

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

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The mutagenic title compound (5,8-DiMeIQx) was synthesized by two different routes: from 2-methyl-4,6-dinitroaniline; and from 4-chloro-2-methyl-6-nitroaniline. The latter and more convenient route involved 2,1,3-benzoselenadiazole intermediates.

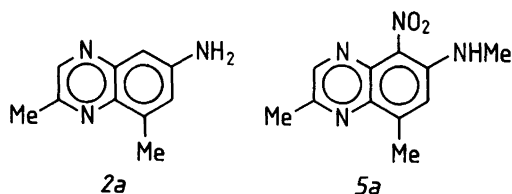
Several heterocyclic amines identified in pyrolysates of amino acids and cooked meat or fish show high mutagenic activity. One group of these are the so-called IQ compounds. They are divided into two classes, imidazo[4,5-*f*]quinolin-2-amines (IQ and MeIQ)¹ and -quinoxalin-2-amines (MeIQx,² 4,8-DiMeIQx³ and 7,8-DiMeIQx⁴). For recently published reviews including the toxicity and metabolism of these substances, see Ref. 5. Convenient synthetic methods which produce these IQ compounds are necessary for reference purposes, and studies of their metabolism and possible carcinogenic or other toxic properties. Recently, we reported the isolation of a new mutagenic DiMeIQx compound in a model system by heating creatinine, fructose and alanine.⁶ This mutagen was tentatively identified as 4,8-DiMeIQx. However, it was not possible to rule out the isomeric 5,8-DiMeIQx since this was expected to have similar spectral characteristics. Thus, the synthesis of the title compound was necessary in order to definitely confirm the identity of the isolated compound.

In this paper, chemical synthesis of 5,8-DiMeIQx (6) by two routes is reported. A brief outline of the first was given recently,⁷ and is shown in Scheme 1, starting from 2-methyl-4,6-dinitroaniline⁸ (1). Using hydrogen or hydrazine hy-

drate and Raney nickel, 1 was reduced to the corresponding triamine which was immediately treated with pyruvaldehyde to produce the isomers 3,8- and 2,8-dimethylquinoxalin-6-amine (2 and 2*a*, respectively). The sequence from 2 to 5,8-DiMeIQx (6) outlined in Scheme 1 is based on the synthesis of IQ and MeIQ reported by Japanese scientists.⁹ Thus, compound 2 was formylated to 3, which, upon reduction, yielded the amine 4. Nitration of 4 yielded 5, which, after hydrogenation and reaction with cyanogen bromide, yielded 6. A ¹⁴C or ¹³C label can be introduced in the last step (5 → 6) through cyclization with [¹⁴C]- or [¹³C]-cyanogen bromide.²

The second route via 2,1,3-benzoselenadiazole intermediates, outlined in Scheme 2, is based on a method devised in our laboratory and advantageously applied to the synthesis of MeIQx and 7,8-DiMeIQx.¹⁰ 4-Chloro-2-methyl-6-nitroaniline¹¹ (7) was hydrogenated to give the diamine 8. Reaction of 8 with selenium dioxide yielded the selenadiazole 9; subsequent nitration gave 10 in high yield. Nucleophilic replacement of the chlorine atom with methylamine converted 10 to 11. After reduction of 11 with hydrogen sulfide in 2-methoxyethanol and ammonia, the resulting diamine was immediately treated with aqueous pyruvaldehyde to yield almost exclusively the 3,8-dimethyl isomer 5. This was identical with 5 obtained according to Scheme 1. However, when the reaction was performed in pyridine, the yield

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was poorer and condensation with pyruvaldehyde produced *N*,3,8- and *N*,2,8-trimethyl-5-nitroquinoxalin-6-amine, **5** and **5a**, in approximate ratio 3:2, respectively. The isomers could be separated by flash chromatography.¹²

