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Isolation and Identification of the Food Mutagens IQ and MeIQx from a Heated Model System of Creatinine, Glycine and Fructose

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ABSTRACT

A mixture of creatinine, D-*fructose and glycine was heated in diethylene glycol containing 14% water for 2 h at* ca. *128°C. The reaction mixture was extracted with 1-butanol and the extract subjected to cation exchange column chromatography, C_{18} reversed-phase Sep-Pak treatment and reversed-phase HPLC. The first of the two HPLC fractions isolated was identified as the known beef mutagen, MeIQx. In the second HPLC fraction, MeIQx and the also known potent fish and beef mutagen, IQ, were co-eluted. HPLC, mass and ^1H NMR spectrometry were used for their identification. These results reinforce the hypothesis that creatinine, sugars and amino acids might be the precursors of these extremely potent mutagens.*

Abbreviations: IQ: 3-Methyl-3*H*-imidazo[4,5-*f*]quinolin-2-amine. MeIQ: 3,4-Dimethyl-3*H*-imidazo[4,5-*f*]quinolin-2-amine. MeIQx: 3,8-Dimethyl-3*H*-imidazo[4,5-*f*]quinoxalin-2-amine. DiMeIQx: Trimethyl-3*H*-imidazo[4,5-*f*]quinoxalin-2-amine.7,8-DiMeIQx: 3,7,8-Trimethyl-3*H*-imidazo[4,5-*f*]quinoxalin-2-amine. HPLC: high performance liquid chromatography.

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INTRODUCTION

Extremely mutagenic imidazo[4,5-*f*]quinolin-2-amines (IQ and MeIQ) and -quinoxalin-2-amines (MeIQx and homologues) have been found in broiled or fried fish and meat, beef extracts and model reaction systems. For a more extensive introduction see Grivas *et al.* (1985), and for recently published reviews and discussions on these food mutagens and their metabolism, see Hatch *et al.* (1984), Felton *et al.* (1984), Miller (1985) and Sugimura (1985). It has recently been proposed that these so-called IQ compounds are formed via Maillard reaction products from creatine, free amino acids and hexoses. High mutagenic activity is produced on heating model systems containing these precursors, all of which are present in fish and meat (Jägerstad *et al.*, 1983*a*). From heated mixtures of water-soluble beef fractions and creatine phosphate, IQ has been isolated (Taylor *et al.*, 1985) and from heated mixtures of creatinine, glycine and D-glucose, MeIQx (Jägerstad *et al.*, 1984) and its methyl derivative 7,8-DiMeIQx (Negishi *et al.*, 1984) have been isolated. The use of D-fructose rather than the less reactive glucose (Haworth & Jones, 1944) as the hexose component of such model mixtures seems to result in a somewhat higher yield of IQ compounds. Thus, another methyl derivative (DiMeIQx) of MeIQx is formed on heating a mixture of creatinine, DL-alanine and fructose, and it is probably accompanied by MeIQ (Grivas *et al.*, 1985). These results indicate that the methyl group in position 4 of DiMeIQx and MeIQ might originate from the methyl group of alanine, as required by the proposed reaction route in Fig. 5. As further support of this route, the formation of the known food mutagens, IQ and MeIQx, on heating a mixture of creatinine, glycine and fructose, is now reported.

EXPERIMENTAL

Solvents were evaporated at reduced pressure below 50 °C. High performance liquid chromatography (HPLC) was performed using Waters Associates equipment under the following conditions: column, Nucleosil C_{18}, 5 μ particle size (250 × 4·6 mm inside diameter); mobile phase, methanol/H_3PO_4 0·010M–NaOH pH 7·3 (1:1·2); flow rate, 1·0 ml/min at room temperature. The ^1H NMR spectra were recorded with a Jeol FX 90 Q instrument operated at 89·55 MHz and 29 °C.

Tetramethylsilane (TMS) was used as internal standard. Mass spectra were recorded with a Finnigan 4021 instrument with electron impact ionization (70 eV, direct insertion) and an ion-source temperature of 250 °C.

Synthetic samples of MeIQx (Grivas & Olsson, 1985) and IQ (Adolfsson & Olsson, 1983) were used as references.

Model system and isolation of the mutagens

Creatinine (3·96 g, 35·0 mmol), D-fructose (3·15 g, 17·5 mmol) and glycine (2·62 g, 35·0 mmol) in diethylene glycol (210 ml) containing 14% (v/v) water were refluxed for 2 h at *ca.* 128 °C. The method of isolation of the mutagens was similar to that described by Grivas *et al.* (1985). In brief, the reaction mixture was adjusted to pH *ca. 8* ($NaHCO_3$) and extracted with 1-butanol. The extract was concentrated, diluted with water, acidified to pH 4·3 and poured through a column packed with cation exchange resin (Dowex, H^+-form). The mutagens were eluted with ethanolic ammonia, treated with C_{18} reversed-phase Sep-Pak and purified by reversed-phase HPLC. Since the respective retention times of authentic MeIQx and IQ were 14 and 17 min, one fraction was collected after 13–20 min. This fraction was rechromatographed under the same conditions and final fractions were collected after 12–16 and 16–19 min.

