N-β-DIMETHYLAMINOETHYLURETHYLANE AS A CHEMICAL MUTAGEN WITH POSSIBLE CARBAMOYLATING ACTION

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The carcinogenic [1], mutagenic [2] and carcinostatic [3] activity of urethane has been attributed to its metabolic activity by N-hydroxylation [3]. These effects are considered specific not only for urethane and its N-hydroxy derivative and alkylating and carboethoxylating action has been proposed as well as conversion to hydroxylamine, nitroxyl or hyponitrous acid [3]. The mutagenic activity of H_2NOH has been studied in detail [4]. In light of the results of Boche et al. [5], subsequent metabolic activation of N-hydroxyurethane may also be proposed by O-phosphorylation which permits electrophilic amination of DNA bases.

However, mutagenic activity was later found for carbamates [6], while the metabolic N-hydroxylation of urethane and other carbamates has not been confirmed [7]. We propose that the biological activity of carbamates may be attributed to their carbamoylating action as found for nitrosomethylureas [8]. In order to check this hypothesis, N-β-dimethylaminoethylurethylane (I) and its hydrochloride salt (III) were synthesized from β-aminoethyldimethylamine (I) [9]. The β-dimethylaminoethyl substituent was selected, in accord with our previous proposals [10], in order to provide a positively charged group in (II) upon the protonation of (II) to increase the local concentration of the reagent in the region of the gene target containing negatively charged phosphate groups.

Cytological and genetic studies on wheat have shown that urethylanes (II) and (III) possess specific aberrational activity (chromosome bridges are induced at a high frequency) and mutagenic activity (5-10 times greater than spontaneous mutation) [11].

$$Me_2NH + \left|\begin{array}{l}\diagdown\\ \diagup\end{array}\right.NH \xrightarrow[Me_2NH\cdot HCl]{} H_2N(CH_2)_2NMe_2 \xrightarrow{MeOCOCl}$$

$$\rightarrow \underset{(II)}{MeOCONH(CH_2)_2NMe_2} \xrightarrow{HCl} \underset{(III)}{MeOCONH(CH_2)_2\overset{+}{N}HMe_2Cl^-}$$

The possibility of the carbamoylating action of urethylane (III) was shown by its model reaction with aniline under mild conditions in water at 20°C and pH 7.

$$(III) \xrightarrow[H_2O]{PhNH_2} PhNHCONH(CH_2)_2NMe_2\cdot HCl \xleftarrow[2.\ HCl]{1.\ PhNCO} (I)$$

The structure of the carbamoylation product was confirmed by convergent synthesis.

EXPERIMENTAL

The PMR spectra were measured on a JNM-C-60 spectrometer at 60 MHz on HMDS and the mass spectra were taken on an MKh-1303 spectrometer at 30 eV.

β-Aminoethyldimethylamine (I). A solution of 43 g (1 mole) ethyleneimine, 90 g (2 moles) Me_2NH and 2 g (24.5 mmoles) $Me_2NH\cdot HCl$ in 300 ml methanol was maintained for 12 h at 20°C and then for 12 h at reflux. After evaporation, the residue was distilled over granulated KOH and then over sodium to give 35 g (40%) (I), bp 105-107°C, n_D^{20} 1.4270, d_4^{20} 0.8040 [9]. PMR spectrum in $CDCl_3$ (δ, ppm): 1.61 br. s (HN), 2.20 s (MeN), 2.31 m and 2.75 m [$(CH_2)_2$, A_2B_2]. Mass spectrum, m/z (rel. intensity, %): M^+ 88 (10), 58 (100), 44 (18), 30 (25).