Experimental

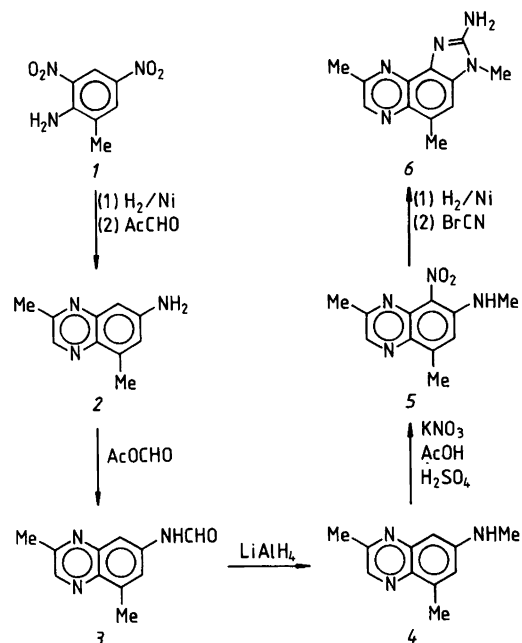
All reactions and purifications were monitored by TLC (UV detection) on silica gel (Merck 60) using dichloromethane/methanol or ethyl acetate (4:1 v/v) as eluent, or by GLC on a 30 m × 0.263 mm i.d. fused silica capillary column coated with DB-1 and heated from 100 to 270 °C at 10 °C/min. Flash chromatography¹² (FC) was performed on silica gel (Merck 60, 230–400 mesh ASTM). Melting points are corrected. The mass spectra (70 eV, direct insertion) were obtained with electron impact ionization and an ion source temperature of 200 °C. Ions containing isotopes other than ³⁵Cl or ⁸⁰Se are not listed. The ¹H NMR spectra were recorded (89.55 MHz) at ca. 30 °C, unless otherwise stated. The data for compounds **7** and **8** were analyzed by computer simulation of the spectra using a program from Jeol (FAS No. 6).

Since some of the IQ compounds have been shown to be carcinogenic in mice and rats,¹³ contact with the reaction mixture or product through the skin or by breathing must be avoided in the final step **5** → **6**. These precautions may be superfluous in the earlier steps, since the imidazole moiety appears to be necessary for their strong mutagenicity in Ames test.¹⁴

Synthesis according to Scheme 1

3,8-Dimethylquinoxalin-6-amine (2). A vigorously stirred mixture of 2-methyl-4,6-dinitroaniline⁸ (**1**) (9.85 g, 50.0 mmol), Raney nickel (two teaspoons) and absolute ethanol (400 ml) was hydrogenated at ambient conditions until no more hydrogen was absorbed and the intense

red-brown colour faded. After about 90 min, the catalyst was filtered off, the solution heated to reflux and 40% aqueous pyruvaldehyde (9.2 ml, 55 mmol) was added dropwise over a period of 5 min. After another 5 min, the solvents were evaporated and the residue, containing a 1:1 mixture of **2** and **2a** was dissolved in ethyl acetate (250 ml). The solution was extracted with 0.5 M citrate buffer (pH 2.5) until GLC indicated no more 3,8-dimethyl isomer in the organic phase (ca. 5×). (The 2,8-dimethyl isomer has a shorter retention time than the 3,8-isomer.) The aqueous extract was neutralized with sodium hydrogen carbonate and extracted with dichloromethane. The dichloromethane extract, now containing a 3:1 mixture of **2** and **2a**, was dried (Na₂SO₄) and evaporated. Crystallization of the residue from toluene yielded **2** (2.60 g, 30%), m.p. 146–150 °C. Anal. C₁₀H₁₁N₃: C, H, N. MS, *m/z* (rel. int.): 173 (100, M), 172 (16), 146 (6), 145 (9), 131 (7), 105 (60), 104 (16), 78 (11), 77 (14), 51 (11). ¹H NMR (CDCl₃): δ 2.67 (3-Me, s), 2.69 (8-Me, d), 4.1 (NH₂, broad s), 6.92 (5-H, d), 6.97 (7-H, dq), 8.45 (2-H, s). |*J*| 2.6 (5-H, 7-H), 0.8 (7-H, 8-Me) Hz. Pure **2a** was obtained when the ethyl acetate residue was subjected to column chromatography (FC, CH₂Cl₂/EtOAc/MeOH, 10:2:1



Scheme 1.

v/v), m.p. 159–162°C. Anal. $C_{10}H_{11}N_3$: C, H, N. MS, m/z (rel. int.): 173 (100, M), 172 (17), 146 (6), 145 (7), 105 (68), 104 (15), 86 (8), 78 (11), 77 (15), 73 (12). 1H NMR ($CDCl_3$): δ 2.69 (2- and 8-Me, s), 3.5 (NH_2 , broad s), 7.00 (5- and 7-H, broad s), 8.54 (3-H, s).

