SYNTHESIS AND MUTAGENICITY OF A NEW MUTAGEN, 2-AMINO-1,7,9-TRIMETHYLIMIDAZO-[4,5-*g*]QUINOXALINE, AND ITS ANALOG

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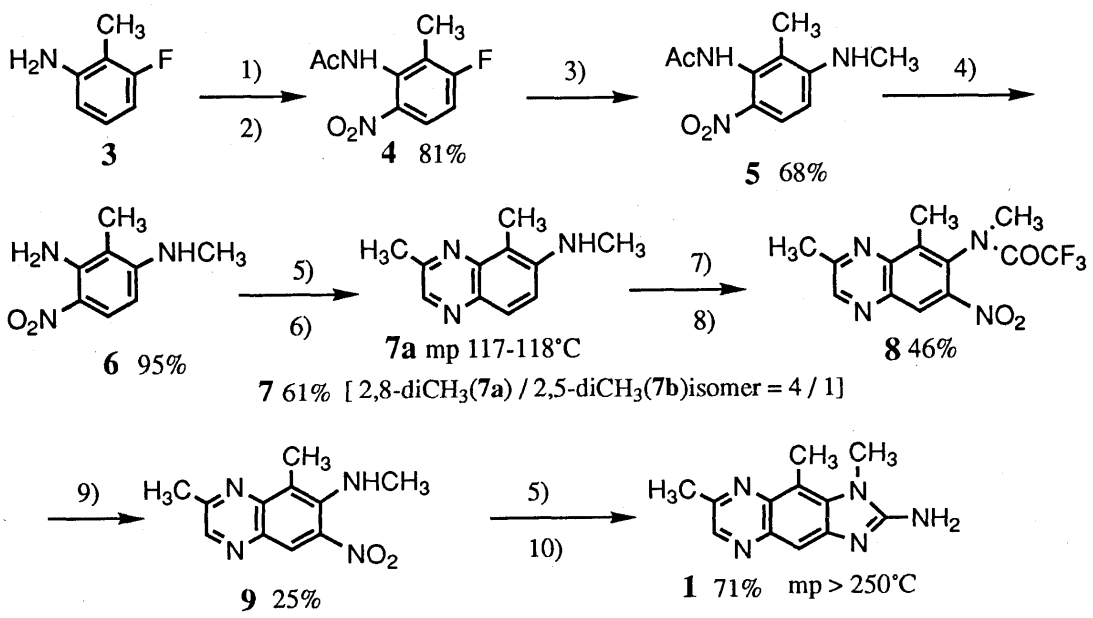
A new mutagen, 2-amino-1,7,9-trimethylimidazo[4,5-*g*]quinoxaline (**1**), isolated from beef extract, was synthesized from 3-fluoro-2-methylaniline *via* an intermediate, 2,8-dimethyl-7-methylaminoquinoxaline (**7a**). Its 2-methylanalog (**2**) was also synthesized from the same intermediate. The synthetic **1** showed the same mutagenic activity as the isolated mutagen. However, **2** was non-mutagenic.

KEYWORDS total synthesis; imidazo[4,5-*g*]quinoxaline derivaive; mutagen; Ames assay

In 1992, H. Nukaya *et al.* isolated a new mutagen from beef extract, and determined its structure to be 2-amino-1,7,9-trimethylimidazo[4,5-*g*]quinoxaline (7,9-DiMeIgQx) (**1**) by X-ray analysis.[1] The new mutagen (**1**) has a unique imidazo[4,5-g]quinoxaline ring, although analogous mutagens reported previously[2] were imidazo[4,5-*f*]quinoxaline derivatives. We planned to synthesize it for synthetic structure elucidation and further investigation of possible carcinogenic property and structure-mutagenicity relationships. We wish to report here the first total synthesis of 7,9-DiMeIgQx (**1**) and 1,2,7,9-tetramethyl-imidazo[4,5-*g*]quinoxaline (**2**), and their mutagenicities.

Synthesis of **1** from commercially available 3-fluoro-2-methylaniline (**3**) was summarized in Chart 1. Substitution of fluorine of **4** with methylamine was achieved under relatively mild conditions in an autoclave. Catalytic reduction of nitro group of **6** with palladium charchol gave 1,2-diaminobenzene derivative, which was treated with pyruvic aldehyde without purification to afford a mixture of quinoxaline derivatives (**7a** : **7b** = 4 : 1).



