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STRUCTURE AND CHEMICAL SYNTHESIS OF ME-IQ, A POTENT MUTAGEN ISOLATED FROM BROILED FISH

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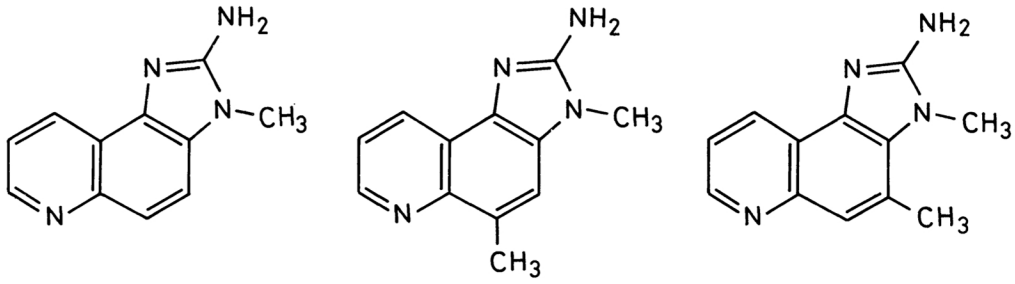
Structure of a mutagenic compound (Me-IQ) isolated from broiled fish was determined to be 2-amino-3,4-dimethylimidazo[4,5-f]quinoline based on the mass-, UV- and ^{1}H-NMR-spectra and chemical synthesis. Me-IQ showed strong mutagenic activity towards *Salmonella typhimurium* TA98 in the presence of S-9 mix.

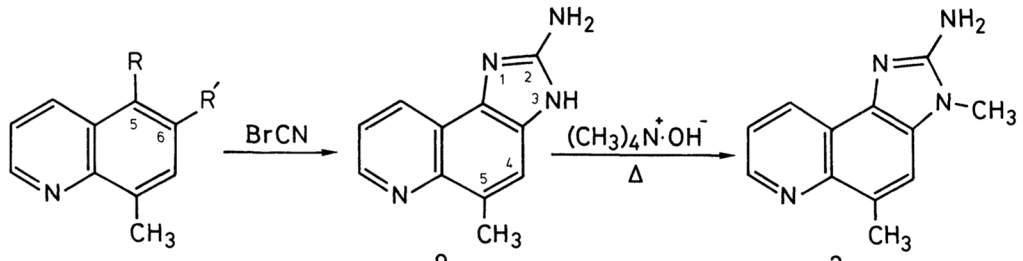
We have isolated two potent mutagens, IQ and Me-IQ, from a methanol extract of sardines broiled under normal domestic cooking conditions, and proposed that the structures of these compounds are 1 and 2, respectively[1]. We have also confirmed the proposed structure of IQ (1) by chemical synthesis[2]. IQ showed strong mutagenic activity towards *Salmonella typhimurium* TA98 (433,000 revertants/μg) with activation by microsomal enzymes, S-9. The mutagen IQ was also isolated from heated beef extract[3], and hamburger[4], suggesting that these mutagens are commonly present in ordinary cooked foods.



1 2 3

In the previous report[1], the structure of Me-IQ was proposed as 2, mainly based on its 270 MHz ^{1}H-NMR- and mass-spectra, determined with small amounts of material (ca. 200 μg). In this communication we report that the structure of Me-IQ is 3, deduced by direct comparison of its spectral data with those of synthetic 2 and 3.

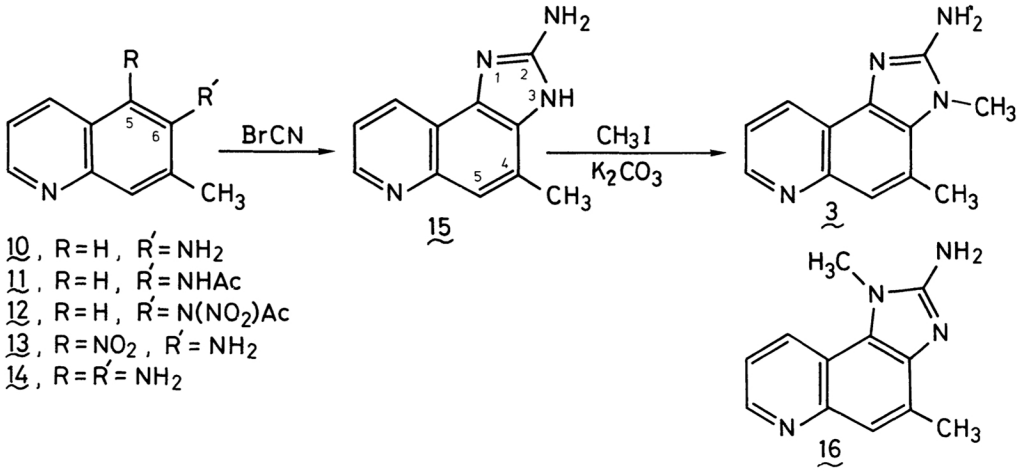
Compound 2 was synthesized via 5,6-diamino-8-methylquinoline (8) as shown in the following scheme. This method is practically the same as that reported for the synthesis of IQ[2]. 6-Amino-8-methylquinoline (4)[5] was converted to the tosyl derivative (5) and nitrated with 61 % HNO_3 to



