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A Convenient Synthesis of the Potent Mutagen 3,4,8-Trimethyl-3*H*-imidazo[4,5-*f*]quinoxalin-2-amine

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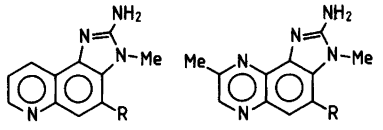
The highly mutagenic title compound (4,8-DiMeIQx) was synthesized in 13 % overall yield from 2-fluoro-5-nitrotoluene in eight operations. The average operation yield was 83 %. The reaction sequence used gave, in addition to the title compound, the isomer 3,4,7-trimethyl-3*H*-imidazo[4,5-*f*]quinoxalin-2-amine (4,7-DiMeIQx).

The *Salmonella*/mammalian-microsome mutagenicity test system (Ames test)[1] has been used on a number of heterocyclic amines. These compounds have been demonstrated in pyrolysates of amino acids, in broiled or fried fish and meat, in commercial beef extracts and in a heated (128 °C) model system comprising creatinine, glycine and glucose.[2,3] One group of these mutagenic heterocyclic amines are imidazoquinoline or -quinoxaline derivatives (*1*, *2* and *3*; Scheme 1) and there is some evidence that they are produced during cooking of animal protein-rich food.[3,4,5] Their carcinogenicity is now being investigated in long term animal studies; to date, *1*("IQ") has been shown to be carcinogenic in mice.[2,6]

Convenient synthetic methods which produce these so-called IQ compounds on a larger scale are necessary for (a) reference purposes, (b) metabolic studies, (c) investigation of their formation mechanism and of their possible carcinogenic or other toxic properties. The title compound *4* (4,8-DiMeIQx) is suspected to be formed in a model system by heating creatinine, threonine and glucose.[7] Thus, its synthesis was necessary in order to definitely confirm its formation in the above mentioned model system. In this paper, a convenient chemical synthesis of 4,8-DiMeIQx and its 3,4,7-trimethyl isomer (*5*) is reported.

By analogy with a synthesis of *3*,[8] 2-fluoro-5-nitrotoluene (*6*) was used as starting material. Thus, *6* was reduced by Raney nickel-catalysed hydrogenation to 4-fluoro-3-methylbenzenamine which was then acetylated[9] (Ac_2O, 0 °C) to *N*-acetyl-4-fluoro-3-methylbenzenamine (*7*), Scheme 2.



1: R=H, "IQ" *3*: R=H, "MeIQx"
2: R=Me, "MeIQ" *4*: R=Me, "348MeIQx"

Scheme 1. Some of the most potent mutagenic heterocyclic amines demonstrated in amino acid pyrolysates, model systems, fried meat and fish, and beef extracts.

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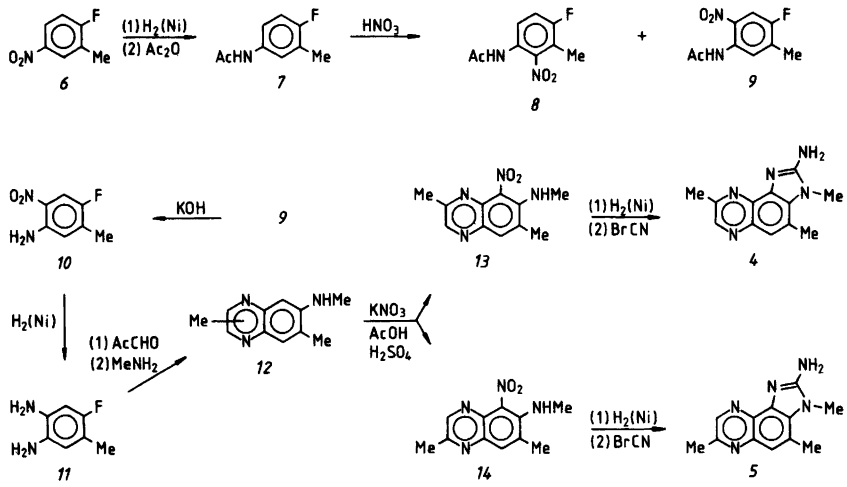

Scheme 2. Synthesis of the title compound *4* and its isomer *5*.

