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Syntheses of N-Alkyl-N-(ω-carboxyalkyl)nitrosamines Related to N-Butyl-N-(3-carboxypropyl)nitrosamine, Principal Urinary Metabolite of a Potent Bladder Carcinogen N-Butyl-N-(4-hydroxybutyl)nitrosamine

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Fourteen N-alkyl-N-(ω-carboxyalkyl)nitrosamines related to N-butyl-N-(3-carboxypropyl)nitrosamine, principal urinary metabolite of a potent bladder carcinogen N-butyl-N-(4-hydroxybutyl)nitrosamine, were prepared for the purpose of mainly characterizing urinary metabolites and in part testing their carcinogenic effects in rats. They were N-alkyl-N-(3-carboxypropyl)nitrosamines, N-alkyl-N-(2-carboxyethyl)nitrosamines, and N-alkyl-N-(carboxymethyl)nitrosamines. They were mostly prepared by the permanganate oxidation of the corresponding N-alkyl-N-(ω-hydroxyalkyl)nitrosamines.

Keywords——N-nitrosamine; N-alkyl-N-(ω-carboxyalkyl)nitrosamine; N-alkyl-N-(3-carboxypropyl)nitrosamine; permanganate oxidation; urinary metabolite; bladder carcinogen; organospecific carcinogenicity

N-Butyl-N-(4-hydroxybutyl)nitrosamine (butyl-butanol-(4)-nitrosamine:BBN) is known to induce urinary bladder tumors selectively when administered orally to rats[2] and mice.[3] The principal urinary metabolite of BBN as well as of N,N-dibutylnitrosamine, which was demonstrated to induce not only bladder tumors but also tumors of the liver and the esophagus

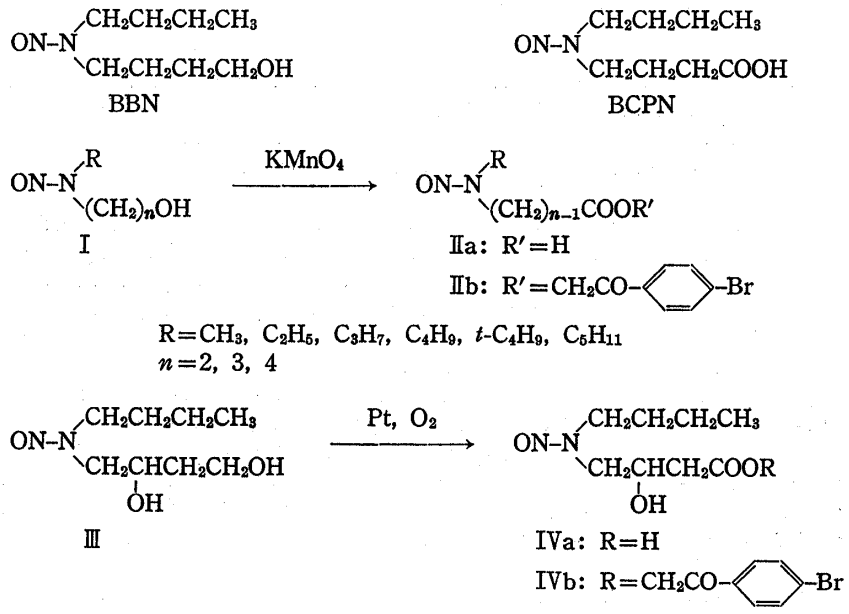

Chart 1

1) Location: *Takada 3-41-8, Toshima-ku, Tokyo, 171, Japan.*
2) H. Druckrey, R. Preussmann, S. Ivancovic, C.H. Schmidt, H.D. Mennel, and K.W. Stahl, *Z. Krebsforsch. Klin. Onkol.*, **66**, 280 (1964); N. Ito, Y. Hiasa, A. Tamai, E. Okajima, and H. Kitamura, *Gann*, **60**, 401 (1969).
3) G. Akagi, A. Akagi, M. Kimura, and H. Otsuka, *Gann*, **64**, 331 (1973); J.S. Bertram and A.W. Craig, *Europ. J. Cancer*, **8**, 587 (1972).