N-Formyl-3,8-dimethylquinoxalin-6-amine (3). A mixture of acetic anhydride (2.5 g, 25 mmol) and absolute formic acid (25 ml, 65 mmol) was added to a solution of 2 (1.73 g, 10.0 mmol) in absolute formic acid (50 ml). The mixture was stirred for 1 h at room temperature and evaporated to dryness. The residue was treated with a little aqueous ammonia and extracted with chloroform. The extract was washed with water and evaporated to dryness. The residue was recrystallized from ethyl acetate, yielding 1.41 g (70%) of 3, m.p. 202–204°C. Anal. $C_{11}H_{11}N_3O$: C, H, N. MS, m/z (rel. int.): 201 (100, M), 173 (53), 172 (24), 146 (17), 145 (17), 105 (68), 104 (24), 78 (19), 77 (31), 51 (23). 1H NMR (CD_3SOCD_3 , 120°C): δ 2.65 (3-Me, s), 2.68 (8-Me, dd), 7.64 (7-H, dq), 7.93 (5-H, dq), 8.55 (CHO, d), 8.65 (2-H, s), 9.9 (NH, broad s). $|J|$ 4.4 (CHO, NH), 2.4 (5-H, 7-H), 1.0 (7-H, 8-Me), 0.6 (5-H, 8-Me) Hz. (cf. Ref. 4)

N,3,8-Trimethylquinoxalin-6-amine (4). A cold (0–5°C) solution of 3 (1.00 g, 5.00 mmol) in dry tetrahydrofuran (50 ml) was added cautiously to a cold solution of lithium aluminium hydride (0.34 g, 9.00 mmol) in dry tetrahydrofuran (25 ml). The reaction mixture was stirred at 0–5°C. After ca. 4 h, ethyl acetate (10 ml) was added, and the reaction mixture poured onto ice-cold dil. sulfuric acid. The organic solvents were evaporated. The remaining suspension was treated with a little water, neutralized with aqueous ammonia and extracted with dichloromethane. The organic phase was washed with water and evaporated to dryness. Crystallization from petroleum ether (b.p. 60–70°C) gave 0.65 g (70%) of 4, m.p. 135–141°C. Anal. $C_{11}H_{13}N_3$: C, H, N. MS, m/z (rel. int.): 187 (100, M), 186 (62), 173 (10), 158 (13), 145 (14), 119 (13), 93 (14), 77 (15), 63 (10), 51 (11). 1H NMR ($CDCl_3$): δ 2.67 (8-Me, d), 2.69 (3-Me, s), 2.96 (NMe, s), 4.2 (NH, broad s), 6.78 (5-H, d), 6.90 (7-H, dq), 8.41 (2-H, s). $|J|$ 2.6 (5-H, 7-H), 0.9 (7-H, 8-Me) Hz. (cf. Ref. 4)

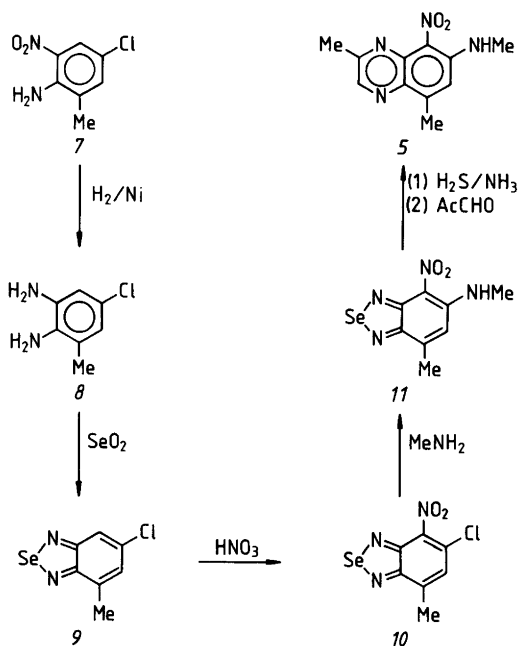
N,3,8-Trimethyl-5-nitroquinoxalin-6-amine (5). Cold concentrated sulfuric acid (5.5 ml) was added to a solution of 4 (560 mg, 3.0 mmol) in glacial acetic acid (5.0 ml). The solution was

cooled to 0°C. Powdered potassium nitrate (310 mg, 3.1 mmol) was added in one portion. The reaction was complete after 90 min stirring at 20°C. The mixture was poured onto ice, neutralized with concentrated ammonia and extracted with dichloromethane. The organic phase was washed with water, dried (Na_2SO_4) and purified by column chromatography (FC, $CH_2Cl_2/EtOAc$, 4:1 v/v). The yield of pure 5 was 450 mg (65%), m.p. 179–181°C. Anal. $C_{11}H_{12}N_4O_2$: C, H, N. MS, m/z (rel. int.): 232 (100, M), 213 (22), 187 (27), 185 (23), 171 (41), 170 (45), 158 (27), 117 (22), 90 (23), 89 (26). 1H NMR ($CDCl_3$): δ 2.74 (3- and 8-Me, s), 3.15 (NMe, s), 7.13 (7-H, s), 7.7 (NH, broad s), 8.48 (2-H, s). (cf. Ref. 3)

