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Syntheses of N-(ω-Acetoxyalkyl and ω-Carbomethoxyalkyl)-N-(α-acetoxyalkyl)nitrosamines, Model Compounds for Possible metabolically Activated N,N-Dialkylnitrosamines

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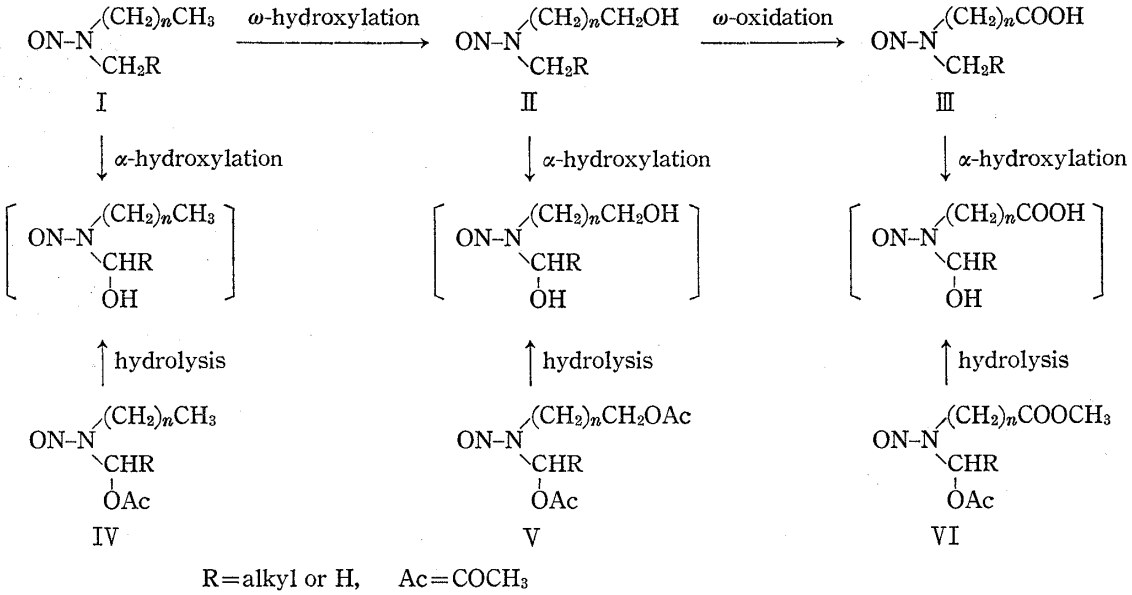
A series of new N-alkyl-N-(ω-acetoxyalkyl or ω-carbomethoxyalkyl)nitrosamines substituted at the α-carbon atom of the alkyl chain with an acetoxy group were synthesized as possible model compounds for metabolically activated N,N-dialkylnitrosamines.

Keywords——N-nitrosamine; N,N-dialkylnitrosamine; N-(ω-hydroxyalkyl or ω-carboxyalkyl)-N-(α-acetoxyalkyl)nitrosamine; model compound for active metabolite; metabolic activation; chemical carcinogen; direct mutagen

In the course of our studies on the correlation of chemical structure and *in vivo* metabolism with organotropic carcinogenicity of N,N-dialkylnitrosamines (I), with special reference to induction of urinary bladder tumors, the metabolic fate of a number of I and their primary metabolites, N-alkyl-N-(ω-hydroxyalkyl)nitrosamines (II) was elucidated in the rat.[2)]

According to these studies, principal urinary metabolites of II (n=1,2,3; R=H, CH_3, C_2H_5, C_3H_7) were identified as the corresponding carboxylic acids, N-alkyl-N-(ω-carboxyalkyl)nitrosamines (III), the urinary excretion of which amounted to more than 40% of the oral dose. N-Alkyl-N-(4-hydroxybutyl)nitrosamines (II, n=3) were demonstrated to induce



R=alkyl or H, Ac=$COCH_3$

Fig. 1. Possible Mechanism of Action of N,N-Dialkylnitrosamines and Their Primary Metabolites

