Studies on \( N \)-Nitroso Derivatives of \( N \)-Methylcarbamate Insecticides (I)

Properties of \( N \)-Nitroso Derivatives of 3-Methylphenyl \( N \)-Methylcarbamate, 3,4-Dimethylphenyl \( N \)-Methylcarbamate and Naphthyl \( N \)-Methylcarbamate

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N-methylcarbamate insecticides are used in place of organochlorine insecticides. The \( N \)-nitroso derivatives of 3-methylphenyl \( N \)-methylcarbamate (MTMC, Tsumacide), 3,4-dimethylphenyl \( N \)-methylcarbamate (MPMC, Meobal) and naphthyl \( N \)-methylcarbamate (NAC, Carbaryl), which are relatively much in the amounts of using in Japan, were crystallized, and their physico-chemical properties and stabilities were studied. It was found that these \( N \)-nitrosocarbamates were rather stable at room temperature in organic solvent in the dark but extremely unstable to light. Especially, 3-methylphenyl \( N \)-methyl-\( N \)-nitrosocarbamate (NO-MTMC) and 3,4-dimethylphenyl \( N \)-methyl-\( N \)-nitrosocarbamate (NO-MPMC) were completely decomposed when their ethanol solutions (10 mM) were exposed to sunlight through glass for 3 hr. Nitrosocarbamates were decomposed with increasing in the acidity of solution. When 20% dimethyl sulfoxide solution of NO-MTMC (10 mM) was kept at 37°C and pH 5.8 or 1.2 for 1 hr, it decreased to 93 or 67% of initial amounts, respectively.

1. Introduction

The formation of nitrosocarbaryl (naphthyl \( N \)-methyl-\( N \)-nitrosocarbamate, NO-NAC) by the interaction of carbaryl (naphthyl \( N \)-methylcarbamate, NAC), one of \( N \)-methylcarbamate insecticides, with sodium nitrite in acidic media was reported.\(^1\) These \( N \)-nitrosocarbamates derived from carbamate insecticides have been demonstrated to be potent mutagens.\(^2\)\(^\text{--}^5\) Moreover Eisenbrand et al.\(^6\) reported that NO-NAC induced polymorphic-cell sarcomas at the injection sites of Wistar rats by single sc dose (1000 mg of NO-NAC).

\( N \)-Methylcarbamate insecticides are widely used recently. Nitrite is contained in various kinds of vegetables and salted vegetables\(^7\) and is found almost everywhere in environment, such as in water, in sewage and in soil.\(^8\) Therefore there might be a possibility that \( N \)-methylcarbamate insecticides react with nitrite to form \( N \)-nitroso derivatives in the environment, especially when carbamate insecticides are sprayed. If the \( N \)-nitroso derivatives are stable, they will remain in environment and also in foods.

However, the properties of nitrosocarbamates were not fully reported except only a few plain

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\( \text{Scheme 1. Compounds tested} \)
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reports presented by Uchiyama et al.4) concerning the mutagenicity of nitrosocarbamates and by Benson and Gajan5) on nitrosation of NAC. In this paper, the physicochemical properties and the stabilities of N-nitroso derivatives of 3-methylphenyl N-methylcarbamate (MTMC, Tsumacide), 3,4-dimethylphenyl N-methylcarbamate (MPMC, Meobal) and NAC, which are relatively much in the amount of using in Japan, were studied (see Scheme 1).

2. Methods

2.1 Apparatus

i) MS spectrometer: JEOL JMS-0ISG-02

ii) IR spectrophotometer: JASCO Model-DS-403G

iii) Spectrophotometer: Shimadzu UV-200

iv) Melting point apparatus: Yamato Scientific Co., Ltd. Type MP-1

2.2 Reagents

i) MTMC, MPMC, and NAC were obtained from Sankyo Co. Ltd., however these were used after purification by recrystallization.

ii) Griess reagent: 1% sulfanilic acid and 0.1% N-1-naphthylethylenediamine dihydrochloride; both solutions were prepared in 30% acetic acid, stored at 4°C and mixed just before use (1:1).10)

iii) Silica gel: Kieselgel HF264+866

iv) Developing solvent: benzene-n-hexane-ethanol (10:10:1)

The other chemicals used were of special grade.

