Communications to the Editor

[Chem. Pharm. Bull.]
[28(11)3447—3448(1980)]

Nitrosation of Ethyl Carbamate: Isolation of Ethyl N-Ethyl-N-nitrosocarbamate

A potent mutagen, ethyl N-ethyl-N-nitrosocarbamate, was isolated from the reaction of ethyl carbamate with NaNO_2 . A possible reaction mechanism was proposed.

Keywords—mutagen; ethyl carbamate; ethyl N-ethyl-N-nitrosocarbamate; nitrous acid; alkylating agent

Despite intensive study for the carcinogenesis of ethyl carbamate (1), controversy still remain.¹⁾ Part of our efforts in this area,²⁾ have involved attempts to explore mutagen formation from 1 under physiological conditions.

This paper is on the isolation and characterization of a mutagen formed by treatment of 1 with sodium nitrite in an acidic condition. The mutagen was confirmed as ethyl N-ethyl-N-nitrosocarbamate (2) and was tested the activity.

The reaction of 1 with NaNO_2 in an acidic medium gave a yellow solution. This fluid indicated the presence of a mutagen by preliminary bacterial assay. TLC analysis (E. Merck, Kieselgel 60 F₂₅₄, ether-hexane (1:1)) showed that the reaction product consisted of five major components. The mutagenic activity was found only on the spot with $R_f=0.78$.

The rate of mutagen formation was dependent on both the pH of the reaction media and the amount of NaNO_2 . The rate increased with increasing amount of NaNO_2 and with decreasing pH. No mutagen was formed at pH 4.5 or above.

A typical isolation method for 2 is as follows. A solution of NaNO_2 (34.5 g, 0.5 mol) in water (100 ml) was added dropwise with stirring to a solution of 1 (89 g, 1 mol) in 10% hydrochloric acid (300 ml) at 0°. The resulting mixture was extracted with hexane. The organic layer was washed with water and dried over anhydrous MgSO_4 . After removal of the solvent under normal pressure, the residue was distilled to give 2 (120 mg, 0.16%) as a yellow oil, bp 55–60° (20 mmHg, bath temp.) [bp 52.5–53.5° (5 mmHg)³⁾]. The spectral data and elemental analysis of the compound agreed with those of the authentic sample prepared by nitro-

Chart 1

- 1) I. Berenblum, D. Ben-Ishai, N. Haran-Ghera, A. Lapidot, E. Simon, and N. Trainin, *Biochem. Pharmacol.*, **2**, 168 (1959); J.A. Miller, J.W. Cramer, and E.C. Miller, *Cancer Res.*, **20**, 950 (1960); E. Boyland and R. Nery, *Biochem. J.*, **94**, 198 (1965); S.S. Mirvish, *Biochim. Biophys. Acta*, **93**, 673 (1964); *idem, ibid.*, **117**, 1 (1966); G.A. Dahl, J.A. Miller, and E.C. Miller, *Cancer Res.*, **38**, 3793 (1978).
- 2) a) K. Tatsumi, H. Yoshimura, Y. Kawazoe, T. Horiuchi, and H. Koga, *Chem. Pharm. Bull.*, **28**, 351 (1980); b) H. Koga, Y. Kawazoe, K. Tatsumi, and T. Horiuchi, *Mutation Res.*, **78**, 145 (1980).
- 3) A.L. Wilder and A.L. Meader, Jr., *J. Org. Chem.*, **13**, 763 (1948).

TABLE I. Comparison of the Mutagenic Activity of Ethyl N-Ethyl-N-nitrosocarbamate (**2**) and Methyl Methanesulfonate (MMS) in *E. coli* WP 2, *E. coli* WP 2 *uvrA* and *Salmonella typhimurium* TA100

Strain	Revertants/nmol	
	2	MMS
WP 2	23	0.42
WP 2 <i>uvrA</i>	16.5	0.12
TA 100	3	0.73

The numbers are results from linear-response curves.

sation of ethyl N-ethylcarbamate.³⁾ The amount of **2** isolated by this method probably do not truly reflect that actual yield since distillation for the isolation of analytically pure sample led unavoidably to losses of material.

Chart 1 depicts a possible reaction mechanism for the formation of **2**. The reaction proceeds *via* initial formation of ethyl N-nitrosocarbamate, subsequent coupling with **1** followed by N-nitrosation affords the tetrazene intermediate capable of undergoing bond reorganization to **2**. The hydrolysis of **2** could lead to the ethyldiazenol, which upon loss of nitrogen forms an alkylating agent.

Table I report the mutagenic activity of **2** in three tester strains, together with that of methyl methanesulfonate (MMS). Mutation assay in this report were carried out by the method described in our previous report.^{2b)} The data in Table I show **2** to be quite mutagenic.

Further investigations are now under progress.

Acknowledgement This investigation was supported in part by Grant-in-Aid for special project research from Ministry of Education, Science and Culture, Japan.

The competent technical assistance of Mr. K. Fukamachi and Mr. K. Morita is also gratefully acknowledged.

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Received July 12, 1980

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