3,5,8-Trimethyl-3H-imidazo[4,5-f]quinoxalin-2-amine (6). A vigorously stirred mixture of 5 (275 mg, 1.19 mmol), Raney nickel (half teaspoon) and absolute ethanol (60 ml) was hydrogenated under ambient conditions. After 60 min, the catalyst was filtered off and the filtrate concentrated to ca. 20 ml. Cyanogen bromide (139 mg, 1.31 mmol) was added. After standing overnight at room temperature, the reaction was complete. Petroleum ether (b.p. 40–60°C, 20 ml) was added and the reaction mixture allowed to cool to 0°C. The hydrobromide of 6 was collected, washed with cold ethanol/petroleum ether (1:1 v/v) and dissolved in warm water (20 ml). Pure 6 separated from the solution when concentrated ammonia (3 ml) was added and the solution allowed to cool. The crystals of 6 were washed with cold methanol. Yield 197 mg (73%), m.p. > 300°C. Anal. $C_{12}H_{13}N_5$: C, H, N. MS, m/z (rel. int.): 227 (100, M), 226 (50), 212 (5), 199 (10), 185 (4), 159 (5), 158 (5), 117 (3), 114 (4), 90 (4). 1H NMR (CD_3SOCD_3): δ 2.69 (8-Me, s), 2.73 (5-Me, s), 3.63 (NMe, s), 6.5 (NH_2 , broad s), 7.63 (4-H, s), 8.67 (7-H, s). (cf. Ref. 3)

Synthesis according to Scheme 2

2-Amino-5-chloro-3-methylaniline (8). A vigorously stirred mixture of 4-chloro-2-methyl-6-nitroaniline¹¹ (7) (5.60 g, 30.0 mmol), Raney nickel (half teaspoon) and absolute ethanol (250 ml) was hydrogenated at ambient conditions until the green colour faded. After about 1 h, the catalyst was filtered off and the filtrate evaporated to dryness. Recrystallization of the residue from carbon tetrachloride yielded pure 8 (4.27 g, 91%), m.p. 61.0–62.5°C. Anal. $C_7H_7ClN_2$: C, H, N.



Scheme 2.

MS, m/z (rel. int.): 156 (100, M), 155 (17), 128 (15), 121 (43), 104 (17), 94 (17), 93 (16), 77 (10), 65 (17), 52 (12). $^1\text{H NMR}$ (CDCl_3): δ 2.15 (Me, dd), 3.3 (NH_2 , broad s), 6.58 (6-H, m), 6.61 (4-H, m). $|J|$ 2.3 (4-H, 6-H), 0.7 (4-H, Me), 0.5 (6-H, Me) Hz. (cf. Ref. 3)

6-Chloro-4-methyl-2,1,3-benzoselenadiazole (9). Compound 8 (3.91 g, 25.0 mmol) was treated with hot 1 M hydrochloric acid (50 ml). To the obtained suspension of the dihydrochloride, a solution of selenium dioxide (2.78 g, 25.0 mmol) in water (20 ml) was added dropwise. Crude 9 precipitated immediately. After stirring for 30 min, the precipitated crystals were collected and washed with water and ethanol. Recrystallization from toluene yielded 5.25 g (91%) of pure 9, m.p. 172–176°C. Anal. $\text{C}_7\text{H}_5\text{ClN}_2\text{Se}$: C, H, N. MS, m/z (rel. int.): 232 (100, M), 205 (22), 197 (25), 170 (18), 125 (33), 117 (41), 115 (51), 90 (87), 64 (54), 63 (68). $^1\text{H NMR}$ (CDCl_3): δ 2.68 (Me, dd), 7.21 (5-H, dq), 7.70 (7-H, dq). $|J|$ 2.0 (5-H, 7-H), 1.2 (5-H, Me), 0.7 (7-H, Me) Hz. (cf. Ref. 15)