Nitration of *7* with fuming HNO_3 at 20 °C produced the two expected isomers *8* (19 %) and *9* (66 %) which were readily separated by "flash" liquid chromatography.[10] The protecting acetyl group of *9* was hydrolysed by KOH dissolved in aqueous methanol at 60 °C for 30 min; basic hydrolysis was preferred to acidic one due to poor solubility of the acid salts. The resulting amine (*10*) was hydrogenated (Raney nickel, ambient conditions) to give *11*. Reaction with aqueous pyruvaldehyde at 80 °C,[11] followed by nucleophilic replacement of the fluorine atom ($MeNH_2$, 175 °C) converted *11* to the isomeric mixture *12* in high yield. Nitration of *12* was accomplished by dissolving the substrate in glacial acetic acid and concentrated sulfuric acid, followed by addition of potassium nitrate at 0 °C (*cf.* Ref. 12). Nitrations with concentrated or fuming nitric acid at 0–20 °C failed, leaving the starting material intact. When higher temperatures were tried, oxidation of the pyrazine methyl group and/or nitration of the methylamino group took place. The presence of sulfuric acid is necessary for the required reaction to take place below 20 °C. The isomers *13* and *14* were readily separated by "TLC mesh flash" chromatography,[13] reduced by Raney nickel-catalysed hydrogenation (ambient conditions) and *4* and *5* were obtained by reaction with cyanogen bromide in ethanol at 20 °C. The overall yields of *4* and *5* from *6* were 13 and 3 % respectively. A ^{14}C- or ^{13}C-label may be introduced in the last step (*13*→*4* or *14*→*5*) through cyclization with [^{14}C]- or [^{13}C]-cyanogen bromide.[8]

EXPERIMENTAL

The starting material, 2-fluoro-5-nitrotoluene was purchased from Aldrich-Chemie and it was of 99.0 % purity. All reactions and purifications were monitored by TLC on silica gel (Riedel-de Haën, SIF) or by GLC on a 25 m×0.24 mm i.d. capillary column coated with CP Sil 5 and heated from 90 to 270 °C at 8 °C/min. UV irradiation was used to detect TLC spots. "Flash" liquid chromatography[10] (FC) and "TLC mesh flash" liquid chromatography[13] (TC) were performed on silica gel. Evaporations were performed under vacuum at 40 °C. The organic solvents used were freshly distilled. Melting points are corrected. The mass spectra (70 eV, direct insertion) were obtained on a Finnigan 4021 instrument with electron

impact ionization and an ion-source temperature of 200 °C. The ^{1}H NMR spectra were recorded on a Jeol FX 90 Q (89.55 MHz) instrument at 29 °C.

Since the IQ compounds may be cancerogenic,[2,6] *contact with the reaction mixture or product through the skin or by breathing should be avoided in the final steps* 13→4 *and* 14→5. These precautions may be superfluous in the earlier steps, since the imidazole ring appears to be necessary for the strong mutagenicity in Ames test.[14]

N-*Acetyl-4-fluoro-3-methylbenzenamine (7).* A vigorously stirred mixture of *6* (17.0 g, 0.11 mol), Raney nickel (three teaspoons) and absolute ethanol (0.8 l) was hydrogenated under ambient conditions. When *ca.* 6.7 l of hydrogen had been absorbed, GLC analysis showed the completion of the reaction. The catalyst was filtered off through a No. 3 sintered filter with filter aid, and the filtrate was evaporated to dryness. The residue was cooled in an ice bath and treated dropwise with acetic anhydride (10.6 ml) over a period of 5 min. Monitoring by GLC and TLC (CH_2Cl_2–EtOAc, 1:1 v/v) indicated completion of the reaction after 50 min. This was diluted with water, neutralized with concentrated ammonia and extracted with dichloromethane. The extract was washed with water, dried over sodium sulfate, and evaporated to dryness. Recrystallization of the residue from CCl_4 yielded 15.8 g (86 %) of pure *7*, m.p. 75.5–76.5 °C. Anal. $C_9H_{10}FNO$: C, H, N. MS, *m/z* (rel. int.) 167 (13, M), 126 (2), 124 (100), 109 (3), 107 (2), 106 (2), 97 (3), 96 (2), 95 (4), 77 (5). ^{1}H NMR ($CDCl_3$): δ 2.14 (NAc, s), 2.23 (3-Me, d, $|J|$ 1.8 Hz), 6.91 (5-H, dd, $|J|$ 8.7 and 8.8 Hz), 7.16–7.39 (2-H, 6-H, NH, m).