in rats,[4] was identified as N-butyl-N-(3-carboxypropyl)nitrosamine (BCPN)[5,6] (Chart 1). In connection with these studies, a number of N-alkyl-N-(ω-hydroxyalkyl)nitrosamines related to BBN were synthesized[7] and their metabolic fate and carcinogenicity in rats were investigated[8] in order to elucidate any possible relationship between chemical structure, the metabolism *in vivo*, and the specific action on the bladder. This paper deals with the syntheses of N-alkyl-N-(ω-carboxyalkyl)nitrosamines related to BCPN, which were required principally for the identification of metabolites and in part for the carcinogenicity test.

Potassium permanganate oxidation of BBN in the usual way gave BCPN in a good yield (80%). The homologs and analogs of BBN, N-alkyl-N-(ω-hydroxyalkyl)nitrosamines (I in Chart 1), the syntheses of which were reported in the previous paper,[7] were treated with the same oxidizing agent to afford the corresponding N-alkyl-N-(ω-carboxyalkyl)nitrosamines (IIa) in fairly good yields (40—80%). They are listed in Table I.[9]

The N-alkyl-N-(ω-carboxyalkyl)nitrosamines synthesized gave a positive reaction for N-nitroso group with diphenylamine and sulfanilic acid–N-[α-naphthyl]ethylenediamine reagents.[7] Data of elemental analysis are given in Table I and ultraviolet (UV) and infrared (IR) spectral data are indicated in Table II.[10] The UV spectra of these compounds show two absorption bands at 230—237 and 348—355 nm, and the first maximum is more distinct than the second.[7,12] The IR absorption bands assignable to the vibrations of the C=O and N=O bonds[7,10] are given in the table.

β-Hydroxy derivative of BCPN, N-butyl-N-(2-hydroxy-3-carboxypropyl)nitrosamine (BHCPN) (IVa) which was obtained as a minor urinary metabolite of BBN in the rat,[5,8] was prepared in a good yield (75%) from N-butyl-N-(2,4-dihydroxybutyl)nitrosamine (III) by the oxidation procedure using platinum as catalyst.[13] It is included in Table I, and its UV and IR spectral data are given in Table II.

All the N-nitroso compounds with an ω-carboxyalkyl chain synthesized in the present work were led to the corresponding crystalline *p*-bromophenacyl esters (IIb, IVb) in fairly good yields (35—74%), which are listed in Table III.[10]

4) H. Druckrey, R. Preussmann, S. Ivankovic, and Schmähl, *Z. Krebsforsch. Klin. Onkol.*, **69**, 103 (1967).
5) M. Okada and E. Suzuki, *Gann*, **63**, 391 (1972).
6) E. Suzuki, J. Aoki, and M. Okada, Proceedings of the 31st Annual Meeting of the Japanese Cancer Association, Nagoya, 1972, p.9; L. Blattmann and R. Preussmann, *Z. Krebsforsch. Klin. Onkol.*, **79**, 3 (1973).
7) M. Okada, E. Suzuki, and M. Iiyoshi, *Chem. Pharm. Bull.* (Tokyo), **26**, 3891 (1978).
8) 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).
9) The following abbreviations are used in the tables: MCPN, N-methyl-N-(3-carboxypropyl)nitrosamine; ECPN, N-ethyl-N-(3-carboxypropyl)nitrosamine; PCPN, N-propyl-N-(3-carboxypropyl)nitrosamine; *t*-BCPN, N-*tert*-butyl-N-(3-carboxypropyl)nitrosamine; ACPN, N-amyl(=pentyl)-N-(3-carboxypropyl)-nitrosamine; ECEN, N-ethyl-N-(2-carboxyethyl)nitrosamine; PCEN, N-propyl-N-(2-carboxyethyl)-nitrosamine; BCEN, N-butyl-N-(2-carboxyethyl)nitrosamine; ACEN, N-amyl-N-(2-carboxyethyl)-nitrosamine; ECMN, N-ethyl-N-(carboxymethyl)nitrosamine; BCMN, N-butyl-N-(carboxymethyl)-nitrosamine; ACMN, N-amyl-N-(carboxymethyl)nitrosamine; BHCPN, N-butyl-N-(2-hydroxy-3-carboxypropyl)nitrosamine.
10) Because of a partial double bond character of the N-N bond, *syn* and *anti* conformers occur in N-nitrosamines which are in dynamic equilibrium.[11] The conformer ratios of the N-alkyl-N-(ω-carboxyalkyl)-nitrosamines and their derivatives (*p*-bromophenacyl and methyl esters, the latters are not described in this paper) were determined by nuclear magnetic resonance and high-pressure liquid chromatography. The results will be reported elsewhere.
11) G.J. Karabatsos and R.T. Taller, *J. Am. Chem. Soc.*, **86**, 4373 (1964); W. Lijinsky, L. Keefer, and J. Loo, *Tetrahedron*, **26**, 5137 (1970).
12) P.N. Magee, R. Montesano, and R. Preussmann, "Chemical Carcinogens," ed. by C.E. Searle, American Chemical Society, Washington, D.C., 1976, Chapter 11.
13) K. Heyns and H. Paulsen, *Adv. Carbohyd. Chem.*, **17**, 169 (1962).