5-Chloro-7-methyl-4-nitro-2,1,3-benzoselenadiazole (10). Compound 9 (3.47 g, 15.0 mmol) was dissolved in concentrated sulfuric acid (15 ml) and cooled in an ice bath. A cold mixture of

concentrated nitric acid (1.5 ml, 20 mmol) and concentrated sulfuric acid (4.5 ml, 85 mmol) was added over a period of 30 min. The reaction mixture was removed from the ice bath, and after stirring for another 30 min, GLC showed completion of the reaction. The solution was poured onto ice (50 g). The precipitate was collected, washed with water and air-dried. The yield of pure 10 was 4.06 g (98%), m.p. 190–191°C. Anal. $\text{C}_7\text{H}_4\text{ClN}_3\text{O}_2\text{Se}$: C, H, N. MS, m/z (rel. int.): 277 (54, M), 247 (74), 140 (100), 139 (50), 124 (62), 115 (74), 88 (57), 64 (73), 63 (54), 62 (44). $^1\text{H NMR}$ (CDCl_3): δ 2.75 (Me, d), 7.37 (6-H, q). $|J|$ 1.2 (6-H, Me) Hz. (cf. Ref. 16)

N,7-Dimethyl-4-nitro-2,1,3-benzoselenadiazole-5-amine (11). A solution of compound 10 (2.76 g, 10.0 mmol) in 2-ethoxyethanol (50 ml) was heated in a water bath (90°C). Methylamine (33% in ethanol, 3.7 ml, 30 mmol) was added in one portion. Dark yellow crystals soon precipitated. After 15 min, the mixture was cooled and the precipitate was collected and washed with water. Recrystallization from pyridine/benzene (1:1 v/v) yielded 2.39 g (88%) of pure 11, m.p. > 300°C. Anal. $\text{C}_8\text{H}_8\text{N}_4\text{O}_2\text{Se}$: C, H, N. MS, m/z (rel. int.): 272 (54, M), 144 (40), 118 (47), 107 (71), 105 (45), 93 (58), 80 (54), 78 (42), 66 (40), 52 (41). $^1\text{H NMR}$ (CDCl_3): δ 2.70 (7-Me, d), 3.31 (NMe, d), 7.18 (6-H, q), 10.4 (NH, broad s). $|J|$ 5.2 (NMe, NH), 1.2 (6-H, 7-Me) Hz.

N,3,8-Trimethyl-5-nitroquinoxalin-6-amine (5). (cf. Ref. 17) A suspension of compound 11 (542 mg, 2.0 mmol) in 2-methoxyethanol (50 ml) and concentrated ammonia (1.0 ml) was heated in a boiling water bath. Hydrogen sulfide was bubbled through the mixture. After 25 min, the dark solution was purged with nitrogen, allowed to cool and filtered. The filtrate was heated to 60°C and 40% aqueous pyruvaldehyde (350 μl , 2.2 mmol) added in one portion. After 5 min, the solution was concentrated to ca. 5 ml, treated with petroleum ether (b.p. 60–70°C, 10 ml) and filtered hot. After cooling in ice, the precipitated crystals were filtered off and washed with cold petroleum ether. The yield of 5 was 348 mg (75%).

N,2,8-Trimethyl-5-nitroquinoxalin-6-amine (5a). Reaction of 11 with hydrogen sulfide was performed as described in the preceding paragraph but using pyridine instead of ammoniacal 2-methoxyethanol as solvent. The products were separated by column chromatography (FC, CH_2Cl_2 /

EtOAc, 16:1 v/v). The first fraction contained only 5, the second a 1:1 mixture of 5 and 5a, and the third pure 5a. After crystallization of the fractions from toluene, 5 was obtained in 20% and 5a in 13% yield, m.p. 213–216°C. Anal. $C_{11}H_{12}N_4O_2$: C, H, N. MS, *m/z* (rel. int.): 232 (100, M), 213 (20), 187 (27), 185 (23), 171 (41), 170 (42), 158 (28), 157 (28), 116 (21), 90 (22). 1H NMR ($CDCl_3$): δ 2.70 (2-Me, s), 2.74 (8-Me, d), 3.15 (NMe, d), 7.19 (7-H, q), 8.0 (NH, broad s), 8.73 (3-H, s). $|J|$ 5.1 (NMe, NH), 1.1 (7-H, 8-Me) Hz.

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