N-*Acetyl-4-fluoro-3-methyl-2-nitrobenzenamine (8)* and N-*acetyl-4-fluoro-5-methyl-2-nitrobenzenamine (9).* Compound *7* (11.5 g, 68.8 mmol) was dissolved in 65 % nitric acid (70 ml), which was cooled in an ice–NaCl bath (3:1), and treated dropwise with 96 % nitric acid (58 ml) over a period of 65 min. TLC monitoring ($CHCl_3$–EtOAc, 6:1 v/v) showed reaction completion 15 min after the addition of all fuming nitric acid. The solution was poured onto ice. The precipitate was collected and washed with cold water. This proved to be only isomer *9*. The washings and filtrate were treated with concentrated ammonia and extracted with dichloromethane. The organic phase was washed with water, dried over sodium sulfate and evaporated to dryness. Column chromatography (FC, CH_2Cl_2–EtOAc, 20:1 v/v) of the residue yielded isomer *8* and a small amount of *9*. After recrystallization from CCl_4 2.78 g (19 %) of pure *8* was isolated. The overall yield of pure *9* was 9.6 g (66 %), after recrystallization from water–ethanol, 8:2 v/v. M.p. of *8*: 139–139.5 °C. Anal. $C_9H_9FN_2O_3$: C, H, N. MS, *m/z* (rel. int.): 212 (13, M), 170 (51), 153 (42), 124 (8), 105 (13), 97 (8), 95 (8), 83 (5), 75 (8), 43 (100). ^{1}H NMR ($CDCl_3$): δ 2.19 (NAc, s), 2.31 (3-Me, d, $|J|$ 2.3 Hz), 7.23 (5-H, dd, $|J|$ 8.9 Hz), 7.95–8.10 (6-H, NH, m, $|J|$ 9.2 and 4.9 Hz). M.p. of *9*: 103–104 °C. Anal. $C_9H_9FN_2O_3$: C, H, N. MS, *m/z* (rel. int.): 212 (20, M), 171 (8), 170 (100), 140 (10), 124 (40), 112 (7), 97 (9), 83 (5), 75 (5), 43 (43). ^{1}H NMR ($CDCl_3$): δ 2.29 (NAc, s), 2.77 (5-Me, ddd, $|J|$ 2.1, 0.7 and 0.4 Hz), 7.88 (3-H, m, $|J|$ 9.2 Hz), 8.66 (6-H, m, $|J|$ 7.2 Hz), 10.2 (NH, broad s).

4-Fluoro-5-methyl-2-nitrobenzenamine (10). Compound *9* (9.7 g, 45.7 mmol) was dissolved in a cold solution of 86 % potassium hydroxide (2 g) in water (40 ml) and methanol (150 ml). The solution was kept in a warm (60 °C) water-bath for 15 min. Water (35 ml) was added and after another 15 min, the reaction was complete (TLC, CH_2Cl_2–CCl_4, 4:1 v/v). The reaction mixture was allowed to cool in an ice bath, and the precipitated crystals were collected and washed with cold water. The yield of pure *10* was 7.55 g (97 %), m.p. 111–112.5 °C. Anal. $C_7H_7FN_2O_2$: C, H, N. MS, *m/z* (rel. int.): 170 (100, M), 153 (4), 140 (8), 124 (59), 122 (15), 112 (13), 97 (39), 96 (11), 95 (21), 77 (23). ^{1}H NMR ($CDCl_3$): δ 2.26 (5-Me, ddd, $|J|$ 2.1, 0.7 and 0.4 Hz), 5.94 (NH_2, broad s), 6.63 (6-H, dm, $|J|$ 6.5 Hz), 7.75 (3-H, d, $|J|$ 9.8 Hz).