3 → **4** 81% → **5** 68% → **6** 95% → **7a** mp 117-118°C → **8** 46% → **9** 25% → **1** 71% mp > 250°C

7 61% [2,8-diCH$_3$(**7a**) / 2,5-diCH$_3$(**7b**)isomer = 4 / 1]

Chart 1. Synthesis of 2-Amino-1,7,9-trimethylimidazo[4,5-*g*]quinoxaline (**1**)

Fortunately, desired 2,8-dimethyl-7-methylaminoquinoxaline (**7a**) was easily separated by silica gel column chromatography. Each structure was supported by ^{1}H-^{13}C long-range coupling in the ^{1}H detected heteronuclear multiple bond connectivity (HMBC) experiment (Fig. 1).

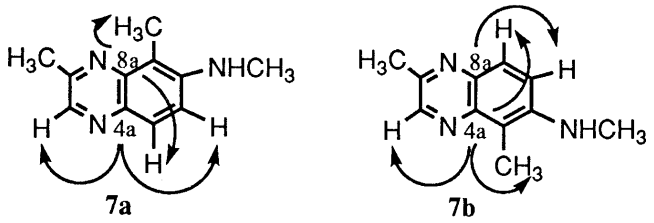
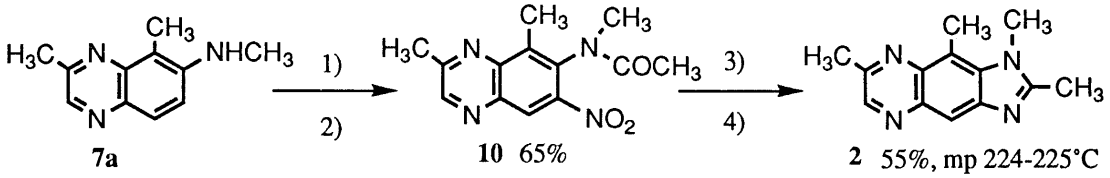


Fig. 1. ^{1}H - ^{13}C Correlations (^{3}J) at C-4a and C-8a from the HMBC Spectra

After *N*-trifluoroacetylation of **7a**, nitration with nitric acid in trifluoroacetic anhydride at -40~-20°C gave 6-nitro compound (**8**) containing a small amount of 5-nitro analog. Hydrolysis with aqueous potassium carbonate in methanol, and then catalytic reduction followed by treatment with cyanogen bromide, gave a final product (**1**). Its ^{1}H-NMR and UV spectra[3] were in agreement with those of the isolated product. The purity was also determined by HPLC analysis using two kinds of columns,[4] where the retention times agreed with those of the isolated product.

The 2-methyl analog (**2**) was easily prepared from **7a** in 4 steps (Chart 2). On the other hand, the synthesis of **1** from 2,8-dimethyl-7-(*N*-methylacetoamido)-6-nitroquinoxaline (**10**) was unsuccessful because of the difficulty of deacetylation without decomposition.



1) Ac_2O, 2) HNO_3/Ac_2O, 3) H_2 / Pd-C, 4) PTS / THF

Chart 2. Synthesis of 1,2,7,9-Tetramethylimidazo[4,5-*g*]quinoxaline (**2**)

The mutagenicity by Ames test of the synthetic **1** showed the same potency within experimental error as that of the product from beef extract with *Salmonella typhimurium* TA 98 in the presence of S9 mix. However, the 2-methyl analog (**2**) showed non- mutagenicity under the same conditions.[5] These facts indicate that the 2-amino group plays an important role in the induction of mutation.

REFERENCES AND NOTES

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3) ^{1}H-NMR (DMSO-d_6) δ: 2.66 (3H, s, 7-CH_3), 3.02 (3H, s, 9-CH_3), 3.86 (3H, s, 1-CH_3), 7.01 (2H, br, NH_2), 7.43 (1H, s, 4-CH), 8.57 (1H, s, 6-CH). UV λmax (MeOH) : 229, 266, 358 nm.

4) Cation exchange column, SP-2SW (30% CH_3CN in 100 mM phosphate buffer, pH 3) and ODS column (120Å) (3-20% CH_3CN in 25 mM phosphate buffer, pH 2)

5) Mutagenicity of **1**: 620 revertants / μg by preincubation method. Numbers of the revertant colonies induced with **2** were below to those on control plates.

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