Table I. N-Alkyl-N-(ω-carboxyalkyl)nitrosamines

Compound[9]	ON-N<R_1 / R_2		Yield (%)	mp (°C)	Formula	Analysis (%) Calcd. (Found)		
	R_1	R_2				C	H	N
MCPN	CH_3	$(CH_2)_3COOH$	80	<25[a]	$C_5H_{10}N_2O_3$	41.09 (41.33	6.90 7.14	19.17 19.23)
ECPN	C_2H_5	$(CH_2)_3COOH$	64	<25	$C_6H_{12}N_2O_3$	44.99 (44.86	7.55 7.70	17.49 17.24)
PCPN	C_3H_7	$(CH_2)_3COOH$	70	<25	$C_7H_{14}N_2O_3$	48.26 (48.22	8.10 8.22	16.08 16.30)
BCPN	C_4H_9	$(CH_2)_3COOH$	80	39—40	$C_8H_{16}N_2O_3$	51.05 (50.90	8.57 8.60	14.88 14.69)
t-BCPN	*t*-C_4H_9	$(CH_2)_3COOH$	74	76—78	$C_8H_{16}N_2O_3$	51.05 (51.20	8.57 8.77	14.88 14.94)
ACPN	C_5H_{11}	$(CH_2)_3COOH$	69	47—48	$C_9H_{18}N_2O_3$	53.44 (53.52	8.97 9.11	13.85 13.94)
ECEN	C_2H_5	$(CH_2)_2COOH$	42	Oil	$C_5H_{10}N_2O_3$	41.09 (41.59	6.90 6.94	19.17 18.82)
PCEN	C_3H_7	$(CH_2)_2COOH$	55	Oil	$C_6H_{12}N_2O_3$	44.99 (45.19	7.55 7.77	17.49 17.27)
BCEN	C_4H_9	$(CH_2)_2COOH$	46	<25	$C_7H_{14}N_2O_3$	48.26 (48.28	8.10 8.39	16.08 15.80)
ACEN	C_5H_{11}	$(CH_2)_2COOH$	40	<25	$C_8H_{16}N_2O_3$	51.05 (50.67	8.57 8.59	14.88 14.56)
ECMN	C_2H_5	CH_2COOH	40	84—85	$C_4H_8N_2O_3$	36.36 (36.18	6.10 6.00	21.20 21.24)
BCMN	C_4H_9	CH_2COOH	45	61	$C_6H_{12}N_2O_3$	44.99 (44.86	7.55 7.47	17.49 17.21)
ACMN	C_5H_{11}	CH_2COOH	60	73—74	$C_7H_{14}N_2O_3$	48.26 (48.18	8.10 8.17	16.08 15.86)
BHCPN	C_4H_9	CH_2CHCH_2COOH | OH	75	45	$C_8H_{16}N_2O_4$	47.05 (47.17	7.90 7.96	13.72 13.59)

a) Crystalline state in a refrigerator, but an oily substance at about 25°.