4-Fluoro-5-methyl-1,2-benzenediamine (11) was prepared in nearly quantitative yield by Raney nickel-catalysed hydrogenation of *10* (5.5 g, 32.3 mmol) in absolute ethanol (500 ml) under ambient conditions. After filtration of the catalyst, the solution was evaporated to

dryness and the residue was recrystallized from CCl_4. The yield of pure *11* was 4.25 g (93 %), m.p. 103.5–104.5 °C. Anal. $C_7H_9FN_2$: C, H, N. MS, *m/z* (rel. int.): 140 (100, M), 139 (78), 124 (14), 123 (6), 122 (15), 112 (23), 111 (9), 96 (11), 84 (4), 70 (7). ^{1}H NMR ($CDCl_3$): δ 2.12 (5-Me, m, $|J|$ 1.9 Hz), 3.80 (NH_2, broad s), 6.40 (3-H, d, $|J|$ 10.4 Hz), 6.48 (6-H, m, $|J|$ 7.4 and 0.4 Hz).

7-Fluoro-2,6-dimethylquinoxaline and *6-fluoro-2,7-dimethylquinoxaline.* To a stirred solution of *11* (5.60 g, 40.0 mmol) in hot (80 °C) water (135 ml), pyruvaldehyde (50 % in water; 6.10 g, 42 mmol) was added in one portion. The reaction was complete after 15 min (TLC, $CHCl_3$–EtOAc, 4:3 v/v). The solution was cooled and extracted with dichloromethane. The organic solvent was washed with water and evaporated to dryness. Column chromatography (FC, CH_2Cl_2–EtOAc, 12:1 v/v) of the residue yielded the isomer mixture (5.28 g, 75 %). MS, *m/z* (rel. int.): 176 (100, M), 150 (6), 149 (72), 135 (4), 134 (8), 109 (6), 108 (24), 107 (41), 88 (5), 81 (5). Its ^{1}H NMR spectrum in $CDCl_3$ showed only one pyrazine proton singlet at δ 8.67 (*cf.* the ^{1}H NMR spectrum of 6- and 7-fluoro-2-methylquinoxaline [8]).

N,*2,7- and* N,*3,7-Trimethyl-6-quinoxalinamine (12).* A solution of the isomer mixture above (5.00 g, 28.4 mmol) in 33 % ethanolic methylamine (157 ml) was heated at 175 °C in a steel autoclave for 19 h. The reaction mixture was cooled and evaporated to dryness. The residue was treated with a little water and extracted with dichloromethane. The organic phase was concentrated to a minimum volume and chromatographed (FC, CH_2Cl_2–EtOAc, 1:1 v/v). Mixture *12* was isolated in 92 % yield (4.90 g). MS, *m/z* (rel. int.): 187 (100, M), 186 (44), 172 (44), 158 (7), 149 (6), 119 (11), 94 (12), 89 (6), 79 (7), 77 (11). Its ^{1}H NMR ($CDCl_3$) spectrum showed a singlet at δ 2.34 (7-Me), a singlet at δ 2.67 (2- or 3-Me), a singlet at δ 3.02 (NMe), a broad singlet at δ 4.16 (NH), a 5-H signal at δ 6.92, a 8-H signal at δ 7.68 and the pyrazine protons at δ 8.40 (2-H) and δ 8.53 (3-H), *cf.* Ref. 8.