1) Location: *Takada 3-41-8, Toshima-ku, Tokyo, 171, Japan.*

2) M. Okada, E. Suzuki, J. Aoki, M. Iiyoshi, and Y. Hashimoto, *Gann Monograph on Cancer Research*, 17, 161 (1975); M. Okada, "Fundamentals in Cancer Prevention," ed. by P.N. Magee, S. Takayama, T. Sugimura, and T. Matsushima, University of Tokyo Press, Tokyo, 1976, pp. 251—266; M. Okada and M. Ishidate, *Xenobiotica*, 7, 11 (1977).

selectively bladder cancer in rats,[3] and this organotropic effect on the urinary bladder of the N-nitroso compounds with a 4-hydroxybutyl chain was ascribed to their urinary principal metabolites with a 3-carboxypropyl group (III, $n=3$). N-Alkyl-N-(3-hydroxypropyl or 2-hydroxyethyl)nitrosamines (II, $n=2$ or 1) did not produce any bladder tumors in rats, although the N-nitrosamines with a 2-hydroxyethyl group were demonstrated to be potent hepatocarcinogens[4] while those with a 3-hydroxypropyl group were found to be non-carcinogenic.[3] It was reasonable, therefore, to conclude that the N-alkyl-N-(2-carboxyethyl or carboxymethyl)nitrosamines (III, $n=2$ or 1), major urinary metabolites of N-alkyl-N-(3-hydroxypropyl or 2-hydroxyethyl)nitrosamines (II, $n=2$ or 1), cannot be involved in the induction of bladder tumors in rats.[2]

In the mutagenesis test using *Salmonella typhimurium* strain TA 1535, on the other hand, N-alkyl-N-(3-carboxypropyl)nitrosamines (III, $n=3$) and N-alkyl-N-(2-carboxyethyl)nitrosamines (III, $n=2$) were demonstrated to be effective without metabolic activation by the S-9 mix, while N-alkyl-N-(carboxymethyl)nitrosamines (III, $n=1$) were found to be inactive with or without metabolic activation by the S-9 mix.[5]

The biological effects of most carcinogens and of many mutagens are mediated through the formation of reactive electrophilic reagents which react with nucleophilic groups in cellular macromolecules.[6] Thus the chemically stable II and III which were found to be carcinogenic[2] or mutagenic[5] should require further metabolic activation to become truly carcinogenic and mutagenic. As illustrated in Fig. 1, a probable pathway by which I are transformed into the biologically effective species is an enzyme-mediated hydroxylation at the α-carbon atom[7,8] giving unstable intermediates N-alkyl-N-(α-hydroxyalkyl)nitrosamines which spontaneously decompose to yield a common reactive alkylating species possibly an alkylcarbonium ion. With longer chain N,N-dialkylnitrosamines (I), a large number of metabolites with hydroxy, oxo, and/or carboxy group, usually in the one alkyl chain formed by ω, ω-1 or ω-2 or β[9]-oxidation were isolated and characterized from the urine of rats, while none of metabolites with an α-hydroxy group has so far been isolated.[2]

In the previous paper,[10] we reported the preparation of acetyl derivatives (IV) of several N-alkyl-N-(α-hydroxyalkyl)nitrosamines as model compounds for active metabolites of I. The α-acetoxy derivatives were stable compounds and underwent a non-enzymic hydrolysis or an enzymic cleavage by esterases to yield highly reactive intermediates similar to those formed from the parent compounds (I) by enzymic hydroxylation. This paper deals with the preparation of N-(ω-acetoxyalkyl)-N-(α-acetoxyalkyl)nitrosamines (V) and N-(ω-carbomethoxyalkyl)-N-(α-acetoxyalkyl)nitrosamines (VI). These α-acetoxy compounds may also be regarded as possible model compounds for metabolically activated N,N-dialkylnitrosamines (II and III).

Nine N-(ω-acetoxyalkyl and ω-carbomethoxyalkyl)-N-(α-acetoxyalkyl)nitrosamines listed in Table I[11] were synthesized according to either of three methods, A,B, and C, outlined

