were added to the residue to react with VMA and HVA at 65° in oil-bath for 15 minutes, and the solution was condensed to dryness under a stream of nitrogen. The residue was dissolved in 0.5 ml of *n*-hexane, 1 μl of which was injected into a Shimadzu 3AE gas chromatograph equipped with an electron capture detector and a 2% OV-17 column. Gas chromatograms of authentic sample and urine specimen from a patient with neuroblastoma are shown in Fig. 1. The peak height ratio of VMA or HVA to HMPE was proportional to the quantity injected from 0.5 to 5 pmoles. These peaks were identified by the use of Shimadzu LKB 9000 mass spectrometer coupled to a gas chromatograph under the same condition as described above. The mass spectrum obtained from the peak due to the derivative of VMA or HVA from patient urines showed the same pattern as the one from the authentic sample, demonstrating the absence of impurity in each peak. The parent peaks supported the structures of the derivatives as shown in Fig. 1.

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Chemical Structure of QA_{II}, One of the Covalently Bound Adducts of Carcinogenic 4-Nitroquinoline 1-Oxide with Nucleic Acid Bases of Cellular Nucleic Acids[1)]

The chemical structure of the quinoline-adenine adduct, QA_{II}, which was isolated from deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) extracted from the cells treated with carcinogenic 4-nitroquinoline 1-oxide was proposed to be either 3-(N^6-adenyl)-4-aminoquinoline 1-oxide or 3-(N^1-adenyl)-4-aminoquinoline 1-oxide.

It has been reported that the cellular deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were chemically modified by treatment of the cells with carcinogenic 4-nitroquinoline 1-oxide (4NQO)[2-7)] or with its reduced metabolite, 4-hydroxyaminoquinoline 1-oxide (4HAQO). The quinoline moiety of 4NQO was thereby bound through a covalent bond to the nucleic acid bases.[4,5)] Four distinct adducts have been isolated by paper chromatography after acid-hydrolysis of DNA extracted from the cells thus treated and three of them were also found in RNA extracted simultaneously.[4,7)] Such a base modification can be produced *in vitro* by treatment of DNA, RNA, or synthetic polynucleotides with 4HAQO with the help

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of its activating enzyme system.[8-10] Three of the adducts have been characterized and designated by Tada and Tada as "quinoline-adenine adduct II" (QA_{II}), "quinoline-guanine adduct I" (QG_{I}) and "quinoline-guanine adduct II" (QG_{II}), respectively.[7] This communication describes the chemical structure of QA_{II} which is the most stable among those so far isolated.

QA_{II} was prepared from polyriboadenylic acid that had been reacted with 4HAQO in the 4HAQO-activating enzyme system[10] and purified by paper chromatography[4] and recrystallization from water. About one milligram of the QA_{II} preparation thus obtained was sufficiently pure after chromatographic and spectroscopic examinations.

Proton magnetic resonance (PMR) spectra of QA_{II} and its hydrochloride were taken in dimethyl sulfoxide.[11] PMR (QA_{II}) δ: 8.59 (singlet, 2-H), 8.37 (doublet, 5-H), 7.60 (triplet, 6-H), 7.75 (triplet, 7-H), 8.53 (doublet, 8-H), 6.62 (4-NH_2), 8.15 (3-NH), 8.18 (singlet, 2- or 8-H of adenine), 8.12 (singlet, 8- or 2-H of adenine). PMR (QA_{II}·HCl) δ: 9.10 (singlet, 2-H), 8.67 (doublet, 5-H), 7.77 (triplet, 6-H). 8.07 (triplet, 7-H), 8.18 (doublet, 8-H), 8.86 (4-NH_2), 8.53 (singlet, 2- or 8-H of adenine), 8.42 (singlet, 8- or 2-H of adenine). PMR data, including the chemical shift and shape of each signal, indicate the presence of both an N-substituted adenine and a 3-substituted 4-aminoquinoline 1-oxide in the molecule. The structure assignment is based on the following characteristics. The signal of the proton-2 on the quinoline ring appeared as a singlet, indicating that a substituent was introduced into position-3 of the quinoline ring. With respect to signals due to all other protons of the quinoline ring, an almost identical spectrum was observed with those due to the corresponding hydrogens in 4-aminoquinoline 1-oxide and 3-anilino-4-aminoquinoline 1-oxide. Presence of the N-oxide group was evidenced by a characteristic lower field shift of the proton-8 resonance in QA_{II} and further confirmed by an unusual shift reversely to the higher field of the same proton resonance by the formation of its salt in the molecule.[12] Presence of one primary and one secondary amino groups was evidenced by observation of characteristic signals in the spectra of QA_{II} and its salt, which disappeared by the addition of deuterium oxide to the solution being examined. No reliable conclusion can be derived as to the presence or absence of another NH function. When the signals thus assigned were artificially erased from the spectrum, two singlets due to one proton each were left in the spectrum, which were assigned to two C–H protons of the adenine ring. This fact is unequivocal evidence for the idea that the binding of adenine ring with 4-aminoquinoline 1-oxide moiety should be bridged with one of the nitrogens but not with any carbons in the adenine moiety.

