_{max}^{MeOH}, ε): 214 (29,000), 274 (42,000), 237 (4,000); NMR (δ_{CDCl_3}, J): 8.74 ppm (1H, s, 8-H), 7.73 (1H, d, 9Hz, 5-H), 7.60 (1H, d, 9Hz, 4-H), 5.10 (2H, s, broad, $-NH_2$), 3.72 (3H, s, $N-CH_3$), 2.77 (3H, s, $C-CH_3$).

Synthetic compound 1 showed strong mutagenic activity towards *Salmonella typhimurium* with metabolic activation by microsomal enzymes (S9), as shown in Fig. 1a. The specific activities of Me-IQx towards TA98 and TA100 were 145,000 and 14,000 revertants/μg respectively under optimal conditions (Fig. 1b). The isomer of Me-IQx, compound 9, also showed strong mutagenic activity towards TA98 and TA100 (146,000 and 26,000 revertants/μg, respectively). The mutagenic activities of these imidazoquinoxaline derivatives are weaker than those of IQ[3] and Me-IQ[5]. Large scale synthesis of Me-IQx for carcinogenic tests and for examination of its other biological activities is now in progress.

Acknowledgements

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