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Syntheses of N-Alkyl-N-(α-acetoxyalkyl)nitrosamines, Model Compounds for metabolically Activated N,N-Dialkylnitrosamines

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Syntheses of a series of new N,N-dialkylnitrosamines monosubstituted at the α-carbon with an acetoxy group were reported. They were prepared by the procedures reported earlier, but a new procedure was provided, according to which N-alkyl-N-(methoxymethyl)-nitrosamines were converted into N-alkyl-N-(acetoxymethyl)nitrosamines in low but satisfactory yield by refluxing them in acetic acid. The (*E*)–(*Z*) conformer ratios of these compounds determined by nuclear magnetic resonance measurement were given.

Keywords——N,N-dialkylnitrosamine; N-alkyl-N-(α-acetoxyalkyl)nitrosamine; N-alkyl-N-(acetoxymethyl)nitrosamine; N-alkyl-N-(methoxymethyl)nitrosamine; metabolic activation; direct mutagen; chemical carcinogen; conformational analysis; (*E*)-(*Z*) conformer

It is now generally accepted that aliphatic N-nitrosamines, potent experimental carcinogens,[2–4)] require metabolic activation to become truly carcinogenic and mutagenic. Probable pathway by which these compounds are transformed into the biologically effective species is illustrated in Fig. 1 for N,N-dialkylnitrosamines. By enzyme-mediated hydroxylation at the α-carbon atom,[2,5)] N,N-dialkylnitrosamines (I) are metabolized to unstable intermediates N-alkyl-N-(α-hydroxyalkyl)nitrosamines (II) which spontaneously decompose to yield a common reactive alkylating species possibly an alkylcarbonium ion (RCH_2^+) and an aldehyde. Because of their high reactivity, none of these intermediates (II) has so far been isolated as such and their role in carcinogenesis and mutagenesis has not been directly investigated. This paper reports the preparation of acetyl derivatives (III) of several N-alkyl-N-(α-hydroxyalkyl)nitrosamines (II) principally consisting of N-alkyl-N-(hydroxymethyl)nitrosamines. These α-acetoxy derivatives are stable compounds and undergo a non-enzymic hydrolysis or an enzymic cleavage by esterases[6)] to yield intermediates (II) similar to those formed from the parent N,N-dialkylnitrosamines (I) by enzymic hydroxylation (Fig. 1).

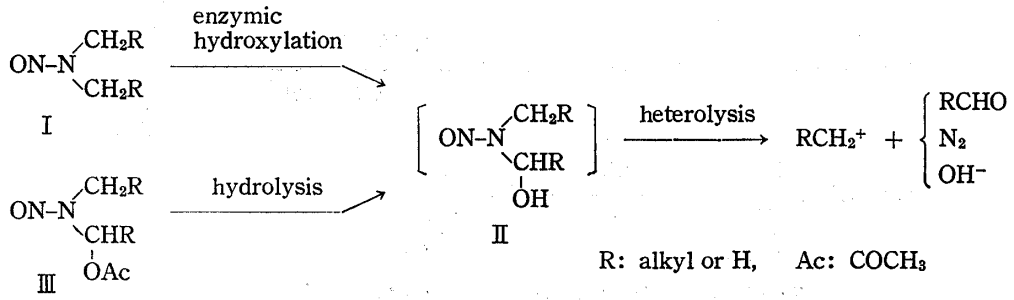

Fig. 1. Probable Mechanism of Action of N,N-Dialkylnitrosamines

1) Location: *Takada 3-41-8, Toshima-ku, Tokyo, 171, Japan.*
2) H. Druckrey, R. Preussmann, S. Ivankovic, and D. Schmähl, *Z. Krebsforsch. Klin. Onkol.*, **69**, 103 (1967).
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Nine N-alkyl-N-(α-acetoxyalkyl)nitrosamines[7] listed in Table I were synthesized according to either of three methods, A, B, and C, outlined in Fig. 2, in order to examine their carcinogenic and mutagenic effects. Method A reported by Roller, *et al.*[6] which was devised for the synthesis of MAMN by modifying the procedure developed by Eiter, *et al.*,[8] was first used in the present work. Thus all the α-acetoxy nitrosamines were obtained by this method in low but satisfactory yield, while method B described by Wiessler[9] was utilized only for the preparation of BABN.

