The 1,6-anhydro-β-rings of **4** and **9** were cleaved with an acetolysis mixture (H_2SO_4–Ac_2O–AcOH, 1: 70: 30, v/v) to give the tetrasaccharide tetradecaacetate (**6**) and hexasaccharide eicosaacetate (**11**), respectively, as anomeric mixtures containing α-anomer predominantly. **6**: amorphous powder, $[\alpha]_D^{22}$ +7.2° ($CHCl_3$), 94.3% yield. ^{1}H-NMR ($CDCl_3$): 1.97, 1.99, 2.03, 2.08, 2.16, 2.19 (42H, all s, OAc×13, NAc), 5.81 (*ca.* 0.3H, d, $J_{1,2}$=8 Hz, H-1, β-Glc), 6.29 (1H, br. s, exchangeable with D_2O, NH), 6.37 (*ca.* 0.7H, d, $J_{1,2}$=3.5 Hz, H-1, α-Glc). **11**: amorphous powder, $[\alpha]_D^{22}$ +12.7° ($CHCl_3$), 93.9% yield. ^{1}H-NMR ($CDCl_3$): 1.93, 1.98, 2.07, 2.16 (60H, all s, OAc×18, NAc×2), 5.62 (1H, d, exchangeable with D_2O, $J_{NH,2''}$ or $_{2''''}$=8 Hz, NH), 6.30 (<1H, d, $J_{1,2}$=3.5 Hz, H-1, α-Glc), 6.40 (1H, d, exchangeable with D_2O, $J_{NH,2''''}$ or $_{2''}$=8 Hz, NH).

De-O-acetylation of **6** and **11** with methanolic MeONa gave **7** (73.5% yield) as a white powder, $[\alpha]_D^{19}$ +11.8° (H_2O) [lit.[6] mp 185—187°, $[\alpha]_D$ +8° (H_2O)], and **12** (80% yield), crystallizable from aq. EtOH as grains, mp 223—225°, $[\alpha]_D^{21}$ +9.1° (no mutarotation, H_2O), respectively.

The data of elemental analysis of all these compounds were in good agreement with the theoretical values.

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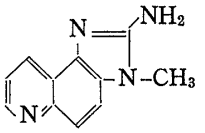
Identification of a Reactive Metabolite of the Mutagen, 2-Amino-3-methylimidazolo[4,5-*f*]quinoline

A reactive major metabolite of the mutagen, 2-amino-3-methylimidazolo[4,5-*f*]-quinoline (IQ), by rat liver microsomes was 2-hydroxyamino-3-methylimidazolo[4,5-*f*]-quinoline (N–OH–IQ). The synthesis and reaction with DNA of N–OH–IQ were discussed.

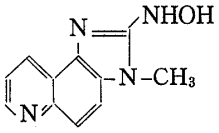
Keywords——mutagen; 2-amino-3-methylimidazolo[4,5-*f*]quinoline; IQ; 2-hydroxyamino-3-methylimidazolo[4,5-*f*]quinoline; metabolic activation; microsomes; hydroxylamine; hydroxyaminoimidazole; carcinogen; DNA modification

Recent studies showed that pyrolysis products of proteins and amino acids contain strong mutagens, and active compounds were isolated and their structures were determined.[1] Among these compounds, 3-amino-5H-pyrido[4,3-*b*]indoles (Trp-P)[1a] from a pyrolysate of tryptophan and 2-aminodipyrido[1,2-*a*: 3′,2′-*d*]imidazoles (Glu-P)[1b] from a pyrolysate of

glutamic acid are very strong mutagens, especially towards *Salmonella typhimurium* TA 98 with metabolic activation. Active metabolites of these mutagens formed by rat liver microsomes were identified, and proved to modify DNA nonenzymatically.[2] The structures of modified nucleic acid bases by the activated mutagens were also determined.[3] Very recently, two new potent mutagens were isolated from broiled sardines: they are novel mutagens with a six-six-five membered ring system, 2-amino-3-methylimidazolo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazolo[4,5-*f*]quinoline (MeIQ).[4] Their mutagenic activity are shown only after metabolic activation. Elucidation of structures of activated forms of these mutagens which would react with nucleic acid must be essential to know the molecular aspect of mutagenic action of these compounds. In this paper, we report the identification, synthesis, and reaction with DNA of a reactive metabolite of IQ.