Then, the structure of QA_{II} was examined by mass spectrometry. It is known that the quinoline 1-oxides readily split the oxygen atom from the molecule during ionization by the electron impact to give a peak of *m/e* (M−16) in the spectrum.[13] The mass spectrum of QA_{II}, taken at 250° with the ionizing energy of 70 eV, showed an intense peak at *m/e* 277 at the highest mass region but the peak at *m/e* 261 (277−16) was missing in the spectrum. It should therefore be regarded that the molecular weight is 293. (277+16), even if the parent ion peak was not observed. The molecular weight of 293 exactly coincides the structure of adenyl-4-aminoquinoline 1-oxide ($C_{14}H_{11}ON_7$) which is proposed for QA_{II} from PMR spectroscopy.

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10) M. Tada and M. Tada, *Nature*, **255**, 510 (1975).
11) The signal assignment of spectra of 4-aminoquinoline 1-oxide and its 3-anilino derivative was performed using the corresponding deuterium-labeled derivatives, *i.e.*, 2-, 5-, and 8-monodeuterio ones. The signal assignment of QA_{II} spectrum was achieved by comparison with spectra of the above reference compounds with the help of spin-decoupling technique.
12) M. Ogata, H. Kano, and K. Tori, *Chem. Pharm. Bull.* (Tokyo), **11**, 1527 (1963); K. Tori, M. Ogata, and H. Kano, *ibid.*, **11**, 681 (1963).
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This conclusion was further supported by the fact that 3-anilino-4-aminoquinoline 1-oxide (mol. wt.=251) showed a peak of *m/e* 235 but not 251 in the highest mass region of the mass spectrum.

The pK_a values of QA_{II} were roughly estimated to be 9.5, 3.5, and 2.0 by the ultraviolet (UV) spectral change due to pH changes of the medium ranging from 1 to 14. The value of 9.5 can be assigned to the dissociation of NH proton in the imidazole of adenine moiety and those of 3.5 and 2.0 may be due to the basic nitrogens in 4-aminoquinoline 1-oxide and adenine moieties. These UV data support the structure of adenyl-4-aminoquinoline 1-oxide and, in addtion, suggest the idea that the quinoline moiety is bound to one of the nitrogens of pyrimidine moiety of adenine, *i.e.*, N^1, N^3 or N^6.

It is well known that the glycosidic bond in 3- and 7-substituted deoxyadenosines is even more unstable than in 7-substituted deoxyguanosine in DNA.[14] QA_{II} fragments were not appreciably split from the treated DNA in neutral aqueous solution at room temperature. Taking into account such a fairly stable character of the glycosidic bond involved, it turns out that QA_{II} can be formulated as either 3-(N^6-adenyl)-4-aminoquinoline 1-oxide (A) or 3-(N^1-adenyl)-4-aminoquinoline 1-oxide (B). Since QA_{II} was stable in alkaline media, the former may be the more probable structure[15] but the latter can not be completely eliminated.

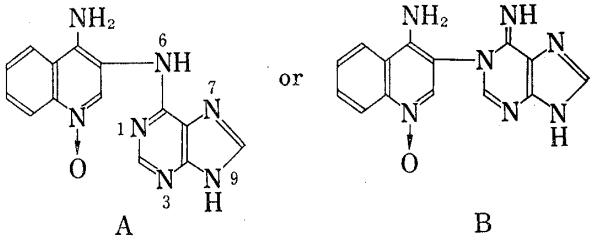

Chart 1. Proposed structure of QA_{II}

It is speculated that the reaction mechanism for production of QA_{II} involves the electrophilic attack of the reactive intermediate to the most nucleophilic center of adenine moiety, N^6 or N^1, the evidence for which will be published in a forthcoming paper.

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