Table I. N-Alkyl-N-(α-acetoxyalkyl)nitrosamines

Compound[7]	ON-N$\langle^{R_1}_{R_2}$		Synthetic method[b]	Yield (%)	bp (mmHg) (°C)	Formula	Analysis (%) Calcd. (Found)		
	R_1	R_2					C	H	N
MAMN	CH_3	CH_2OAc[a]	A	18	100—101.5 (22)[c]	$C_4H_8N_2O_3$	—	—	—
EAMN	C_2H_5	CH_2OAc	A	21	85—86 (6.5)	$C_5H_{10}N_2O_3$	41.09 (41.09	6.90 6.95	19.17 19.03)
PAMN	C_3H_7	CH_2OAc	A	20	81—83 (4.5)	$C_6H_{12}N_2O_3$	44.99 (44.74	7.55 7.59	17.49 17.53)
i-PAMN	$CH(CH_3)_2$	CH_2OAc	A	11	98—99 (13)	$C_6H_{12}N_2O_3$	44.99 (45.38	7.55 7.57	17.49 17.57)
BAMN	C_4H_9	CH_2OAc	A C	25 39	91—92 (4)	$C_7H_{14}N_2O_3$	48.26 (48.11	8.10 8.24	16.08 16.08)
i-BAMN	$CH_2CH(CH_3)_2$	CH_2OAc	A C	6 18	76—77 (2.5)	$C_7H_{14}N_2O_3$	48.26 (48.42	8.10 8.17	16.08 16.15)
s-BAMN	$CH(CH_3)C_2H_5$	CH_2OAc	A C	18 22	86—87 (3.5)	$C_7H_{14}N_2O_3$	48.26 (48.25	8.10 8.15	16.08 15.81)
t-BAMN	$C(CH_3)_3$	CH_2OAc	A C	15 14	84.5—85 (2)[d]	$C_7H_{14}N_2O_3$	—	—	—
BABN	C_4H_9	$CH(OAc)C_3H_7$	A B	18 15	89—93 (0.45)	$C_{10}H_{20}N_2O_3$	55.53 (55.73	9.32 9.47	12.95 12.92)

a) Ac=$COCH_3$.
b) *cf.* Fig. 2.
c) Reported[6]: 113 (32).
d) Reported[9]: 58 (0.05).