9 2

4, R=H, R′=NH_2
5, R=H, R′=NHTs
6, R=NO_2, R′=NHTs
7, R=NO_2, R′=NH_2
8, R=R′=NH_2

afford compound 6, which was hydrolyzed with H_2SO_4 to 6-amino-5-nitro-8-methylquinoline (7). Compound 7 was reduced to the diamine, compound 8, with Fe-HCl mixture. Compound 8 was then treated with cyanogen bromide to afford the cyclized derivative (9). The tetramethylammonium salt of compound 9 was heated under reduced pressure to give the N-3-methyl derivative (2) as a major product [MS: M^+, m/e 212, M^+-CH_3, m/e 197; UV (λ_{max}^{MeOH}, ε): 213 (24,900), 265 (45,600), 354 (3,600) nm; NMR (δ_{CDCl_3}, J): 8.91 ppm (H-7, d, 4.0 Hz, 1H), 7.48 (H-8, dd, 4.0, 8.2, 1H), 8.67 (H-9, d, 8.2, 1H), 7.44 (H-4, s, 1H), 6.07 ($-NH_2$, s, broad, 2H), 3.67 ($N-CH_3$, s, 3H), 2.87 ($C-CH_3$, s, 3H)].



15 3 16

10, R=H, R′=NH_2
11, R=H, R′=NHAc
12, R=H, R′=N(NO_2)Ac
13, R=NO_2, R′=NH_2
14, R=R′=NH_2

For synthesis of compound 3, an intermediate compound, 6-amino-5-nitro-7-methylquinoline (13) was prepared as follows. 6-Amino-7-methylquinoline (10)[6] was converted to the 6-acetamido derivative (11), since its conversion to the tosyl derivative was unsuccessful. Nitration of compound 11 with KNO_3-H_2SO_4 mixture gave a N-nitro derivative 12. Acid hydrolysis of compound 12 gave the 5-nitro derivative 13. In this reaction, the nitro group on nitrogen migrated to the neighboring C-5 position of the quinoline nucleus. Compound 13 was reduced to 5,6-diamine (14)