Table II. UV and IR Spectral Data of N-Alkyl-N-(ω-carboxyalkyl)nitrosamines

Compound[9]	UV $\lambda_{max}^{95\%EtOH}$ nm (ε)		IR ν_{max}^{KBr} cm^{-1}	
			C=O	N=O
MCPN	230.5 (7400)	348 (92)	1723	1420
ECPN	233 (7500)	352 (84)	1720	1440 (broad)
PCPN	234 (7200)	352 (85)	1711	1455
BCPN	234.5 (7400)	352 (87)	1720	1470
t-BCPN	231.5 (7000)	355 (59)	1730	1443
ACPN	234.5 (7100)	352.5 (86)	1700	1465
ECEN	232.5 (6800)	353 (82)	1725	1430 (broad)
PCEN	234 (7200)	353 (88)	1720	1438
BCEN	234 (6900)	353 (79)	1718	1457
ACEN	234.5 (7100)	353 (86)	1715	1458
ECMN	235 (6400)	349.5 (83)	1725	1422
BCMN	237 (5900)	350 (79)	1737	1420
ACMN	236 (6200)	350.5 (86)	1740	1410
BHCPN	236 (7200)	353 (93)	1720 3400 (OH)	1460

Table III. *p*-Bromophenacyl Esters of N-Alkyl-N-(ω-carboxyalkyl)nitrosamines

p-Bromophenacyl ester of[9]	mp (°C)	Formula	Analysis (%) Calcd. (Found) C	H	Br	N	UV $\lambda_{max}^{95\%EtOH}$ nm (ε)	IR ν_{max}^{KBr} cm^{-1} –COO	C=O
MCPN	76	$C_{13}H_{15}BrN_2O_4$	45.50 (45.36	4.41 4.23	23.29 23.49	8.16 7.96)	256 (20200)	1748,	1705
ECPN	70—71	$C_{14}H_{17}BrN_2O_4$	47.07 (46.99	4.80 4.76	22.37 22.58	7.84 7.72)	256 (28200)	1745,	1703
PCPN	67	$C_{15}H_{19}BrN_2O_4$	48.53 (48.22	5.16 5.00	21.52 21.72	7.55 7.44)	256 (22700)	1740,	1697
BCPN	66	$C_{16}H_{21}BrN_2O_4$	49.89 (49.52	5.49 5.38	20.74 21.04	7.27 7.12)	256.5 (25400)	1745,	1703
t-BCPN	90—91	$C_{16}H_{21}BrN_2O_4$	49.89 (50.13	5.49 5.38	20.74 20.63	7.27 6.98)	256.5 (17900)	1748,	1708
ACPN	75—76	$C_{17}H_{23}BrN_2O_4$	51.13 (51.01	5.82 5.77	20.01 19.95	7.02 7.12)	256 (20600)	1743,	1703
ECEN	101—102	$C_{13}H_{15}BrN_2O_4$	45.50 (45.86	4.41 4.33	23.29 23.04	8.16 7.87)	256.5 (16100)	1742,	1700
PCEN	101	$C_{14}H_{17}BrN_2O_4$	47.07 (46.99	4.80 4.61	22.37 22.59	7.84 7.67)	256 (22500)	1740,	1702
BCEN	104	$C_{15}H_{19}BrN_2O_4$	48.53 (48.22	5.16 4.96	21.52 21.65	7.55 7.33)	256.5 (17300)	1743,	1705
ACEN	107	$C_{16}H_{21}BrN_2O_4$	49.89 (49.39	5.49 5.38	20.74 20.73	7.27 7.09)	256 (20000)	1740,	1700
MCMN	99—101	$C_{11}H_{11}BrN_2O_4$	41.93 (41.91	3.52 3.42	25.36 25.30	8.89 8.72)	257 (20300)	1750,	1702
BCMN	38—39	$C_{14}H_{17}BrN_2O_4$	47.07 (46.98	4.80 4.65	22.37 22.52	7.84 7.71)	257 (20400)	1753,	1698
ACMN	77—78	$C_{15}H_{19}BrN_2O_4$	48.53 (48.57	5.16 5.10	21.52 21.39	7.55 7.64)	257 (24800)	1748,	1697
BHCPN	80—81	$C_{16}H_{21}BrN_2O_5$	47.89 (47.77	5.28 5.14	19.91 20.05	6.98 6.54)	256.5 (15800)	3470 (OH) 1740,	1697