2.3 Thin-layer chromatography

Thin-layer plates were prepared in conventional manner with 250 µ layer of Kieselgel HF254+366. Five to ten micrograms of standard nitrosocarbamates and 20~30 µl aliquots of the sample solutions were spotted. After development with the solvent system described above in the dark, darkly colored spots were observed on the plate under UV light (254 and 366 nm). After Griess reagent was sprayed, the plate was irradiated under UV light (without filter) for 2~5 min, and red-violet spots, which appeared within this period, were observed.10)

Spots of carbamate insecticides could be detected only with short wave UV lamp (254 nm). Spots of nitrosocarbamates could be detected with both of long and short wave lamps (254 and 366 nm) and with Griess reagent.

2.4 Preparation of N-nitrosocarbamates

N-nitrosocarbamates were prepared in the similar manner as described for NO-NAC by Benson and Gajan.4)

Each carbamate insecticide (0.1 mol) was dissolved in glacial acetic acid (120~310 ml). To this solution, a solution of 34.5 g (0.5 mol) of sodium nitrite in 100 ml of water was added dropwise over 20 min with gentle stirring at room temperature. After the addition, the reaction vessel was stoppered and allowed to stand overnight at room temperature in the dark. The orange-yellow precipitate was separated out from the reaction mixture of NAC, and the yellow-orange oil was separated out from the reaction mixture of MTMC and from that of MPMC. When NAC was nitrosated, precipitate obtained was washed with water, and recrystallized from ether—petroleum ether in this experiment, instead of aqueous ethanol employed in Benson and Gajan’s procedure. When MTMC or MPMC was nitrosated, separated oil was extracted with ether, and the ether layer was washed with water and dried over anhydrous sodium sulfate. After evaporation of the solvent, the product was recrystallized with ether—petroleum ether. The yields of NO-MTMC, NO-MPMC, and NO-NAC were about 77, 86, and 82%, respectively.

In order to avoid the effect of light, all procedures were carried out in the dark as much as possible.

The chemical structures of these nitrosocarbamates were confirmed by elemental analysis, IR, NMR, and Mass spectral data (see 3.1).

2.5 Decomposition of NO-MTMC in aqueous solution in the dark

NO-MTMC was dissolved in 20% dimethyl sulfoxide in the concentration of 1.5 mM. The solution of NO-MTMC was divided into 5 tubes wrapped completely with aluminum foil. The solutions in 4 tubes among 5 were acidified appropriately by addition of different drops of concentrated hydrochloric acid or 10% hydrochloric acid, and 5 tubes were kept at 37°C or allowed to stand at room temperature for 1 hr. The pH value of each 5 solution was measured using a part of the solution during the incubation at 37°C or standing at room temperature. After the incubation or standing, 5 ml of each solution was extracted with equal volume of benzene.
The extract was dried with anhydrous sodium sulfate, and the optical density was measured. Two trials were carried out as to each extraction.  

2.6 Stability of N-nitrosocarbamates in organic solvents in the dark  
Nitrosocarbamates were dissolved in ethanol and benzene, and left at room temperature in the dark for the period shown in Table 3. The variations of optical density at 422 nm for NO-MTMC and NO-MPMC, and at 422.5 nm for NO-NAC, and absorption spectra from 350 to 450 nm were measured.  

2.7 Photodecomposition of N-nitrosocarbamates under sunlight  
N-Nitrosocarbamates were dissolved in ethanol (about 10 mM) in test tubes, and exposed to sunlight through glass for 3 hr (January~February, around at noon). Immediately after the exposure, absorption spectra of the solutions were measured at 350~450 nm and thin-layer chromatography (TLC) was carried out.  