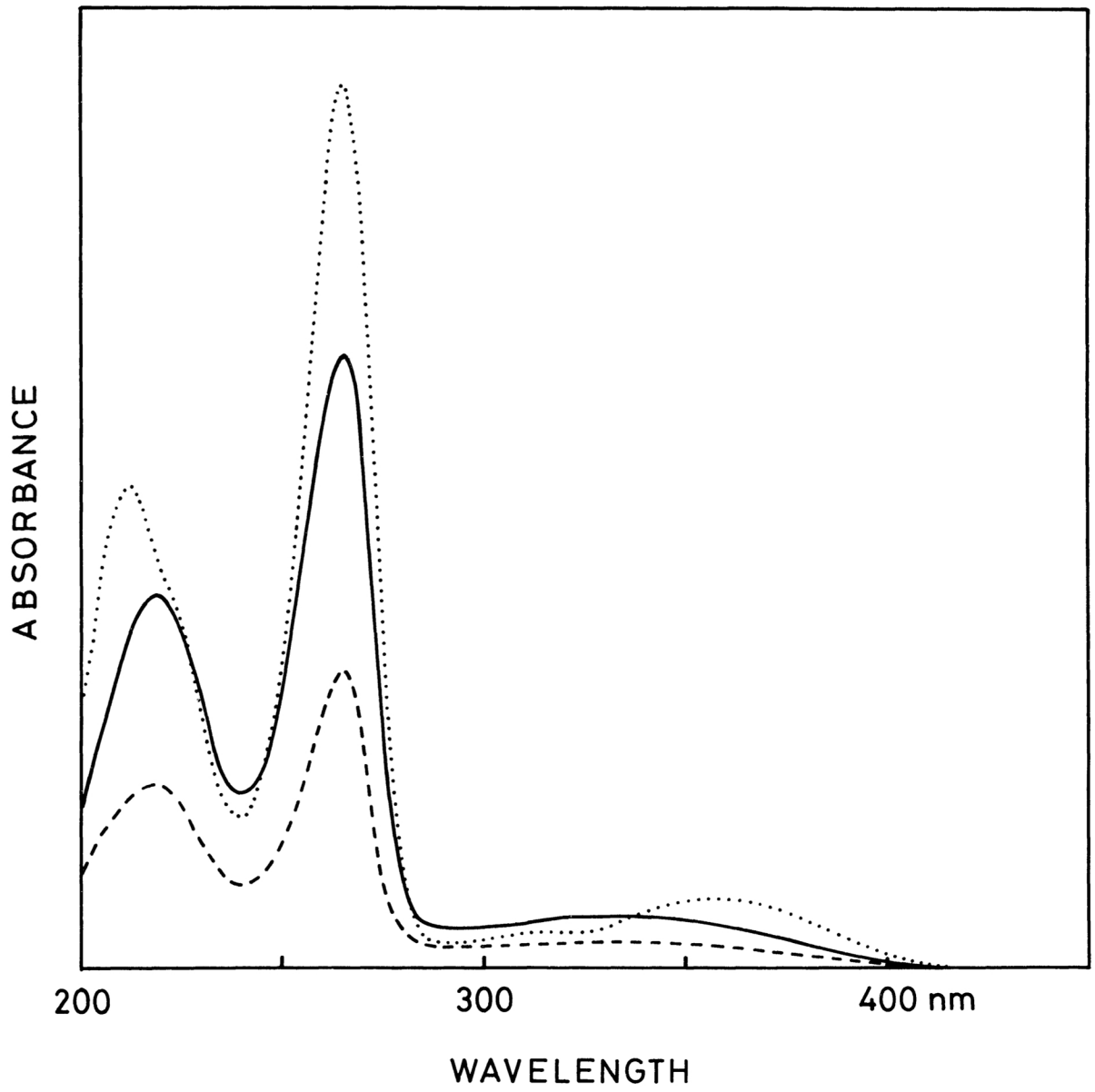


Fig. 1. UV spectra of synthetic 2 (·····), synthetic 3 (———) and Me-IQ from broiled fish (- - - - -).

with Fe-HCl mixture. Compound 14 was treated with cyanogen bromide to afford the cyclized derivative 15, which was methylated with CH_3I in K_2CO_3-DMSO mixture to give a N-3-methyl derivative (3) and N-1-methyl derivative (16). Compound 3 was separated from compound 16 by high pressure liquid chromatography. Structural assignments for these products were based on observation of NOE between the N-methyl group and C-methyl group (3 %) in compound 3 and NOE between the N-methyl group and H-9 (15 %) in compound 16. Compound 3, MS: M^+, m/e 212, M^+-CH_3, m/e 197, UV (λ_{max}^{MeOH}, ε): 219 (23,200), 265 (38,200), 332 (3,200); NMR (δ_{CDCl_3}, J): 8.82 ppm (H-7, d, 4 Hz, 1H), 7.41 (H-8, dd, 4, 8, 1H), 8.65 (H-9, d, 8, 1H), 7.60 (H-5, s, 1H), 6.05 ($-NH_2$, s, broad, 2H), 3.89 ($N-CH_3$, s, 3H), 2.84 ($C-CH_3$, s, 3H).

The mass-, UV- (Fig. 1) and ^{1}H-NMR-spectra of Me-IQ isolated from broiled fish were completely identical with those of synthetic compound 3 but not with those of compound 2. Thus the structure of Me-IQ was established as 3. In the previous report[1] structure 2 was proposed for Me-IQ, because 1) no long-range coupling between the 7.53 ppm signal (now assigned to H-5) and 8.63 ppm signal (H-9) was observed; and 2) structure 2 was sterically more probable than structure 3. Synthetic compound 3 showed potent mutagenic activity towards TA98 (663,000 revertants/μg) in the presence of S-9 mix. It should be mentioned that compound 2 also showed strong mutagenic activity on TA98 (142,000 revertants/μg). It is possible that compound 2 is present as well as compounds 1 and 3 in cooked foods. Studies are in progress on the detection and quantitative measurement of these compounds in cooked foods using the GC/MS technique.

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