The carcinogenic effect of BCPN and BCPN, the principal urinary metabolite of BBN and N-ethyl-N-(4-hydroxybutyl)nitrosamine (EHBN)[7,8] respectively, was examined in rats. Selective induction of bladder cancer was observed with BCPN[14] and ECPN[15,16] as effectively as the original compounds, BBN and EHBN, thus demonstrating that these compounds having a 3-carboxypropyl chain are responsible for the organospecific action of the compounds with a 4-hydroxybutyl group. BCMN with a carboxymethyl group, on the other hand, which was identified as a minor urinary metabolite of BBN[6,8] and the principal metabolite of a potent hepatocarcinogen N-butyl-N-(2-hydroxyethyl)nitrosamine,[7,17] was found to be non-carcinogenic.

Of 14 N-nitrosamines reported in the present work, 9 compounds (MCPN, ECPN, PCPN, BCPN, *t*-BCPN, ACPN, BCEN, BCMN, and BHCPN) were tested on their mutagenic effects using *Salmonella typhimurium* strain TA 1535.[18] Seven compounds except for *t*-BCPN and BCMN were shown to be mutagenic. Moreover, BCPN and its homologs with a 3-carboxypropyl chain were found to be mutagenic without metabolic activation by the S-9 mix.

14) Y. Hashimoto, E. Suzuki, and M. Okada, *Gann*, **63**, 637 (1972).
15) Y. Hashimoto, M. Iiyoshi, and M. Okada, *Gann*, **65**, 565 (1974).
16) M. Okada, E. Suzuki, and Y. Hashimoto, *Gann*, **67**, 825 (1976).
17) M. Okada and Y. Hashimoto, *Gann*, **65**, 13 (1974).
18) M. Nagao, E. Suzuki, K. Yasuo, T. Yahagi, Y. Seino, T. Sugimura, and M. Okada, *Cancer Res.*, **37**, 399 (1977).

Experimental[19]

Oxidation of N-Alkyl-N-(ω-hydroxyalkyl)nitrosamines (I) to N-Alkyl-N-(ω-carboxyalkyl)nitrosamines (IIa)——The oxidation of I[7] to IIa with $KMnO_4$ was carried out in the usual way. For example, BCPN was prepared from BBN as follows: To a solution of BBN (5 g) and KOH (1.4 g) in water (100 ml) was added powdered $KMnO_4$ (9 g) little by little with stirring and under ice-cooling. After adding $KMnO_4$, the resulting solution was stirred at room temperature for 40 min. The solution was extracted with ether and the aqueous layer was made pH 4 with 10 N HCl. The acidified aqueous layer was then extracted with EtOAc (100 ml × 3) and the organic layer was washed with water and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a pale yellow crystalline residue (4.2 g) which was recrystallized from CCl_4 to afford BCPN as colorless tablets.

Purification of the compounds which were unable to crystallize was made by column chromatography of silica gel (E. Merck AG) using $CHCl_3$–MeOH (39: 1 or 49: 1) as eluting solvent. Yields, mp and data of elemental analysis are given in Table I, and UV and IR spectral data are indicated in Table II.

