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Synthesis of N-Nitrosamino Aldehydes, Metabolic Intermediates possibly involved in the Induction of Tumors in Rats by N-Butyl-N-(ω-hydroxyalkyl)nitrosamines

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Three N-nitrosamino aldehydes, N-butyl-N-(ω-formylalkyl)nitrosamines, which are necessary metabolic intermediates situated between N-butyl-N-(ω-hydroxyalkyl)-nitrosamines and N-butyl-N-(ω-carboxyalkyl)nitrosamines were synthesized for the purpose of investigating their mutagenic effects.

Keywords——N-nitrosamine; N-nitrosamino aldehyde; N-butyl-N-(ω-formylalkyl)-nitrosamine; pyridinum chlorochromate; chromium trioxide–pyridine complex; metabolic intermediate; carcinogenicity; mutagenicity

The metabolic fate of N-butyl-N-(ω-hydroxyalkyl)nitrosamines (I) and their analogs in the rat was elucidated in a series of our studies[2] on the correlation of chemical structure and *in vivo* metabolism with organotropic carcinogenicity of N,N-dialkylnitrosamines. According to these studies, principal urinary metabolites of I (n=1,2,3) were identified as the corresponding carboxylic acids, N-butyl-N-(ω-carboxyalkyl)nitrosamines (III: n=1,2,3), the urinary excretion of which amounted to more than 40% of the oral dose of the N-nitrosamino alcohols (I).

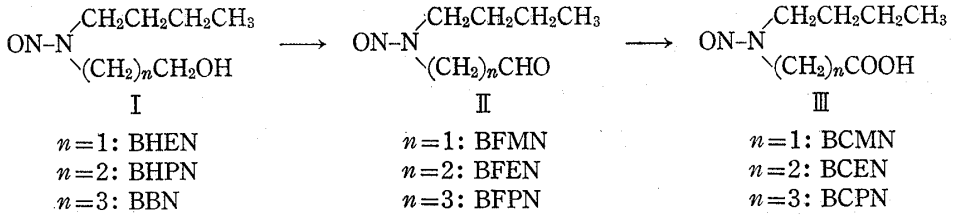

Chart 1

N-Butyl-N-(4-hydroxybutyl)nitrosamine (butyl-butanol-(4)-nitrosamine, BBN: I, n=3)[3] and its major urinary metabolite N-butyl-N-(3-carboxypropyl)nitrosamine (BCPN: III, n=3)[4] were demonstrated to be potent selective bladder carcinogens in rats. N-Butyl-N-(3-hydroxypropyl)nitrosamine (BHPN: I, n=2) did not produce any tumors,[5,6] while N-butyl-N-(2-hydroxyethyl)nitrosamine (BHEN: I, n=1) induced hepatoma as well as papilloma in the esophagus.[5] Principal urinary metabolite of BHEN, N-butyl-N-(carboxymethyl)nitrosamine (BCMN: III, n=1) was found to be non-carcinogenic.[6] Although carcinogenic effect of N-butyl-N-(2-carboxyethyl)nitrosamine (BCEN: III, n=2), the major urinary metabolite of

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).
3) H. Druckrey, R.Preussmann, S. Ivankovic, C.H. Schmidt, H.D. Mennel, and K.W. Stahl, *Z. Krebsforsch. Klin. Onkol.*, **66**, 280 (1964).
4) Y. Hashimoto, E. Suzuki, and M. Okada, *Gann*, **63**, 637 (1972).
5) M. Okada and Y. Hashimoto, *Gann*, **65**, 13 (1974).
6) M. Okada, E. Suzuki, and Y. Hashimoto, *Gann*, **67**, 825 (1976).

BHPN, was not examined, it was reasonably presumed that BCEN should be non-carcinogenic.[2]

In the mutagenesis test using *Salmonella typhimurium* strain TA 1535, on the other hand, N-butyl-N-(ω-hydroxyalkyl)nitrosamines (I)(BHEN, BHPN, BBN) were demonstrated to be effective with metabolic activation by the S-9 mix, while BCEN and BCPN were effective without metabolic activation and BCMN was found to be inactive with or without metabolic activation.[7]