IQ



N-OH-IQ

Chart 1

Three mg of IQ (HBr salt)[5] in 10 ml of 0.05 M Bis-Tris (pH 7.5)[6] was incubated at 37° for 1 min in the presence of 10 mg of NADPH and 30 mg of microsomal proteins freshly prepared from livers of male Wistar rats treated with polychlorinated biphenyls.[7] The incubation mixture was cooled and extracted with cold ethyl acetate promptly. The organic layer was analyzed by liquid chromatography (Fig. 1). The metabolite, peak I, was only observed when incubation time was short: incubation for 10 min did not give this metabolite. The

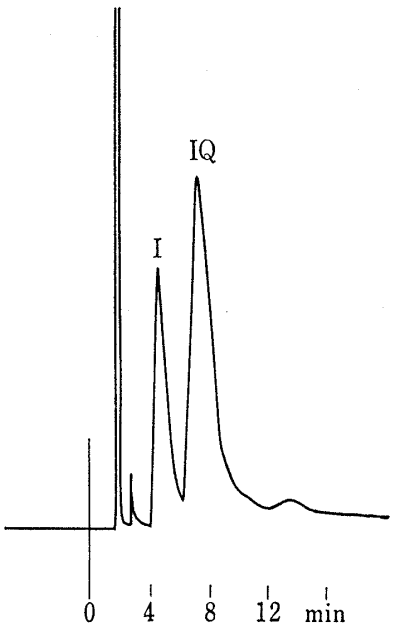


Fig. 1. High Performance Liquid Chromatography of Microsomal Metabolites of IQ

column; Polygosil $_5C_{18}$, 4.6ϕ × 250 mm,
solvent; 23% CH_3CN in 0.02M KH_2PO_4,
flow rate; 0.9 ml/min,
detection; Abs. at 254 nm.

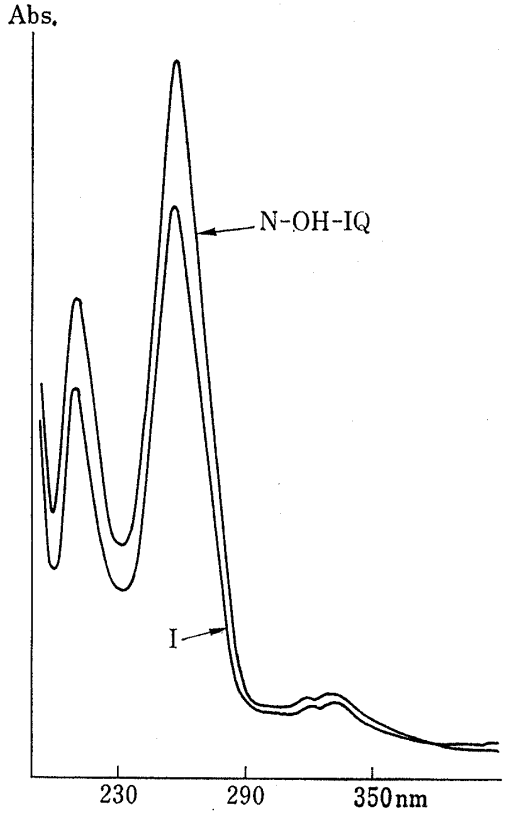


Fig. 2. UV Spectra of Peak I and Synthetic N-OH-IQ

metabolite was very unstable in the incubation mixture and in the organic extract, the half life being about 1 min at room temperatures. This major unstable metabolite was identified as 2-hydroxyamino-3-methylimidazolo[4,5-*f*]quinoline (N-OH-IQ).

N-OH-IQ was prepared from 2-nitro-3-methylimidazolo[4,5-*f*]quinoline (NO_2IQ), which was obtained by substitution of diazotized IQ. IQ (HBr salt, 900 mg) in dil. HCl (pH 3.0, 450 ml) was treated with $NaNO_2$ (3 g) and $CuSO_4 \cdot 5H_2O$ (12 g) for 24 hr at room temperature. The mixture was neutralyzed with K_2CO_3 and extracted with ethyl acetate, organic layer was evaporated, and the residue was recrystallyzed from ethyl acetate to give NO_2IQ (370 mg, 50% yield) as yellow needles, mp 258—260°. The structure was deduced from mass spectroscopy, IR, UV, and elemental analysis. Reduction of NO_2IQ was performed by treatment with aluminum amalgamate in moist tetrahydrofuran at 0° for 10 min. The residue obtained by evaporation of the organic solution at −5° was washed with small amounts of water and methanol, giving crude N-OH-IQ (80% purity) in 40—50% yield. Purification was done by liquid chromatography using a reversed phase column (ODS, CH_3CN–0.02 M KH_2PO_4). Fractions containing N-OH-IQ were extracted with cold ethyl acetate and organic layer was evaporated at −5°, giving a chromatographically pure N-OH-IQ. The structure was deduced from UV [in methanol, nm, (log ε): 212 (4.49), 258 (4.68), 320—330 (3.46)], further reduction to IQ, and derivation by treatment with nitrosobenzene to 2-phenyl-ONN-azoxy-3-methylimidazolo[4,5-*f*]quinoline (azoxyIQ, 80% yield), which was correctly analyzed. The rate of decomposition of the pure N-OH-IQ was rapid (a half life in a solution was about 30 min) but it is storable at −80°.