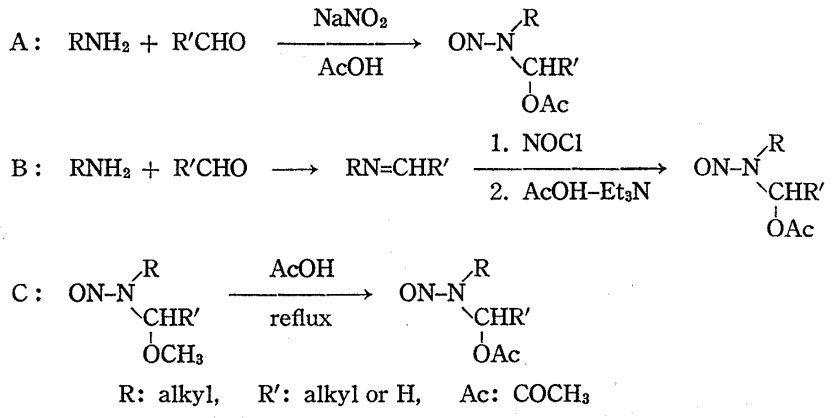

Fig. 2. Synthetic Methods for N-Alkyl-N-(α-acetoxyalkyl)nitrosamines

7) The abbreviations used are: MAMN, N-methyl-N-(acetoxymethyl)nitrosamine; EAMN, N-ethyl-N-(acetoxymethyl)nitrosamine; PAMN, N-propyl-N-(acetoxymethyl)nitrosamine; i-PAMN, N-isopropyl-N-(acetoxymethyl)nitrosamine; BAMN, N-butyl-N-(acetoxymethyl)nitrosamine; i-BAMN, N-isobutyl-N-(acetoxymethyl)nitrosamine; *s*-BAMN, N-*sec*-butyl-N-(acetoxymethyl)nitrosamine; *t*-BAMN, N-*tert*-butyl-N-(acetoxymethyl)nitrosamine; BABN, N-butyl-N-(1-acetoxybutyl)nitrosamine.
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In method C which was developed in the present work, N-alkyl-N-(methoxymethyl)nitrosamines prepared according to the procedure reported previously[8] were refluxed in acetic acid for about 1.5—6 hr to give the corresponding N-alkyl-N-(acetoxymethyl)nitrosamines in satisfactory yield. This method was used for the syntheses of BAMN, i-BAMN, *s*-BAMN, and *t*-BAMN.[10] In case of the method A, we frequently found difficulty in obtaining the desired product in pure state owing to the formation of unidentified by-products which were hard to separate from the desired product, while the method C gave a mixture consisting of only the desired product and the starting material which were easily separable by fractional distillation. Recently, similar procedures to the method C for the preparation of α-acetoxy and related derivatives of N,N-dialkylnitrosamines were reported.[11]

Ultraviolet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectral data of the N-alkyl-N-(α-acetoxyalkyl)nitrosamines synthesized are given in Table II. They showed two characteristic absorption bands at 228—235 and 367—381 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 having no acetoxy group at the α-carbon atom.[4,12] Their NMR spectra in deuteriochloroform showed two sets of signals indicating mixtures of the (*E*)- and (*Z*)-conformers, similarly to those of N,N-dialkylnitrosamines not substituted at the α-carbon atom with an acetoxy group.[13] By NMR integration, the approximate conformer ratio was determined and indicated in the table.

Table II. UV, IR, and NMR Spectral Data

Compound[7]	UV λ_{max} nm (ε)		IR ν_{max} cm^{-1}		NMR[a] (δ)					
					Ratio (%)		NCH_2O		$COCH_3$	
			C=O	N=O	*E*	*Z*	*E*	*Z*	*E*	*Z*
MAMN[6]	228 (6600)	367 (87)	1755	1475	96	4	6.20	5.38	2.15	2.07
EAMN	231 (6900)	372 (82)	1750	1475	86	14	6.19	5.39	2.14	2.06
PAMN	232 (6600)	372 (87)	1750	1480	84	16	6.20	5.39	2.14	2.06
i-PAMN	233 (6700)	375 (90)	1750	1480	65	35	6.19	5.36	2.12	2.04
BAMN	232.5 (7000)	372 (86)	1750	1480	90	10	6.18	5.38	2.14	2.05
i-BAMN	233.5 (7400)	372 (97)	1755	1485	81	19	6.18	5.37	2.13	2.04
s-BAMN	234 (5900)	375 (74)	1755	1485	58	42	6.19	5.35	2.12	2.05
t-BAMN	235 (6700)	381 (74)	1750	1480	3	97	6.26	5.45	2.12	2.03
BABN[b]	232 (6700)	369 (72)	1750	1470			7.02[c]		2.09	

a) Determined in 10% $CDCl_3$ solution after standing for about 20 min. Data concerning only protons at the carbon atom with the acetoxy group and those of the acetyl group were indicated.
b) (*E*)- and (*Z*)-isomers of BABN were indistinguishable on the basis of its NMR spectrum obtained in the present work.
c) $NC\underset{|}{H}O$.

Mutagenic or DNA-modifying effects of the 9 N-alkyl-N-(α-acetoxyalkyl)nitrosamines synthesized in the present work were investigated using *Salmonella typhimurium* strain TA 1535 and by the *rec*-assay respectively.[10,14] All the compounds except *t*-BAMN were shown

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to be effective in these assays. In the mutagenesis test, they were found as expected to be active without metabolic activation by the S-9 mix. Details of the structure–activity relationships of these model compounds for metabolically activated N,N-dialkylnitrosamines will be reported elsewhere.

Experimental[15]

Preparation of N-Alkyl-N-(α-acetoxyalkyl)nitrosamines by Method A——MAMN, EAMN, PAMN, i-PAMN, BAMN, i-BAMN, *s*-BAMN, *t*-BAMN, and BABN were prepared according to the procedure reported earlier[6] using corresponding alkylamines and aldehydes. Yields, bp, and data of elemental analysis are given in Table I. UV, IR, and NMR spectral data are shown in Table II.

Preparation of BABN by Method B——BABN was obtained by this method according to the procedure described previously[9] using butylamine and butyraldehyde. Yield, bp, and elemental analytical data are given in Table I, and UV, IR, and NMR spectral data are indicated in Table II.

