benzene.[12] Among the minor products, 2,2′-dihydroxy-5,5′-dimethoxy-3,3′-di-*t*-butylbiphenyl (II) was identified by comparison of the retention time and *Rf* value with those of an authentic specimen.[11]

Monophenolic compounds are known to undergo nitrosation by nitrite under acidic conditions. Phenol,[13] *p*-cresol[14] and sesamol[15] are nitrosated at the *o*- or *p*-position. Tocopherol is oxidized to the corresponding quinone by nitrogen dioxide.[16] The profile of the reaction of BHA with nitrite was different from those of the above phenols. Ready transformation of BHA into the nitrophenol (I) by nitrite under mild acidic conditions prevented the formation of the nitrosamine in the reaction between dimethylamine and nitrite. Although I has no mutagenicity in rec-assay,[17] it might be metabolically transformed into toxic substances, such as hydroxylamine derivatives.

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A Molecular Orbital Study on the Approach of Hydride Ion to NAD^+ as a Coenzyme

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An *ab initio* molecular orbital study on the approach of a hydride ion (H^-) to NAD^+ as a coenzyme was performed. The nicotinamide ring (NA) of NAD^+ is attacked by H^- at the 4-position in an enzyme such as lactate dehydrogenase. It was found that the order of the electrophilic reactivity was 4-position>2-position>6-position, considering the total π electron densities and the frontier electron densities. Thus, it appears that the reactivity of the 4-position of NA of NAD^+ may be due to its electronic nature rather than to steric factors involving amino acid residues of the enzyme.

Keywords——MO; structure; molecular orbital; *ab initio*; hydride ion; NAD; NADH; nicotinamide; coenzyme; electronic structure

The mechanism of reduction of NAD^+ to NADH has been studied by many researchers. It has been shown that hydrogen is transferred to the nicotinamide ring (NA) of NAD^+ as a hydride ion in experiments using model compounds of NAD^+.[2–4] When NAD^+ is reduced by hydrosulfate, NADH is formed by the addition of H^- at the 4-position of NA.[5] In relation to the reactivity of the 4-position of NA, CN^- reacts with NAD^+ only at this position.[6] How-

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ever, $NaBH_4$ reduces the 1-propyl-3-carbamide pyridinium cation at the 2-position as well as the 4-position,[7] and this cation is a model compound of NAD^+. On the other hand, Dubb *et al.* have shown that base-catalyzed hydrogen exchange occurs at the 2- and 6-positions.[8] The proton at the 2-position exchanges much faster than that at the 6-position.[8] Thus, it is of interest to study the electrophilic reactivity of NA from a quantum chemical point of view.

NAD^+ bound to L-lactate dehydrogenase (LDH) or other enzymes is activated at the 4-position of NA,[9] which is attacked by a hydride ion from substrate L-lactate. Although the hydride ion of L-lactate attacks the 4-position of NA, the 2- and 6-positions of NA might escape attack only because of the steric effects of amino acid residues.[9] However, we considered that the 4-position of NA of NAD^+ might have the largest electrophilic reactivity for H^-. The H^- addition compound of NA (dihydronicotinamide ring) was shown to be planar by X-ray diffraction analysis,[10] and theoretically the planarity of the dihydronicotinamide ring was considered to be due to hyperconjugation.[11] Thus, calculations in which H^- approaches the 2-, 4- or 6-position of 1-hydronicotinamide, as shown in Fig. 1, from a direction perpendicular to the NA plane were carried out. Although eletronic distributions for the nicotinamide moiety of NAD^+ or NADH have been calculated, no previous work has been carried out on the problems of H^- attack on NAD^+.[12]

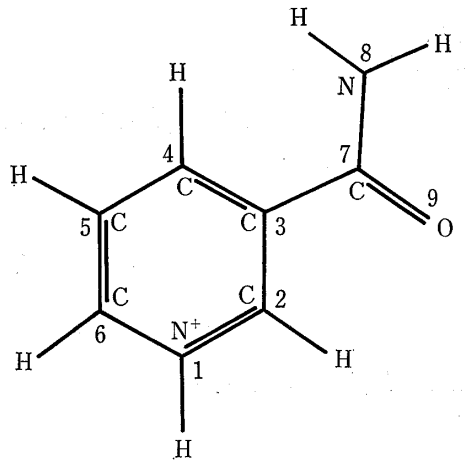

Fig. 1. Structure of 1-Hydronicotinamide

All the calculations were carried out within the closed shell LCAO-SCF approximation using the *ab initio* method. The GAUSSIAN 70 program was used.[13] The basis set was STO-3G.[14] Calculations were carried out using the M-180 computer at the Institute for Molecular Science. The geometry of 1-hydronicotinamide was obtained from the data of Voet.[15]

The results are shown in Table I. When H^- approached the 4-position of 1-hydronicotinamide, the interaction energy was largest. The next largest interaction energy was in the case of approach to the 2-position. These results are in good agreement with the experimental results mentioned above.[2-7] Table II shows the electron densities of 1-hydronicotinamide. The total π electron density at the 4-position (column (2)) was smallest. The electrostatic repulsion energy between H^- and 1-hydronicotinamide will be smallest near the 4-position. The frontier electron density (column (3)) was largest at the 4-position. Since the molecular orbital electron densities of the 2- and 6-positions above the lowest unoccupied MO (LUMO) were larger than that of the 4-position, the MO above LUMO will also play a significant role in nucleophilic attack on NA. The total electron density at the 2-position was smaller than

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Table I. Interaction Energies between 1-Hydronicotinamide and H^-

	(1)	(2)	(3)
1-Hydronicotinamide	—	−409.6212	—
1,2-Dihydronicotinamide	1.150	−409.1777	4.8
1,4-Dihydronicotinamide	1.138	−409.1854	0
1,6-Dihydronicotinamide	1.153	−409.1744	6.9

(1) Distance in Å between C^4 of the NA ring and H^-. H^- approaches from a direction perpendicular to the 1-hydronicotinamide ring.
The geometry of 1-hydronicotinamide did not change.
(2) Total energy in atomic units.
(3) Energy (kcal/mol) relative to 1,4-dihydronicotinamide.

Table II. Electron Densities of 1-Hydronicotinamide

	(1)	(2)	(3)	(4)
1	7.226	1.438	0.323	0.010
2	5.863 (0.830)	0.863	0.190	0.384
3	6.001	0.982	0.050	0.280
4	5.983 (0.870)	0.838	0.397	0.000
5	6.042 (0.864)	0.978	0.015	0.287
6	5.875 (0.846)	0.899	0.267	0.199
7	5.675	0.932	0.003	0.032
8	7.442	1.821	0.001	0.018
9	8.254	1.248	0.014	0.072

(1) Total electron densities. The total electron density of hydrogen is shown in parentheses.
(2) Total π electron densities.
(3) Orbital electron densities at the −0.040 a.u. energy level (LUMO).
(4) Orbital electron densities at the 0.011 a.u. energy level.

those at the 4-, 5- and 6-positions, and that at the 6-position was next smallest. These results are in agreement with experimental results on the base-catalyzed hydrogen-exchange reaction.[8]

The results in Table I show that H^- can be expected to attack the 4-position of 1-hydronicotinamide selectivly simply on the basis of the electronic character of NA.