The N-nitrosamino aldehydes(II) are necessary metabolic intermediates situated between the alcohols (I) and the carboxylic acids (III) (Chart 1). In particular, it has been proposed that N-butyl-N-(3-formylpropyl)nitrosamine (BFPN: II, $n=3$) may be a critical intermediate in the induction of bladder cancer in rats by N,N-dibutylnitrosamine and BBN.[8] This paper deals with the preparation of three N-nitrosamino aldehydes (II), N-butyl-N-(formylmethyl)-nitrosamine (BFMN; $n=1$), N-butyl-N-(2-formylethyl)nitrosamine (BFEN; $n=2$) and BFPN, in order to investigate their biological effects in comparison with those of I and III. Recently, synthesis of several N-nitrosamino aldehydes including BFPN has been reported.[9]

The oxidation of the N-nitrosamino alcohols (I)[10] to the aldehydes (II) was carried out first by using pyridinium chlorochromate.[11] By this oxidizing agent, however, fairly good yield (46%) was obtained only with BFEN. BBN provided BFPN in a satisfactory yield (32%) with the aid of an improved procedure for oxidations with the chromium trioxide-pyridine complex.[12] Treatment of BHEN with pyridinium chlorochromate, on the other hand, did not give the desired BFMN, being the greater part of the starting material recovered unchanged. Moreover, oxidation of BHEN with chromium trioxide–pyridine complex using the improved procedure gave N-methyl-N-butylnitrosamine as a major product. The preparation of BFMN was finally achieved according to the scheme indicated in Chart 2. Thus, N-butyl-N-(formylmethyl)amine diethyl acetal (V) prepared from chloroacetaldehyde diethyl acetal (IV) and butylamine was nitrosated with sodium nitrite and acetic acid yielding BFMN diethyl acetal (VI) which was deacetalized to afford BFMN. Treatment of VI as well as BFMN with 2,4-dinitrophenylhydrazine gave the identical hydrazone (VII).

As reported recently,[9] the N-nitrosamino aldehydes synthesized in the present work were also relatively stable compounds. The most unstable compound BFMN did not produce

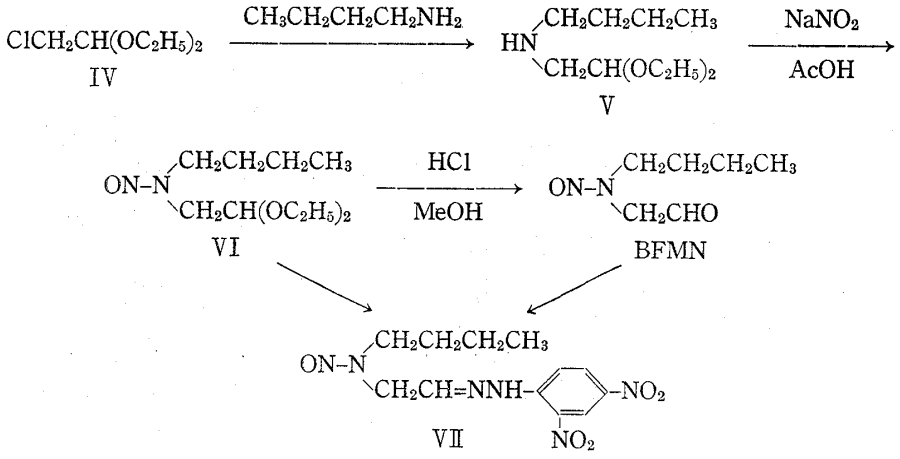

Chart 2

7) M. Nagao, E. Suzuki, K. Yasuo, T. Yahagi, Y. Seino, T. Sugimura, and M. Okada, *Cancer Res.*, 37, 399 (1977).
8) R. Schoental, *Brit. J. Cancer*, **28**, 436 (1973).
9) S.S. Hecht, C.B. Chen, and D. Hoffmann, *Tetrahedron Lett.*, **1976**, 593.
10) M. Okada, E. Suzuki, and M. Iiyoshi, *Chem. Pharm. Bull.* (Tokyo), **26**, 3891 (1978).
11) E.J. Corey and J.W. Suggs, *Tetrahedron Lett.*, **1975**, 2647.
12) R. Ratcliffe and R. Rodehorst, *J. Org. Chem.*, **35**, 4000 (1970).