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N-β-Dimethylaminoethylurethylane (II). A solution of 24 g (0.25 mole) methyl chloroformate in 50 ml abs. ether was added with stirring over 0.5 h to a solution of 22 g (0.25 mole) (I) and 30 g (0.3 mole) Et_3N in 150 ml abs. ether at from −5 to +5°C. The mixture was warmed to 20°C and stirred for 1 h. The solvent was evaporated and the precipitate was separated. Distillation of the residue in vacuum gave 29.2 g (80%) (II), bp 88-90°C (5 mm), n_D^{20} 1.4490, d_4^{20} 1.4505. PMR spectrum in $CDCl_3$ (δ, ppm, J, Hz): 2.19 s (MeN), 2.35 t (CH_2, ^3J = 6.3), 3.25 d. t (CH_2NH, $^3J_{HCCH} = {}^3J_{HCNH}$ = 6.3), 3.64 s (MeO), 6.00 br. s (HN). Mass spectrum: M^+ 146 (12), 115 (18), 72 (15), 90 (20), 59 (40), 58 (100), 44 (20). Found, %: C 49.35; H 9.57; N 19.29. $C_6H_{14}N_2O_2$. Calculated, %: C 49.29; H 9.65; N 19.16.

Hydrochloride Salt of N-β-Dimethylaminoethylurethylane (III). This salt was obtained in quantitative yield by treating (II) with dry HCl in ether, mp 139-140°C (from ethanol–ether). PMR spectrum in D_2O (δ, ppm, J, Hz): 2.80 s (MeN), 3.12 t and 4.10 t [$(CH_2)_2$, A_2B_2 spectrum, $^3J_{HH}$ = 6.3], 4.00 s (MeO).

N-β-Dimethylaminoethyl-N^1-phenylurea (IV). A mixture of 0.18 g (1 mmole) (III) and 1 g (11 mmoles) aniline in 5 ml phosphate buffer (pH 7) was maintained for 12 h at 20°C. After evaporation and azeotropic drying with benzene, the residue was extracted with chloroform. Removal of the solvent and crystallization from 10:1 ethanol–ether gave 0.12 g (50%) (IV), mp 169-172°C which was identified with a sample obtained by convergent synthesis from (I) and phenylisocyanate in $CHCl_3$ with subsequent treatment by dry HCl. PMR spectrum in D_2O (δ, ppm, J, Hz): 2.60 s (MeN), 3.00 t and 3.90 t [$(CH_2)_2$, A_2B_2 spectrum, ^3J = 6.5], 7.10-7.30 m (Ph).

CONCLUSION

N-β-Dimethylaminoethylurethylane and its hydrochloride salt were synthesized by acylation of β-aminoethyldimethylamine by chloromethyl formate. The mutagenic activity of these products is attributed to their carbamoylating action.

LITERATURE CITED

1. A. Nettleship and P. S. Henshaw, J. Natl. Inst. Cancer, 4, 309 (1943).
2. F. Oehlkers, Z. Induktive, Abstrammunge und Vererbungslehre, 81, 313 (1943); I. A. Rapoport, Byull. Éksp. Biol. Med., 23, 198 (1946); Dokl. Akad. Nauk SSSR, 59, 1183 (1948); Genetika, 130 (1965); M. Vogt, Experientia, 4, 68 (1948).
3. E. Boyland and R. Nery, Biochem. J., 94, 198 (1965); R. Nery, Biochem. J., 106, 1 (1968).
4. E. I. Budowsky, in: Progress in Nucleic Acid Research and Molecular Biology, Vol. 15, Academic Press, New York–London (1976), p. 125.
5. G. Boche, R. H. Sommerlade, and F. Bosold, Angew. Chem. Int. Ed. Engl., 25, 562 (1986).
6. Y. É. Bartoshevich, L. M. Filippova, and R. G. Kostyanovskii, Genetika, 147 (1966).
7. J. T. Marsden and J. W. Gorrod, in: Biological Oxidation of Nitrogen in Organic Molecules, J. W. Gorrod, L. A. Damani, and E. Horwood (eds.), Chemistry, Toxicology and Pharmacology (1985), Chap. 20.
8. A. M. Serebryanyi, M. A. Smotryaeva, K. E. Kruglyakova, and R. G. Kostyanovskii, Dokl. Akad. Nauk SSSR, Ser. Khim., 185, 847 (1969).
9. G. I. Braz, Dokl. Akad. Nauk SSSR, 55, 319 (1947); 59, 489 (1948).
10. É. I. Budovskii, M. A. Zalesskaya, V. P. Leshchinskaya, and R. G. Kostyanovskii, Bioorg. Khim., 11, 989 (1985).
11. T. V. Sal'nikova and O. I. Dosmailova, Genetika, 17, 1837 (1981).