RESULTS

The final HPLC fractions were rechromatographed using an electrochemical detector (Grivas & Nyhammar, 1985). Judging from the retention times and peak areas, the first fraction contained almost pure MeIQx, while the second one contained MeIQx and IQ in the approximate ratio 2:1 as well as one minor impurity. The identity of MeIQx and IQ was supported by co-injection with authentic samples. The total amount of MeIQx in both fractions was 45–50 μg and that of IQ, *ca.* 7 μg.

The first fraction (12–16 min) was subjected to mass and ^1H NMR spectrometry. Its mass spectrum was similar to that of authentic MeIQx (Fig. 1). The ^1H NMR spectra of the isolated and synthetic MeIQx are shown in Fig. 2. The singlets a, b, e and f are clearly seen in both spectra. The shifts for a, b and e agree within 0·01 ppm. An AB quartet corresponding to signals c and d can also be seen, although with some

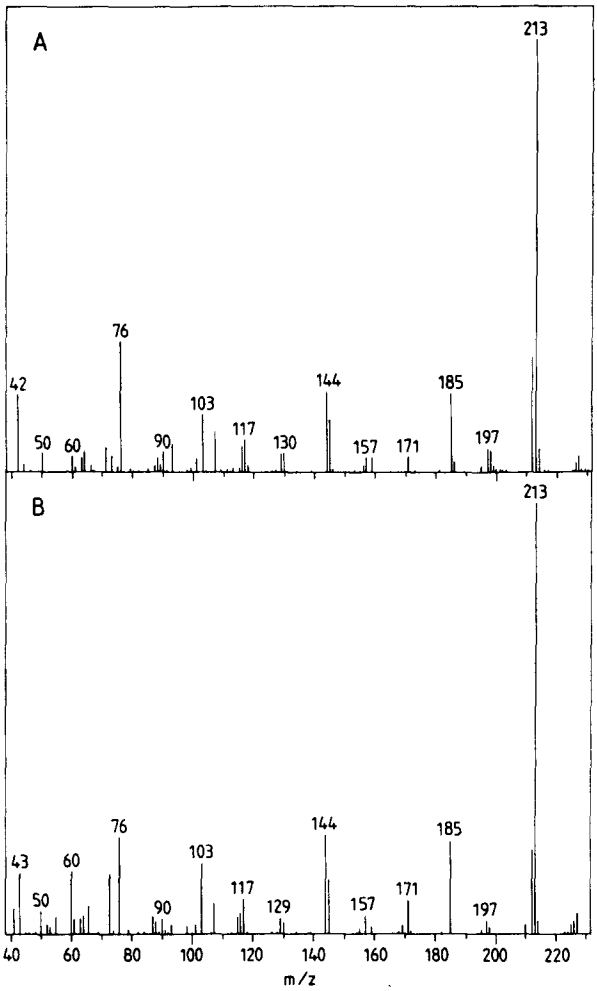

Fig. 1. Mass spectra of isolated (A) and synthetic (B) MeIQx.

difficulty. The peaks below 2·80 ppm are probably due to impurities in the HPLC solvents. The amount of material eluted in the second fraction (16–19 min) was much less than that in the first fraction. Thus, ^{1}H NMR analysis (Fig. 3) only showed the *N*-Me singlets at δ 3·71 (IQ) and δ 3·72 (MeIQx), the *C*-Me singlet of MeIQx at δ 2·82 and the pyrazine proton singlet at δ 8·68. Mass spectral analysis of the second HPLC fraction (Fig. 4) confirmed the HPLC results based on retention times of co-injected samples, and the NMR indications. Both MeIQx and IQ could