Table I. N-Butyl-N-(ω-formylalkyl)nitrosamines

Compound	ON–N(C4H9)(CH2)nCHO	n	Yield (%)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
BFMN[a]		1	72[b]	$C_{10}H_{22}N_2O_3$	55.02	10.16	12.83	55.12	10.14	12.82
BFEN		2	46	$C_7H_{14}N_2O_2$	53.14	8.92	17.71	53.35	8.98	17.61
BFPN		3	32	$C_8H_{16}N_2O_2$	55.79	9.36	16.27	55.89	9.33	16.16

a) As diethyl acetal (VI).
b) Yield of nitrosation.

detectable impurities when stored in methylene chloride under nitrogen and at −20° for several months or more.

Ultraviolet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectral properties of the three N-nitrosamino aldehydes are given in Table II and III. The NMR spectra showed two sets of signals indicating mixtures of *E*- and *Z*-isomers. The approximate ratio *E*/*Z* as determined by NMR[13] was indicated in the Table.

Table II. UV and IR Spectral Properties of N-Butyl-N-(ω-formylalkyl)nitrosamines

Compound	UV $\lambda_{max}^{95\%\ EtOH}$ nm (ε)		IR ν_{max}^{film} cm^{-1} CHO	N=O
BFMN	236 (6900)	352 (86)	2710, 1735	1450
BFEN	234.5(7300)	351.5(94)	2730, 1725	1455
BFPN	234 (7400)	351.5(89)	2730, 1724	1457

Table III. NMR Spectral Data[a] of N-Butyl-N-(ω-formylalkyl)nitrosamines

Compound	n	Ratio (%) E	Z	Chemical shifts, δ ppm >N–CH2(CH2)2CH3 E	Z	>N–(CH2)nCHO E	Z	–CH2CHO E	Z	–CHO E	Z
BFMN	1	10	90	3.5—3.8	4.23 (t, J=7.0)	4.97	4.19 (s)			9.79	9.40 (s)
BFEN	2	30	70	3.3—3.8	4.18 (t, J=7.0)	4.37	3.79 (t, J=7.0)	3.06	2.74 (t, J=7.0)	9.89	9.76 (s)
BFPN	3	50	50	3.55	4.13 (t, J=7.5)	4.13	3.58 (t, J=7.5)	2.47	(t, J=7.0)	9.77—9.82	(m)

a) Determined in 5% $CDCl_3$ solution after standing for about 10 min. *E*-isomer was defined as oxygen atom of the nitroso group and the formylalkyl chain in *trans* position.

The three N-nitrosamino aldehydes were found to be mutagenic on *Salmonella typhimurium* strain TA 1535 with metabolic activation by the S-9 mix.,[14] similarly to the N-nitrosamino alcohols (I).[7] It is worthy of note that spontaneous decomposition product(s) of BFMN which has not been characterized yet showed a very potent mutagenic effect without metabolic activation. Details of the biological activities of these necessary metabolic intermediates situated between the alcohols (I) and the carboxylic acids (III) will be the subject of another paper.

13) G.J. Karabatsos and R.A. Taller, *J. Am. Chem. Soc.*, **86**, 4373 (1964).
14) E. Suzuki, Y. Akaike, and M. Okada, Abstracts of Papers, The 6th Annual Meeting of the Environmental Mutagen Society of Japan, Osaka, November, 1977, p. 24.

Experimental[15]

N-Butyl-N-(2-formylethyl)nitrosamine (BFEN) (II: $n=2$)——To a suspension of pyridinium chlorochromate (970 mg) in CH_2Cl_2 (10 ml) was rapidly added BHPN[10] (480 mg) and the mixture was allowed to stand at room temperature for 2.5 hr. The black reaction mixture was diluted with anhydrous ether (50 ml), the solvent was decanted, and the black solid was washed with ether. The combined ether solution was filtered through Florisil and the solvent was evaporated at reduced pressure. The residue (394 mg) was submitted to column chromatography on silica gel (20 g) with elution by hexane–ether–CH_2Cl_2 (4: 3: 2) to give BFEN (220 mg) as a pale yellow oil. Yield and data of elemental analysis are given in Table I. UV and IR spectral properties and NMR spectral data are indicated in Table II and III, respectively.