3. Results and Discussion  
3.1 Physico-chemical properties of N-nitrosocarbamates  
The physico-chemical data of nitroso derivatives of MTMC, MPMC, and NAC were shown in Tables 1 and 2. NO-MTMC, NO-MPMC, and NO-NAC were recrystallized from etherpetroleum ether instead of the method employed by Benson et al. or by Uchiyama et al. The melting points of all carbamates were lowered by nitrosation (melting

<table>
<thead>
<tr>
<th>Table 1. Properties of N-Nitrosocarbamates (I)</th>
<th>Melting point, elemental analysis, infrared spectroscopy and mass spectrometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compd.</td>
<td>Mol. formula</td>
</tr>
<tr>
<td>--------</td>
<td>--------------</td>
</tr>
<tr>
<td>NO-MTMC</td>
<td>C₇H₁₀N₂O₄</td>
</tr>
<tr>
<td>NO-MPMC</td>
<td>C₈H₁₁N₂O₄</td>
</tr>
<tr>
<td>NO-NAC</td>
<td>C₅H₁₀N₂O₄</td>
</tr>
</tbody>
</table>

* All melting points are uncorrected.  
** KBr tablet.

<table>
<thead>
<tr>
<th>Table 2. Properties of N-Nitrosocarbamates (II)</th>
<th>Absorption maxima</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO-MTMC</td>
<td>NO-MPMC</td>
</tr>
<tr>
<td>λₓₓₓ (nm)</td>
<td>ε</td>
</tr>
<tr>
<td>Absorption in ethanol</td>
<td>237 (7900)</td>
</tr>
<tr>
<td>Absorption in dichloromethane</td>
<td>387.5 (93)</td>
</tr>
<tr>
<td>Absorption in benzene</td>
<td>388 (109)</td>
</tr>
</tbody>
</table>
Fig. 1. Absorption spectrum of 3,4-dimethylphenyl N-methyl-N-nitrosocarbamate (NO-MPMC)*
* 5.5 mM of NO-MPMC in benzene

points of MTMC, MPMC, and NAC were 76~77, 79~80, and 142°C, respectively.11)

The NO bands in the infrared spectrum of each nitrosocarbamate were confirmed by the comparison with that of corresponding carbamate insecticides. The IR data were agreed with those reported by Benson and Gajan concerning NO-NAC.°'

\[ \lambda_{\text{max}} \text{ and } \varepsilon \text{ of NO-MTMC, NO-MPMC, and NO-NAC were shown in Table 2. The absorption spectra of nitrosocarbamates showed triplet peaks in the neighborhood of 400 nm. The absorption spectrum of NO-MPMC at 350~450 nm was shown in Fig. 1.} \]

3.2 Stability of N-nitrosocarbamates

3.2.1 Decomposition of nitrosocarbamates in aqueous solutions in the dark.

Nitrosocarbamates were decomposed very rapidly under alkaline conditions like other N-nitrosamides and also were decomposed with increasing in the acidity of solution.

The decomposition of NO-MTMC in 20% dimethyl sulfoxide at various pH and 37°C or room temperature was shown in Fig. 2. When the pH of the solution was 1.2, NO-MTMC decreased to 67% of initial amount after 1 hr incubation at 37°C, and when the pH of the solution was 5.8 (the solution was not acidified with hydrochloric acid), NO-MTMC decreased to 93% after 1 hr incubation at 37°C.

In a stomach, nitrosocarbamates would decompose with increasing the acidity of contents. But their instability in a stomach dose not necessarily suggest the possibility of their safety for human health.