3) M. Okada, E. Suzuki, and Y. Hashimoto, *Gann*, **67**, 825 (1976).
4) M. Okada and Y. Hashimoto, *Gann*, **65**, 13 (1974).
5) M. Nagao, E. Suzuki, K. Yasuo, T. Yahagi, Y. Seino, T. Sugimura, and M. Okada, *Cancer Res.*, **37**, 399 (1977).
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10) M. Mochizuki, T. Anjo, and M. Okada, *Chem. Pharm. Bull.* (Tokyo), **26**, 3905 (1978).
11) The abbreviations used are: AEAMN, N-(2-acetoxyethyl)-N-(acetoxymethyl)nitrosamine; APAMN, N-(3-acetoxypropyl)-N-(acetoxymethyl)nitrosamine; ABAMN, N-(4-acetoxybutyl)-N-(acetoxymethyl)-nitrosamine; CMMAMN, N-(carbomethoxymethyl)-N-(acetoxymethyl)nitrosamine; CMEAMN, N-(2-carbomethoxyethyl)-N-(acetoxymethyl)nitrosamine; CMPAMN, N-(3-carbomethoxypropyl)-N-(acetoxymethyl)nitrosamine; CMMABN, N-(carbomethoxymethyl)-N-(1-acetoxybutyl)nitrosamine; CMEABN, N-(2-carbomethoxyethyl)-N-(1-acetoxybutyl)nitrosamine; CMPABN, N-(3-carbomethoxypropyl)-N-(1-acetoxybutyl)nitrosamine.

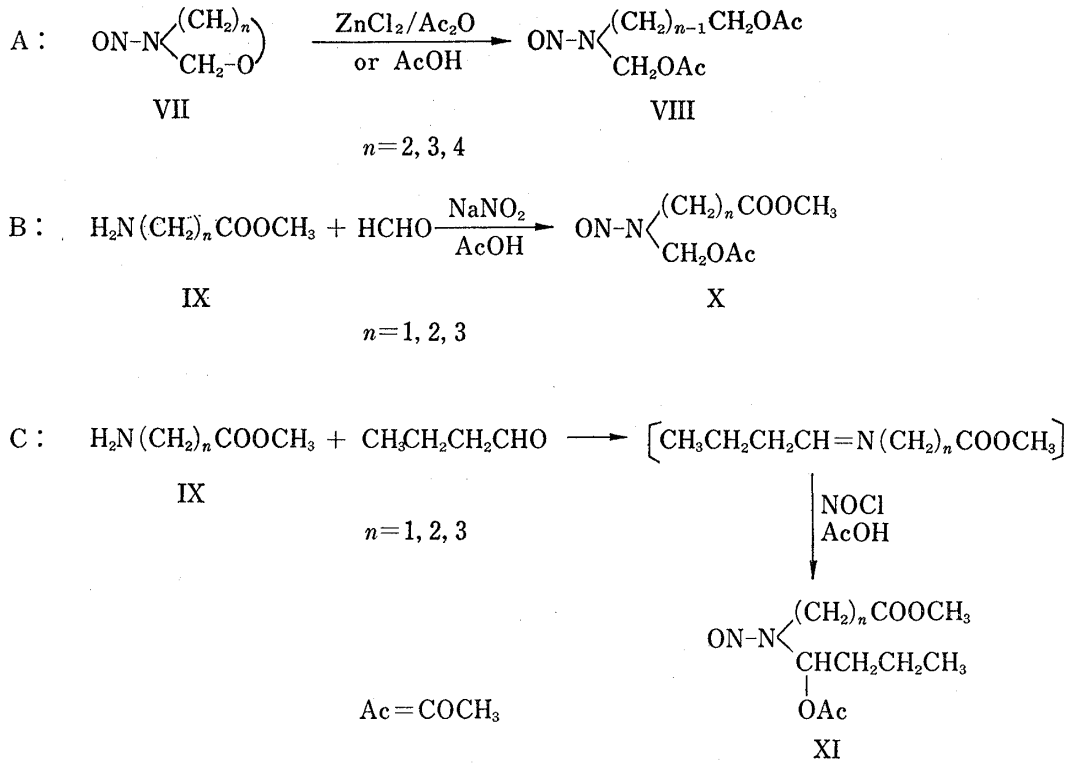

Fig. 2. Synthetic Methods for N-(ω-Acetoxyalkyl or ω-Carbomethoxyalkyl)-N-(α-acetoxyalkyl)nitrosamines

TABLE I. N-(ω-Acetoxyalkyl or ω-Carbomethoxyalkyl)-N-(α-acetoxyalkyl)nitrosamines