Identification of the metabolite, peak I, with synthetic N-OH-IQ was performed by comparing the retention times of liquid chromatography and UV spectra (Fig. 2). Addition of nitrosobenzene to the microsomal incubation mixture after precipitation of protein yielded a corresponding amount of azoxyIQ, which was identified with the synthetic sample in comparison with the retention times and the characteristic UV spectra.

N-OH-IQ was found to bind rapidly with calf thymus DNA at 5° in a neutral condition. More than 200 μmol/mol P (analysis of a hydrolysate of the modified DNA) of the mutagen bound to DNA. The same modified base was detected in the hydrolysate of modified DNA with IQ in the presence of microsomes. Since N-OH-IQ also reacted with 5′-guanylic acid to give the same modified base after hydrolysis, the site of modification of DNA is plausibly the guanine moiety. We are now trying to elucidate the structure of the modified base.

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5) IQ was prepared by the following pathway:

$$\text{6-aminoquinoline} \xrightarrow[87\%]{HCOOCOCH_3} \text{6-formylaminoquinoline} \xrightarrow[89\%]{LiAlH_4}$$

$$\text{6-methylaminoquinoline} \xrightarrow[81\%]{NO_2^+} \text{5-nitro-6-methylaminoquinoline}$$

$$\xrightarrow[78\%]{Pd/C,\ H_2} \text{5-amino-6-methylaminoquinoline} \xrightarrow[59\%]{BrCN} \text{IQ (29\% from 6-aminoquinoline)}$$

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1,2-Dihydrocyclobuta[*c*]quinoline[1)]

Using 1,2-dihydrocyclobuta[*c*]quinolin-3(4H)-one (III) obtained through the photoaddition of 4-methoxy-2-quinolone (I) to ethylene and subsequent base treatment of the resulted cycloadduct (II) as a key intermediate, 1,2-dihydrocyclobuta[*c*]quinoline (V), a new aza-analogue of naphthocyclobutene, was synthesized.

Keywords——photochemical synthesis; photochemical 2+2 cycloaddition; 3-chloro-1,2-dihydrocyclobuta[*c*]quinoline; 1,2,2a,3,4,8b-hexahydrocyclobuta[*c*]quinoline; aza-analogue of naphthocyclobutene; 1,2-dihydrocyclobuta[*c*]quinolin-3(4H)-one

1,2-Dihydrocyclobuta[*b*]quinoline, one of the possible heterocyclic analogues of naphthocyclobutene, has been synthesized previously either from a sealed tube reaction of anthranil with cyclobutanone in the presence of mercuric sulphate,[2)] or *via* a Friedlander synthesis using cyclobutanone and *o*-aminobenzaldehyde.[3)] In this paper, we describe the synthesis and characterization of 1,2-dihydrocyclobuta[*c*]quinoline (V), a new heterocyclic analogue of naphthocyclobutene.

Irradiation of 4-methoxyquinolin-2(1H)-one (I) in a mixture of methanol and acetone (2: 3 v/v) under bubbling of ethylene by high pressure mercury lamp (Toshiba 400P) through a Pyrex filter afforded a 2+2 cycloadduct (II, mp 172.5—173.5°) in 90% yield as a sole isolable product. The adduct (II) was treated with potassium hydroxide in methanol at reflux to give 1,2-dihydrocyclobuta[*c*]quinolin-3(4H)-one (III, mp 223.5—224.5°) in a quantitative yield. Comparison of the spectral data of II and III with those of the cycloadducts of I with substituted olefins and their methanol elimination products[4)] confirmed correctness of the assigned structures. Chlorination of III (reflux in phosphorous oxychloride) led to 3-chloro-1,2-dihydrocyclobuta[*c*]quinoline (IV, mp 115.5—116.5°) in a quantitative yield. The structure of IV was established on the basis of combustion analysis,[5)] UV spectrum [λ_{max}^{MeOH} nm (log ε): 210 (4.60), 229.5 (4.67), 234 (4.68), 277.5 (3.63), 306 (3.60), and 319 (3.66)] which is quite similar to that of 2-chloro-4-methylquinoline [λ_{max}^{MeOH} nm (log ε): 208 (4.59), 229 (4.65), 277 (3.65), 304 (3.54), and 317.5 (3.59)], and finally of its pmr spectrum.[6)] Thus, the four methylene