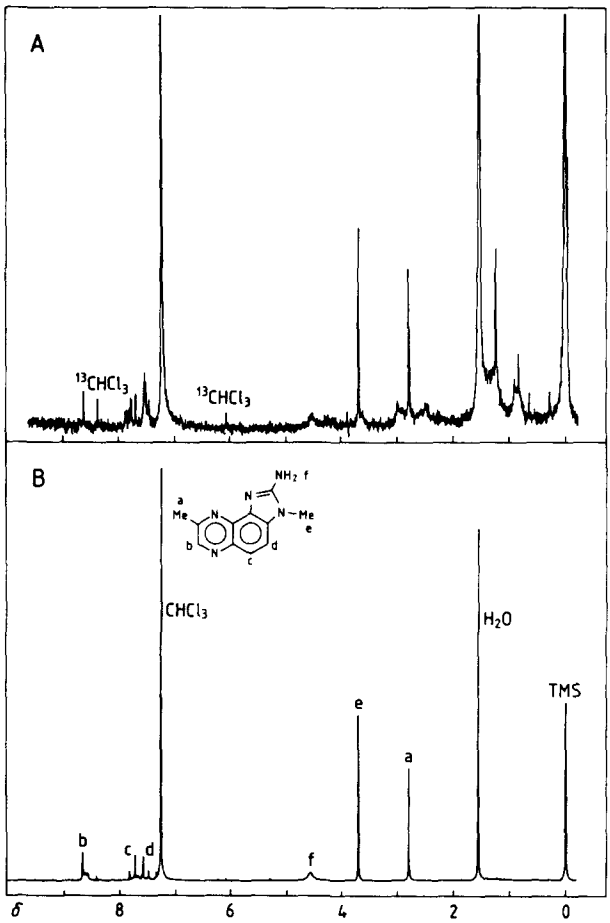

Fig. 2. ^1H NMR spectra of isolated (A) and synthetic (B) MeIQx in $CDCl_3$.

be identified. IQ is volatilized earlier than MeIQx, as shown in Fig. 4A–C. Thus, the mass spectra of relatively pure IQ and MeIQx could be obtained by background subtraction, as shown in Fig. 4D–E.

DISCUSSION

The procedure for isolation of the mutagens has been discussed previously where a new mutagenic DiMeIQx was isolated from a heated mixture of creatinine, D-fructose and DL-alanine (Grivas *et al.*, 1985).

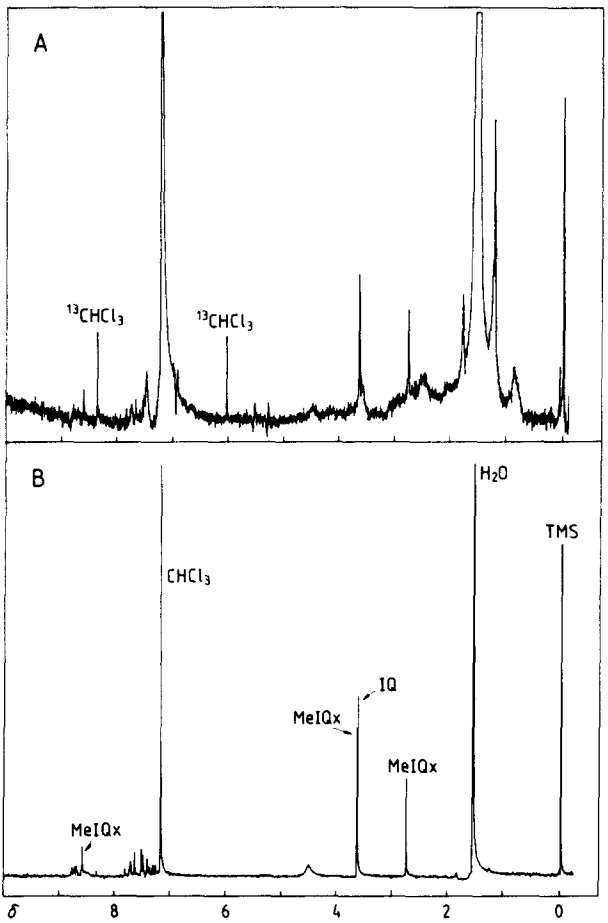

Fig. 3. ^1H NMR spectra of isolated (A) and synthetic (B) mixture of IQ and MeIQx in $CDCl_3$.

Recently, MeIQx (Jägerstad *et al.*, 1984) and the new mutagen 7,8-DiMeIQx (Negishi *et al.*, 1984) were isolated from a heated mixture of creatinine, glucose and glycine, but no IQ was detected. More MeIQx and IQ were formed in the present experiment, perhaps because more solvent and the more reactive sugar fructose were used. According to Bjeldanes *et al.* (1983), the mutagenic activity of the basic fraction extracted from fried ground beef increases with the initial water content, suggesting that adequate water is required as a reaction medium for water-soluble intermediates.