Compound[11]	ON-N<R_1 R_2		Synthetic method	Yield (%)	Formula	Analysis (%) Calcd. (Found)		
	R_1	R_2				C	H	N
AEAMN	CH_2CH_2OAc[a]	CH_2OAc	A	3	$C_7H_{12}N_2O_5$	41.17 (41.11	5.92 5.85	13.72 13.53)
APAMN	$CH_2CH_2CH_2OAc$	CH_2OAc	A	24	$C_8H_{14}N_2O_5$	44.03 (44.10	6.47 6.50	12.84 13.00)
ABAMN	$CH_2CH_2CH_2CH_2OAc$	CH_2OAc	A	9	$C_9H_{16}N_2O_5$	46.54 (46.34	6.94 6.97	12.06 11.76)
CMMAMN	CH_2COOCH_3	CH_2OAc	B	5	$C_6H_{10}N_2O_5$	37.90 (38.02	5.30 5.27	14.73 14.69)
CMEAMN	$CH_2CH_2COOCH_3$	CH_2OAc	B	5	$C_7H_{12}N_2O_5$	41.17 (41.15	5.92 6.00	13.72 13.48)
CMPAMN	$CH_2CH_2CH_2COOCH_3$	CH_2OAc	B	8	$C_8H_{14}N_2O_5$	44.03 (43.73	6.47 6.43	12.84 12.97)
CMMABN	CH_2COOCH_3	$CHCH_2CH_2CH_3$ (OAc)	C	3	$C_9H_{16}N_2O_5$	46.54 (47.01	6.94 7.08	12.06 12.21)
CMEABN	$CH_2CH_2COOCH_3$	$CHCH_2CH_2CH_3$ (OAc)	C	8	$C_{10}H_{18}N_2O_5$	48.77 (48.99	7.37 7.55	11.38 11.34)
CMPABN	$CH_2CH_2CH_2COOCH_3$	$CHCH_2CH_2CH_3$ (OAc)	C	10	$C_{11}H_{20}N_2O_5$	50.75 (51.15	7.75 7.98	10.76 10.69)

a) $Ac=COCH_3$.

in Fig. 2, in order to examine their mutagenic and carcinogenic effects. They are consisted of three N-(ω-acetoxyalkyl)-N-(acetoxymethyl)nitrosamines (VIII; AEAMN, APAMN, ABAMN), three N-(ω-carbomethoxyalkyl)-N-(acetoxymethyl)nitrosamines (X; CMMAMN, CMEAMN, CMPAMN), and three N-(ω-carbomethoxyalkyl)-N-(1-acetoxybutyl)nitrosamines (XI; CMMABN, CMEABN, CMPABN).

The first three compounds (VIII, n=2,3,4) were synthesized by method A, in which starting materials, cyclic N-nitroso compounds (VII), were obtained in fairly good yields according to the method reported by Eiter, *et al.*[12] The cyclic N-nitroso compounds (VII) (n=2,3,4) were treated with acetic anhydride and zinc chloride at −10° or with acetic acid under refluxing[10] to give the corresponding N-(ω-acetoxyalkyl)-N-(acetoxymethyl)nitrosamines (VIII). The second three compounds (X) (n=1,2,3) with carbomethoxyalkyl chain were prepared by method B described by Roller, *et al.*[10,13] using methyl esters (IX) of ω-amino acids, formaldehyde and sodium nitrite. The last three compounds (XI) (n=1,2,3) having ω-carbomethoxyalkyl chain and 1-acetoxybutyl group, on the other hand, were synthesized by method C of Wiessler[14] using IX, butyraldehyde and nitrosyl chloride as nitrosating agent. Yields and data of elemental analyses of these 9 new compounds are given in Table I. The yields were low but satisfactory for our present purpose of examining mutagenic effects of these model compounds for metabolically activated N-nitrosamines.

Ultraviolet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectral data of these compounds are given in Table II. Their UV spectra showed two characteristic absorption bands at 230—232 and 366—372 nm, the first maximum being more distinct than the second which exhibited, on the other hand, a bathochromic shift of about 20 nm as compared with N-nitrosamines with no acetoxy group at the α-carbon atom.[15,16] A similar shift, however, was observed previously[10] with N,N-dialkylnitrosamines (I) substituted at the α-carbon atom with an acetoxy group. The NMR spectra in deuteriochloroform except for those of XI showed two sets of signals indicating mixtures of the (E)-and (Z)-isomers.[17] As reported previously,[10] (E)- and (Z)-isomers of N-butyl-N-(1-acetoxybutyl)nitrosamine were also indistinguishable by its NMR spectrum. By NMR integration, the approximate isomer ratio was determined and indicated in the table.