N-Butyl-N-(3-formylpropyl)nitrosamine (BFPN) (II: $n=3$)——Chromium trioxide (6.0 g) was added to a magnetically stirred solution of anhyd. pyridine (9.5 ml) in CH_2Cl_2 (150 ml), and the solution was stirred for 15 min at room temperature. A solution of BBN[10] (1.7 g) in CH_2Cl_2 (50 ml) was then added in one portion. After stirring an additional 15 min at room temperature, the solution was decanted from a tarry, black deposit, which was washed with ether. The combined organic solutions were washed successively with 5% NaOH, 5% HCl, 5% $NaHCO_3$ and saturated NaCl solution, and dried over anhyd. Na_2SO_4. Evaporation of the solvent at reduced pressure afforded a residue (980 mg) which was submitted to column chromatography on silica gel (50 g) with elution by hexane–ether–CH_2Cl_2 (4: 3: 2). BFPN (550 mg) was obtained as a pale yellow oil. Yield and elemental analytical data are given in Table I, and UV and IR spectral properties are shown in Table II. NMR spectral data are indicated in Table III.

N-Butyl-N-(formylmethyl)nitrosamine (BFMN) (II: $n=1$)——(i) N-Butyl-N-(formylmethyl)amine Diethyl Acetal (V): A mixture of chloroacetaldehyde diethyl acetal (IV) (15.2 g) and butylamine (25.2 g) was refluxed for 5 hr. After removal of butylamine by distillation *in vacuo*, cooled 40% NaOH (50 ml) was added to the residue to give two layers. The upper layer was extracted with ether and the organic layer was washed with saturated NaCl solution and dried over anhyd. Na_2SO_4. The solvent was evaporated and the oily residue was subjected to fractional distillation at reduced pressure to give two fractions: fraction 1 (IV), bp 57.5—59° (23 mmHg), 6.6 g; fraction 2 (V), bp 100—104° (23 mmHg), 5.6 g.

(ii) N-Butyl-N-(formylmethyl)nitrosamine Diethyl Acetal (VI): A mixture of V (5.6 g), $NaNO_2$ (2.6 g) in water (7 ml), and AcOH (1.8 ml) was warmed at 60° for 30 min. The reaction mixture was poured into water (50 ml) and then extracted with ether. The organic layer was washed with 5% $NaHCO_3$ and water, and dried over anhyd. Na_2SO_4. Evaporation of the solvent afforded an oily residue which was distilled at reduced pressure to give VI (4.65 g), bp 115—118° (6 mmHg). Yield and data of elemental analysis are given in Table I.

(iii) N-Butyl-N-(formylmethyl)nitrosamine (BFMN): To a solution of VI (1.044 g) in MeOH (2.4 ml) was added 6 N HCl (4.8 ml) and the mixture was allowed to stand at room temperature for 30 min. Ether (300 ml) was added to the mixture and the organic layer was washed with 5% $NaHCO_3$ and water, and dried over anhyd. Na_2SO_4. Evaporation of the solvent below 25° at reduced pressure gave an oily residue (750 mg) which was chromatographed on a column of silica gel (30 g) by eluting with hexane–ether–CH_2Cl_2 (4: 3: 2) to afford BFMN (450 mg). UV and IR spectral properties of BFMN are given in Table II, and its NMR spectral data are indicated in Table III.

(iv) 2,4-Dinitrophenylhydrazone of BFMN (VII): a) To a solution of VI (300 mg) in EtOH (6 ml) was added 12 ml of 2,4-dinitrophenylhydrazine solution (2,4-dinitrophenylhydrazine 400 mg, 95% H_2SO_4 2 ml, H_2O 3 ml, EtOH 10 ml) and the mixture was allowed to stand at roon temperature. A yellow crystalline precipitate was filtered and washed with 50% EtOH and then subjected to column chromatography on silica gel (20 g) with elution by hexane–ether–CH_2Cl_2 (4: 3: 2). After recrystallization from MeOH VII (358 mg) was obtained as yellow tablets. mp 97°. *Anal.* Calcd. for $C_{12}H_{16}N_6O_5$: C, 44.44; H, 4.97; N, 25.92. Found: C, 44.16; H, 4.84; N, 25.76.

b) BFMN (40 mg) was treated with 2,4-dinitrophenylhydrazine solution in the same way as described above to give yellow tablets (37 mg), mp 98°, after recrystallization from MeOH. The melting point of the mixture with the sample prepared from VI as described above showed no depression.

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15) Melting points were determined on a micro hot-stage apparatus and are uncorrected. UV spectra were measured in 95% 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 an internal standard. The coupling constants (J) are expressed in cycles per second: s, singlet; triplet; m, multiplet. For column chromatography Kieselgel 60, 230—400 mesh (E. Merck AG.) was used.