MUTAGENICITY OF α-ACETOXY-DIALKYLNITROSAMINES: MODEL COMPOUNDS FOR AN ULTIMATE CARCINOGEN*1

A large number of metabolites retaining the nitrosamino moiety have been separated and characterized by Okada et al.10) from the urine of rats given dibutyl nitrosamine (DBN), N-butyl-N-(4-hydroxybutyl)nitrosamine (BB-N), and related compounds. These metabolites were oxidation products with hydroxy, oxo, and/or carboxy group, usually in the one alkyl chain formed by α- and β-oxidation, or by (α-1)- and/or by (α-2)-oxidation, while no metabolites hydroxylated at α-carbon atom could be detected. Several of these metabolites including N-butyl-N-(3-carboxypropyl)nitrosamine (BCPN), the principal urinary metabolite of BBN as well as of DBN, which was demonstrated by Hashimoto et al.6,7) to be the “proximate” form of these nitrosamines as a urinary bladder carcinogen, have been nonmutagenic by themselves as were the original nitrosamines. In view of the α-hydroxylation hypothesis2) concerning metabolic activation of dialkyl nitrosamines, several α-acetoxy-dialkyl nitrosamines related to DBN were synthesized and their mutagenic effects were examined by means of rapid microbial assay methods. This communication describes the first example of the mutagenic action on microorganisms of dialkyl nitrosamine derivatives without using any metabolic activation system.

N-Nitroso compounds used in the present experiment were N-butyl-N-(1-acetoxybutyl)nitrosamine (I), N-butyl-N-(acetoxy methyl)nitrosamine (IIa), N-sec-butyl-N-(acetoxy methyl)nitrosamine (IIa), and N-tert-butyl-N-(acetoxy methyl)nitrosamine (IVa). I was prepared according to the method described by Wiessler.11) IIa, IIIa, and IVa were obtained by refluxing acetic acid solution of the corresponding methoxymethyl compounds (IIb, IIIb, and IVb) which were synthesized using the procedure reported by Eiter et al.5)

As indicated in Table I, compounds Ia, IIa, and IIIa had a positive effect in the so-called “re-assay” method developed by Kada et al.,9) using Marburg strains of Bacillus subtilis 17A (rec+) and 45T (rec−). Salmonella typhimurium strain TA1535*2 was used, on the other hand, for quantitative testing for mutagenicity of the compounds as described by Ames et al.1) The results are given in Fig. 1, demonstrating a good linear relationship between the number of revertant colonies and the concentration of the test compounds. It was observed in these assay methods that the structure of the butyl chain in the N-butyl-N-(acetoxy methyl)nitrosamines profoundly affected the activity in the order of butyl (IIa) > sec-butyl (IIIa) > tert-butyl (IVa), the last

Table I. re-Assay of α-Acetoxy-dialkyl nitrosamines

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µmol/plate)</th>
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<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>I</td>
<td>+</td>
</tr>
<tr>
<td>IIa</td>
<td>+</td>
</tr>
<tr>
<td>IIIa</td>
<td>+</td>
</tr>
<tr>
<td>IVa</td>
<td>−</td>
</tr>
</tbody>
</table>

* More than 3 mm difference between lengths of inhibition zones with rec+ and rec− strains was judged as positive. N-Methyl-N-nitro-N-nitrosoguanidine was positive at the concentration of 1 µmol/plate. Formaldehyde and acetic acid, possible degradation products of the compounds, had no effect at various concentrations tested.

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*2 The compounds gave negative results for mutagenicity with S. typhimurium strains TA1536, TA1537, and TA1538.
being inactive. DBN, BBN, BCPN, and the methoxymethyl compounds (IIb and IVb) were found to be inactive in these tests.

The present results on the mutagenicity of \(\alpha\)-acetoxy-dialkyl-nitrosamines may strongly support the \(\alpha\)-hydroxylation hypothesis concerning the metabolic activation of dialkyl-nitrosamines leading to the ultimate alkylating species probably being a carbonium ion. Moreover, based on this hypothesis, the results could aptly explain the following previous findings: (1) Butylmethylnitrosamine\(^3\) and butylethynitrosamine\(^4\) are potent carcinogens, while the corresponding tert-butyl compounds\(^5,6\) are noncarcinogenic.

(2) N-Alkyl-N-(4-hydroxybutyl)nitrosamines (alkyl: methyl, ethyl, propyl, and butyl)\(^7\) selectively induced urinary bladder cancer in rats while N-tert-butyl derivative induced neither bladder tumors nor any tumors in other organs (unpublished result).

Further works relating to the mutagenicity and chemical reactivities of the \(\alpha\)-acetoxy-dialkynitrosamines are in progress.

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**Fig. 1. Mutation test with \(\alpha\)-acetoxy-dialkynitrosamines using *Salmonella typhimurium* strain TA1535**

The number of revertant colonies (his\(^0\)) on each plate was counted after 2 days of incubation at 37\(\text{o}\). All the compounds were added as 0.1 ml of dimethylsulfoxide solution.

**Concentration of the test compound (\(\mu\)mol/plate)**

**REFERENCES**