Preparation of *p*-Bromophenacyl Esters (IIb) of N-Alkyl-N-(ω-carboxyalkyl)nitrosamines (IIa)——They were prepared in the usual way, taking the *p*-bromophenacyl ester of BCPN as an example, as follows: To an aqueous solution (0.5 ml) of BCPN (100 mg) was added 10% NaOH to make pH of the solution 7. After addition of one drop of 1 N HCl, EtOH (3 ml) and *p*-bromophenacyl bromide (148 mg) were added and the mixture was refluxed for 1 hr. After evaporation of the solvent *in vacuo*, the residue was extracted with $CHCl_3$ and the organic layer was washed with water and dried over anhyd. Na_2SO_4. A residue obtained after evaporation of the solvent, was subjected to column chromatography of silica gel using $CHCl_3$ as eluting solvent. Recrystallization from EtOH–H_2O gave the ester as colorless plates. All the other *p*-bromophenacyl esters (IIb) were prepared in the same way. mp, data of elemental analysis, UV and IR spectral data are given in Table III.

N-Butyl-N-(2-hydroxy-3-carboxypropyl)nitrosamine (BHCPN) (IVa)——(i) N-Butyl-N-(2,4-dihydroxybutyl)nitrosamine (BDHBN) (III): To 1,3-dihydroxy-4-chlorobutane diacetate[20] (41.8 g) was added butylamine (88 g) with stirring and under ice-cooling and the mixture was allowed to stand for 48 hr at room temperature. The mixture was then refluxed for 1 hr and the excess of the amine was removed *in vacuo*. The residue was made alkaline (pH 10) with 28% NH_4OH and extracted with EtOAc (250 ml × 3). The organic layer was dried over anhyd. Na_2SO_4 and then the solvent was evaporated under reduced pressure. The residue was distilled to afford two fractions: fraction 1, bp 96—106° (2 mmHg), 5.1 g, N-butylacetamide; fraction 2, bp 122—126° (2 mmHg), 5.3 g, N-butyl-N-(2,4-dihydroxybutyl)amine. The fraction 2 was nitrosated in the way described previously[7] to afford BDHBN in 70% yield. For elemental analysis it was purified by column chromatography of silica gel using $CHCl_3$–MeOH (9: 1) as eluting solvent. *Anal.* Calcd. for $C_8H_{18}N_2O_3$; C, 50.51; H, 9.54; N, 14.72. Found: C, 50.19; H, 9.77; N, 14.44. UV λ_{max} nm (ε): 236.5 (6500), 352 (85).

(ii) BHCPN (IVa): To a solution of BDHBN (1 g) in acetone (10 ml) was added Pt catalyst suspension in water (20 ml) which was prepared from 890 mg of $PtO_2 \cdot H_2O$ by hydrogenation, and the solution was stirred under oxygen at room temperature for 24 hr. After adding Pt catalyst (prepared from 914 mg of $Pt_2O \cdot H_2O$), the stirring under oxygen was continued for further 48 hr. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure to remove acetone. The concentrate was made alkaline (pH 8) with 2 N NaOH and extracted with EtOAc (80 ml × 2). The aqueous layer was then made acidic (pH 4) by 5 N HCl and extracted with EtOAc (70 ml × 4). The latter organic layer was washed with water, dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a plae yellow crystalline residue (804 mg) which was purified by column chromatography of silica gel using $CHCl_3$–MeOH (19 :1) as eluting solvent. BHCPN was obtained as pale yellow crystals. The yield, mp and data of elemental analysis are given in Table I, and UV and IR spectral data are indicated in Table II.

p-Bromophenacyl ester (IVb) of BHCPN was prepared in the same manner as described above. Melting point, elemental analytical data, UV and IR spectral data are given in Table III.

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19) Melting points were determined on a micro hot-stage apparatus and are uncorrected. UV spectra were measured in 95% EtOH solution. IR spectra were determined in KBr disks on Hitachi EPI-S2 spectrophotometer.

20) M.I. Farbevov and B.F. Ustavshihikov, *Zh. Obshch. Khim.*, **25**, 2071 (1955) [*C.A.* **50**, 8647*f* (1956)].