Table II. UV, IR, and NMR Spectral Data

Compound[11]	UV λ_{max}^{EtOH} nm (ε)		IR ν_{max} cm^{-1}		NMR[a] (δ)						
					Ratio (%)		NCH_2O			$COCH_3$	CO_2CH_3
			C=O	N=O	E	Z	E	Z			
AEAMN	231(6200)	372(82)	1745	1485	85	15	6.25	5.42	2.15(α)	2.05(ω)	
APAMN	231(7000)	372(88)	1740	1480	87	13	6.20	5.37	2.14(α)	2.06(ω)	
ABAMN	232(6600)	372(81)	1740	1475	86	14	6.18	5.36	2.12(α)	2.03(ω)	
CMMAMN	230(6300)	369(92)	1755	1480	93	7	6.27	5.40	2.12		3.71
CMEAMN	230(6600)	371(84)	1740	1480	90	10	6.26	5.40	2.13		3.69
CMPAMN	232(7400)	372(100)	1740	1480	86	14	6.17	5.37	2.13		3.69
CMMABN[b]	230(6300)	366(76)	1755	1470			7.12[c]		2.08		3.70
CMEABN[b]	231(6600)	369(80)	1740	1470			7.05[c]		2.09		3.69
CMPABN[b]	232(6800)	369(75)	1740	1465			7.05[c]		2.09		3.69

a) Determined in 10% $CDCl_3$ solution after standing for about 20 min. Data concerning only protons at the carbon atom with an acetoxy group adjacent to the nitrogen atom and those of the acetyl and the carbomethoxy groups were indicated.
b) (E) and (Z) conformers were indistinguishable by their NMR spectra obtained in the present work.
c) NCHO.

12) K. Eiter, K.-F. Hebenbrock, and H.-J. Kabbe, *Ann. Chem.*, **765**, 55 (1972).
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16) M. Okada, E. Suzuki, and M. Iiyoshi, *Chem. Pharm. Bull.* (Tokyo), **26**, 3891, 3909 (1978).
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Mutagenic or DNA-modifying effects of the new 9 N-(ω-acetoxyalkyl or ω-carbomethoxyalkyl)-N-(α-acetoxyalkyl)nitrosamines synthesized in the present work were tested using *Salmonella typhimurium* strain TA 1535 and other bacterial strains, and by the *rec*-assay.[18] All the compounds were shown to be more or less effective in these assays. In the mutagenicity test, they were found to be active without metabolic activation. Details of the structure–activity relationships of these possible model compounds for metabolically activated N,N-dialkylnitrosamines will be the subject of another paper.

Experimental[19]

N-(2-Acetoxyethyl)-N-(acetoxymethyl)nitrosamine (AEAMN) (VIII, $n=2$)——A solution of N-nitroso-1,3-oxazolidine (VII, $n=2$) (1 g) prepared by the method reported previously[12] in acetic acid (10 ml) was heated under refluxing for 2 hr. The solvent was removed under reduced pressure and an oily residue was purified by silica gel column chromatography using ether as eluting solvent to give AEAMN and unchanged starting material (540 mg).

N-(3-Acetoxypropyl)-N-(acetoxymethyl)nitrosamine (APAMN) (VIII, $n=3$)——N-Nitroso-tetrahydro-1,3-oxazine (VII, $n=3$) (5 g) synthesized according to the procedure described by Eiter, *et al.*[12] was dissolved in acetic anhydride (30 ml) and the solution was cooled to $-10°$. To this solution was added freshly fused $ZnCl_2$ (1.85 g) and the mixture was stirred for 4 hr at $-10°$ and then at 0° overnight. The mixture was poured into ice-water and stirred for 3 hr and then extracted with CH_2Cl_2. The organic layer was washed with water and dried over anhyd.Na_2SO_4. Evaporation of the solvent under reduced pressure afforded an oily residue which was subjected to fractional distillation to give APAMN, bp 122—123° (0.42 mmHg), and 3-(hydroxymethyloxy)-1-propanol diacetate as a by-product.