Preparation of N-Alkyl-N-(acetoxymethyl)nitrosamines by Method C——(i) Preparation of N-Alkyl-N-(methoxymethyl)nitrosamines (Alkyl: Butyl, Isobutyl, *sec*-Butyl and *tert*-Butyl): These compounds were prepared by the method of Either, *et al.*[8]: N-butyl-N-(methoxymethyl)nitrosamine, yield 77%, bp 91—93° (16 mmHg), *Anal.* Calcd. for $C_6H_{14}N_2O_2$: C, 49.30; H, 9.65; N, 19.16. Found: C, 49.28; H, 9.64; N, 19.34. IR ν_{max} cm^{-1}: 1465 (N=O). By NMR integration, the compound exists as a mixture of (*E*):(*Z*) isomers (approximately 94: 6). NMR (10% solution in $CDCl_3$) δ: 5.46 (s, (*E*)-$NC\underline{H}_2O$), 4.83 (s, (*Z*)-$NC\underline{H}_2O$), 3.31 (s, (*E*)-$OC\underline{H}_3$), 3.23 (s, (*Z*)-$OC\underline{H}_3$). N-Isobutyl-N-(methoxymethyl)nitrosamine, yield 63%, bp 74—75° (11 mmHg). *Anal.* Calcd. for $C_6H_{14}N_2O_2$: C, 49.30; H, 9.65; N, 19.16. Found: C, 49.04; H, 9.91; N, 19.40. IR ν_{max} cm^{-1}: 1470 (N=O), NMR (10% solution in $CDCl_3$) δ: 5.48 (s, (*E*)-$NC\underline{H}_2O$), 4.83 (s, (*Z*)-$NC\underline{H}_2O$), 3.31 (s, (*E*)-$OC\underline{H}_3$), 3.24 (s, (*Z*)-$OC\underline{H}_3$), (*E*): (*Z*) = 91: 9. N-*sec*-Butyl-N-(methoxymethyl)nitrosamine, yield 41%, bp 75.5—76.5° (14 mmHg). *Anal.* Calcd. for $C_6H_{14}N_2O_2$: C, 49.30; H, 9.65; N, 19.16. Found: C, 49.06; H, 9.72; N, 19.40. IR ν_{max} cm^{-1}: 1460 (N=O), NMR (10% solution in $CDCl_3$) δ: 5.47 (s, (*E*)-$NC\underline{H}_2O$), 4.79 (s, (*Z*)-$NC\underline{H}_2O$), 3.35 (s, (*E*)-$OC\underline{H}_3$), 3.28 (s, (*Z*)-$OC\underline{H}_3$), (*E*): (*Z*) = 77: 23. N-*tert*-Butyl-N-(methoxymethyl)-nitrosamine, yield 7%, bp 94—96° (37 mmHg) (reported[9]: bp 52° (0.05 mmHg)), IR ν_{max} cm^{-1}: 1470 (N=O), NMR (10% solution in $CDCl_3$) δ: 5.52 (s, (*E*)-$NC\underline{H}_2O$), 4.85 (s, (*Z*)-$NC\underline{H}_2O$), 3.32 (s, (*E*)-$OC\underline{H}_3$), 3.26 (s, (*Z*)-$OC\underline{H}_3$), (*E*): (*Z*) = 11: 89.

(ii) Conversion of N-Alkyl-N-(methoxymethyl)nitrosamines to the Corresponding N-Alkyl-N-(acetoxymethyl)nitrosamines: The above N-alkyl-N-(methoxymethyl)nitrosamines were refluxed in AcOH for 340 (butyl), 150 (isobutyl), 90 (*sec*-butyl) and 135 min (*tert*-butyl). After evaporation of the solvent under reduced pressure, the oily residues were subjected to fractional distillation. If necessary, further purification was made by column chromatography of silica gel 60 (E. Merck AG) using mixtures of hexane, ether and CH_2Cl_2. Yields are given in Table I. Starting material was recovered in 44, 73, 66 and 11% with butyl, isobutyl, *sec*-butyl and *tert*-butyl derivative respectively. Prolongation of the reaction period of time did not give rise to any increase in the yield.

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15) 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.