3.2.2 Stability of nitrosocarbamates in organic solvents in the dark

Benzene or ethanol solutions of nitrosocarbamates were left at room temperature in the dark. The decrease of optical densities at 422 nm for NO-MTMC and NO-MPMC, and at 422.5 nm for NO-NAC were shown in Table 3. After standing for 3 hr, the optical densities of ethanol solutions of nitrosocarbamates decreased slightly, but the changes of shapes of absorption spectra at 350~450 nm were not observed. No variation was observed in the absorptions of benzene solutions of nitrosocarbamates at 350~450 nm after standing even for 42 hr.

It appears that nitrosocarbamates were relatively stable in organic solvents in the dark, though they decomposed in aqueous media as described above.

3.2.3 Photodecomposition of nitrosocarbamates

Three nitrosocarbamates tested were extremely
Table 3. Stability of N-Nitrosocarbamates in Ethanol or Benzene Solutions at Room Temperature in the Dark

<table>
<thead>
<tr>
<th>Solution</th>
<th>Nitrosocarbamate</th>
<th>Concentration (mM)</th>
<th>Variation of O.D.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Start</td>
</tr>
<tr>
<td>Benzene</td>
<td>NO-MTMC</td>
<td>2.42</td>
<td>0.441</td>
</tr>
<tr>
<td></td>
<td>NO-MPMC</td>
<td>1.72</td>
<td>0.312</td>
</tr>
<tr>
<td></td>
<td>NO-NAC</td>
<td>1.60</td>
<td>0.276</td>
</tr>
<tr>
<td>Ethanol</td>
<td>NO-MTMC</td>
<td>2.16</td>
<td>0.259</td>
</tr>
<tr>
<td></td>
<td>NO-MPMC</td>
<td>3.22</td>
<td>0.399</td>
</tr>
<tr>
<td></td>
<td>NO-NAC</td>
<td>6.89</td>
<td>0.861</td>
</tr>
</tbody>
</table>

* Optical densities were measured at 422 nm for NO-MTMC and NO-MPMC, and at 422.5 nm for NO-NAC

![Absorption spectra](image)

Fig. 3. Absorption spectra, in ethanol, of 3-methylphenyl N-methyl-N-nitrosocarbamate (NO-MTMC) before and after exposure to sunlight for 3 hr
*In the concentration of 10 mM
**Sunlight through glass

unstable to light.

When the absorption spectrum of ethanol solution of NO-MTMC was measured and TLC of the solution was carried out immediately after the exposure to sunlight, the absorption spectrum was varied as shown in Fig. 3, and the spot of NO-MTMC disappeared on a thin-layer chromatogram (Fig. 4). But when the solution of NO-MTMC was allowed to stand after sunlight exposure, another spot, which was colored to red-violet by UV light irradiation after spraying Griess reagent, appeared besides the spots shown in Fig. 4.

By exposure to sunlight, NO-MPMC gave a almost similar result to NO-MTMC. The exposure of NO-NAC to sunlight gave 6~7 spots of degradation products which were detected under UV light (254 nm) on a thin-layer chromatogram. However, NO-NAC was a little more stable to sunlight compared with NO-
MTMC or NO-MPMC. After the exposure of NO-NAC to sunlight for 3 hr, NO-NAC was still detected on a thin-layer chromatogram.

In these experiments, nitrosocarbamates were exposed to sunlight through glass, but the degradation might further proceed when they were directly exposed to sunlight. It is desirable that nitrosocarbamates were dissolved in water or at least in aqueous organic solvent if an environmental condition was considered. But nitrosocarbamates were dissolved in ethanol in this experiment, because they were not dissolved enough in water and they decomposed slowly in water or in 20% dimethyl sulfoxide even in the dark.

Nitrosocarbamates might scarcely remain in foods because of their instability to sunlight, even if carbamate insecticides are nitrosated with nitrite in environment, such as in soil or in water for agriculture, when carbamate insecticides were sprayed.

Although the possibility of the accumulation or persistence of nitrosated carbamate in the environment could be ignored, N-nitroso formation from carbamate insecticides in a human stomach is still likely when carbamate insecticides are accidentally ingested during spraying.

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References