N-(4-Acetoxybutyl)-N-(acetoxymethyl)nitrosamine (ABAMN) (VIII, $n=4$)——N-Nitroso-hexahydro-1,3-oxazepine (VII, $n=4$) (3.7 g) obtained according to the method reported earlier[12] was treated in the same manner as described above with APAMN to yield ABAMN, bp 150° (3 mmHg), and 4-(hydroxymethyloxy)-1-butanol diacetate as a by-product.

N-(Carbomethoxymethyl)-N-(acetoxymethyl)nitrosamine (CMMAMN) (X, $n=1$)——Glycine methyl ester hydrochloride (1.27 g) and 37% formaldehyde aqueous solution (1 ml) in glacial acetic acid (30 ml) was treated with $NaNO_2$ under ice-cooling in the similar way as reported.[13] The product was purified by silica gel column chromatography using hexane–ether–CH_2Cl_2 (3 : 2 : 5) as eluting solvent to give CMMAMN.

N-(Carbomethoxyethyl)-N-(acetoxymethyl)nitrosamine (CMEAMN) (X, $n=2$)——β-Alanine (4.5 g) was esterified in the usual way by refluxing its methanolic solution containing HCl. An oily residue obtained by evaporating the solvent under reduced pressure was treated in the same way as described above with CMMAMN. The product was purified by silica gel column chromatography with hexane–ether (1 : 1).

N-(Carbomethoxypropyl)-N-(acetoxymethyl)nitrosamine (CMPAMN) (X, $n=3$)——γ-Aminobutyric acid (4 g) was converted into the methyl ester which was treated in the same manner as described above. The product was purified by silica gel column chromatography using hexane–ether–CH_2Cl_2 (3 : 1 : 1) as solvent to afford CMPAMN.

N-(Carbomethoxymethyl)-N-(1-acetoxybutyl)nitrosamine (CMMABN) (XI, $n=1$)——To a solution of glycine methyl ester hydrochloride (3.1 g, 25 mmol) in acetic acid (45 ml) was added a solution of potassium acetate in acetic acid (5 g/25 ml). The mixture was stirred well to precipitate KCl formed and CH_2Cl_2 (15 ml) was added. After cooling the mixture to 0°, butyraldehyde (2.25 ml) in CH_2Cl_2 (10 ml) was added dropwise. Then the mixture solution was cooled to about $-10°$, and nitrosyl chloride (25 mmol) in CH_2Cl_2 was dropwise added. Stirring of the reaction mixture was made for 30 min at about the same temperature and then it was left to warm to room temperature. Water was added and the resulting solution was extracted with CH_2Cl_2. The organic layer was washed with water and dried over anhyd.Na_2SO_4. After evaporation of the solvent, an oily residue was purified by silica gel column chromatography using hexane–ether–CH_2Cl_2 (6 : 3 : 2) as eluting solvent to afford CMMABN.

N-(Carbomethoxyethyl)-N-(1-acetoxybutyl)nitrosamine (CMEABN) (XI, $n=2$)——β-Alanine (2.2 g) was esterified in the usual way and the methyl ester hydrochloride was treated without further purification

18) E. Suzuki, M. Mochizuki, T. Anjo, Y. Akaike, and M. Okada, Proceedings of the 36th Annual Meeting of the Japanese Cancer Association, Tokyo, 1977, p. 45.

19) UV spectra were measured in EtOH solution. IR spectra were obtained in liquid film with a Hitachi EPI-S2 spectrometer. NMR spectra were taken in deuteriochloroform at 60 MHz, using a Hitachi R-20A spectrometer. Chemical shifts are expressed in δ (parts per million) with tetramethylsilane as internal standard. Silica gel used for column chromatography were either Kieselgel 60, less than 230 mesh, or Kieselgel 60, 230—400 mesh, of E. Merck AG.

in the similar way as described above to give CMEABN. It was purified by silica gel column chromatography with the same solvent system used above with CMMABN.

N-(Carbomethoxypropyl)-N-(1-acetoxybutyl)nitrosamine (CMPABN) (XI, $n=3$)——γ-Aminobutyric acid methyl ester hydrochloride prepared from γ-aminobutyric acid (5 g) in the usual way was treated in the same way as described above to yield CMPABN which was purified by silica gel column chromatography with the same solvent described above.

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