Communications to the Editor

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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 F_{254}, ether–hexane (1 : 1)) showed that the reaction product consisted of five major components. The mutagenic activity was found only on the spot with $Rf=0.78$.

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-

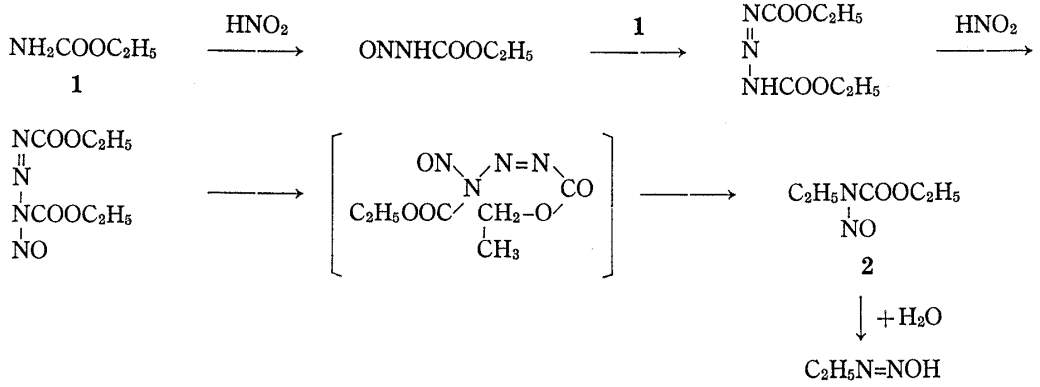

Chart 1

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 *uvrA*	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 truely 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.

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