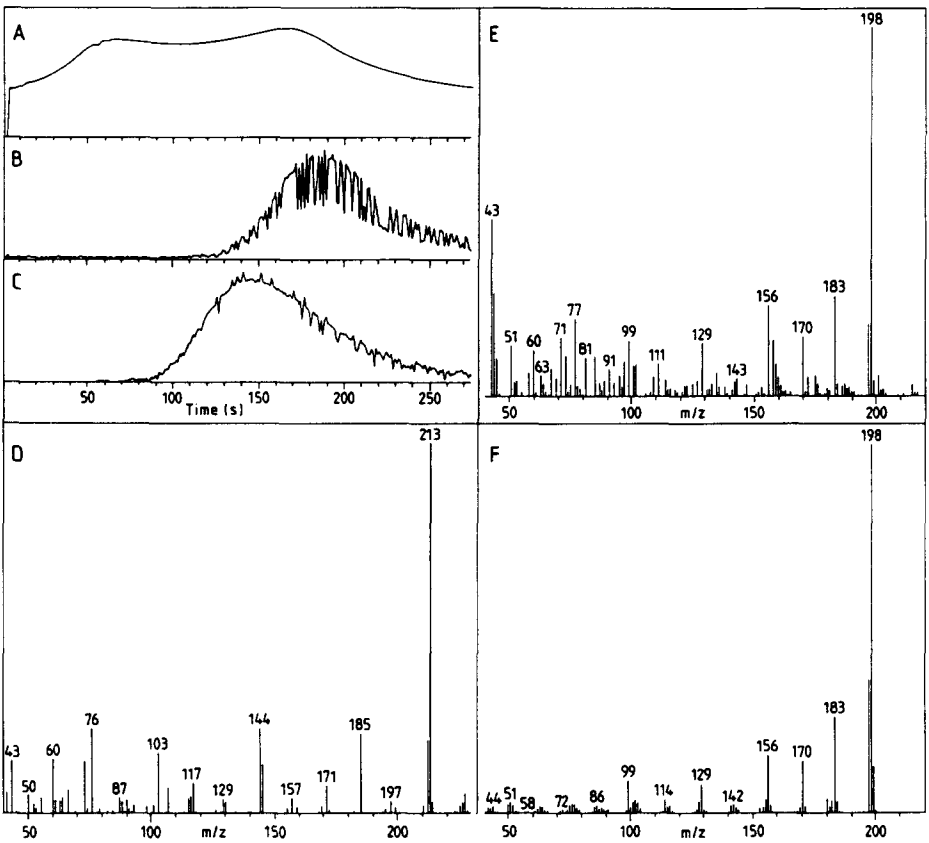

Fig. 4. Mass spectral analysis of the second HPLC fraction (IQ + MeIQx). A. Total ion current profile. B. Mass chromatogram at *m/z* 213 (molecular ion of MeIQx). C. Mass chromatogram at *m/z* 198 (molecular ion of IQ). D. Background-subtracted mass spectrum after 185–198 s (MeIQx). E. Background-subtracted mass spectrum after 129–138 s (IQ). F. Mass spectrum of synthetic IQ.

Jägerstad *et al.* (1983*a*) have proposed that the IQ compounds are formed from a pyridine or pyrazine derivative, an aldehyde (or a related Schiff base) and creatinine, as outlined in Fig. 5. The initial steps are probably related to the aldol condensation. Methyl derivatives of pyridine (Maga, 1981) and pyrazine (Maga, 1982) are often present in cooked foods and may be formed in the Maillard reaction between hexoses and amino acids (Mauron, 1981). Aldehydes are also formed in this reaction through Strecker degradation of the amino acids (Mauron, 1981; Nyhammar *et al.*, 1983). This hypothesis is supported by the

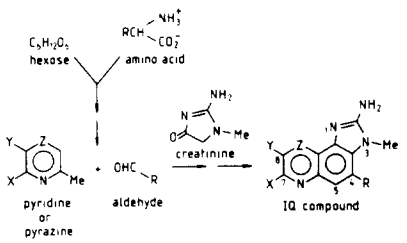

Fig. 5. Proposed formation route of the IQ compounds. R, X and Y may be H or Me; Z may be CH or N.

presence of free hexoses, amino acids and creatin(in)e in animal protein-rich foods, e.g. meat and fish (Sulser, 1978; Laser Reuterswärd *et al.*, 1981; Jägerstad *et al.*, 1983*a*,*b*). Secondly, in the model systems, glycine (R = H) gives rise to IQ, MeIQx (Jägerstad *et al.*, 1984) or 7,8-DiMeIQx (Negishi *et al.*, 1984), whereas alanine (R = Me) gives rise to MeIQ or 4,8-DiMeIQx (Grivas *et al.*, 1985), as required by the route shown in Fig. 5.

Of course, the validity of the hypothesis should be checked by isotopic labelling experiments, but at present the yields of the IQ compounds are far too low for such experiments. The possible formation of IQ compounds along other routes from other precursors should also not be excluded. Thus, Yoshida *et al.* (1984) recently isolated IQ from a heated mixture of proline and creatine, containing no sugar. However, when the sugar was omitted from our model systems, only about 5 % of the original mutagenic activity was produced. No doubt, further work to elucidate the formation mechanism of the IQ compounds is required before precautions to limit their formation during cooking might be successful.

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