N,*3,7-Trimethyl-5-nitro-6-quinoxalinamine (13).* The isomeric mixture *12* (1.26 g, 6.73 mmol) was first dissolved in glacial acetic acid (10 ml) and then cold concentrated sulfuric acid (12 ml) was added. The solution was cooled to 0 °C. Powdered potassium nitrate (700 mg, 7.0 mmol) was immediately added to the dark brown solution. The reaction was complete after 90 min stirring at 20 °C (TLC, $CHCl_3$–EtOAc, 5:1 v/v). It was poured on to ice, neutralized with concentrated ammonia and extracted with dichloromethane. The organic phase was washed with water, dried over sodium sulfate and evaporated to dryness. Column chromatography (FC, CH_2Cl_2–EtOAc, 6:1 v/v) was used to isolate the isomeric mixture of *13* and *14.* The two isomers were readily separated by TC (CH_2Cl_2–EtOAc, 11:1 v/v). Evaporation of the first fraction to dryness and recrystallization from toluene yielded pure *13* (764 mg, 49 %). M.p. 157.0–158.5 °C. Anal. $C_{11}H_{12}N_4O_2$: C, H, N. MS, *m/z* (rel. int.): 232 (100, M), 198 (26), 185 (45), 171 (49), 157 (53), 142 (21), 131 (22), 116 (40), 103 (20), 89 (52). ^{1}H NMR ($CDCl_3$): δ 2.40 (7-Me, s), 2.69 (3-Me, s), 3.08 (NMe, d, $|J|$ 5.6 Hz), 4.44 (NH, broad s), 7.76 (8-H, s), 8.48 (2-H, s).

N,*2,7-Trimethyl-5-nitro-6-quinoxalinamine (14).* Evaporation of the other fraction (see preceding paragraph) to dryness and recrystallization from ethyl acetate yielded pure *14* (164 mg, 10.5 %), m.p. 206.5–207.5 °C. Anal. $C_{11}H_{12}N_4O_2$: C, H, N. MS, *m/z* (rel. int.): 232 (100, M), 198 (19), 185 (49), 171 (43), 157 (51), 146 (20), 131 (20), 116 (35), 103 (20), 89 (48). ^{1}H NMR ($CDCl_3$): δ 2.41 (7-Me, s), 2.69 (2-Me, s), 3.06 (NMe, d, $|J|$ 5.5 Hz), 4.51 (NH, broad s), 7.73 (8-H, s), 8.61 (2-H, s).

*3,4,8-Trimethyl-3*H*-imidazo[4,5-f]quinoxalin-2-amine (4).* A vigorously stirred mixture of *13* (358 mg, 1.54 mmol), Raney nickel (half teaspoon) and absolute ethanol (80 ml) was hydrogenated under ambient conditions. After 20–25 min, *ca.* 125 ml of hydrogen had been absorbed. TLC monitoring ($CHCl_3$–EtOAc, 2:1 v/v) indicated completion of the reaction. The catalyst was filtered off and the filtrate was concentrated to 25 ml. Cyanogen bromide (180 mg, 1.70 mmol) was dissolved in the filtrate, and 90 min afterwards the first crystals of the hydrobromide of *4* began to precipitate. TLC monitoring ($CHCl_3$–MeOH, 4:1 v/v) indicated completion of reaction after 3.5 h. The reaction mixture was allowed to cool at

0 °C. The hydrobromide of *4* was collected, washed with cold methanol and dissolved in warm water (30 ml). Pure *4* separated from the solution when concentrated ammonia (4 ml) was added and the solution allowed to cool. The crystals of *4* were washed with cold methanol. Yield 263 mg (75 %). M.p. >300 °C. Anal. $C_{12}H_{13}N_5$: C, H, N. MS, *m/z* (rel. int.): 227 (100, M), 226 (18), 212 (15), 199 (82), 198 (33), 185 (15), 171 (11), 159 (9), 144 (8), 131 (17), 116 (10). ^{1}H NMR ($CDCl_3$): δ 2.79 (8-Me, s), 2.83 (4-Me, d, $|J|$ 0.9 Hz), 3.92 (3-Me, s), 4.87 (NH_2, broad s), 7.46 (5-H, d, $|J|$ 0.9 Hz), 8.63 (7-H, s).

3,4,7-Trimethyl-3H-imidazo[4,5-f]quinoxalin-2-amine (5) was prepared from *14* exactly as described for *13*→*4*. M.p. >300 °C. Anal. $C_{12}H_{13}N_5$: C, H, N. MS, *m/z* (rel. int.): 227 (100, M), 226 (78), 212 (20), 199 (21), 159 (12), 158 (11), 144 (5), 131 (8), 117 (9), 114 (16). ^{1}H NMR (CD_3SOCD_3): δ 2.63 (7-Me, s), 2.80 (4-Me, s), 3.84 (3-Me, s), 6.66 (NH_2, broad s), 7.26 (5-H, s), 8.65 (8-H, s).

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