[Chem. Pharm. Bull. 28(3) 979—983 (1980)]

Nuclear Magnetic Resonance Spectra of N-Alkyl-N-(hydroxy- and oxo-alkyl)nitrosamines and Chromatographic Separation of Their (*Z*)- and (*E*)-Conformers

Emako Suzuki, Minoru Iiyoshi, and Masashi Okada

Tokyo Biochemical Research Institute[1]

(Received September 5, 1979)

Nuclear magnetic resonance spectral data for a series of N-alkyl-N-(hydroxy- and oxo-alkyl)nitrosamines are presented. The (*Z*)- and (*E*)-conformers of these N-nitrosamines were separated by high-pressure liquid chromatography and thin-layer chromatography.

Keywords——chemical carcinogen; bladder carcinogen; N-nitrosamine; N-alkyl-N-(hydroxyalkyl)nitrosamine; N-alkyl-N-(oxoalkyl)nitrosamine; NMR of N-nitrosamine; HPLC of N-nitrosamine; TLC of N-nitrosamine; (*E*)- and (*Z*)-conformers; separation of (*E*)- and (*Z*)-conformers

The great majority of N-nitroso compounds are carcinogenic to experimental animals and are likely candidates as environmental carcinogens.[2–6]

In the course of our studies[7] on a possible relationship between chemical structure, *in vivo* metabolism, and organotropic carcinogenicity of N,N-dibutylnitrosamine and N-butyl-N-(4-hydroxybutyl)nitrosamine, a number of N-alkyl-N-(hydroxy- and oxo-alkyl)nitrosamines structurally related to these N-nitrosamines and their metabolites were prepared.[8] This paper reports nuclear magnetic resonance (NMR) spectral data for N-alkyl-N-(hydroxy- and oxo-alkyl)nitrosamines and describes the separation of (*Z*)- and (*E*)-conformers[9] of the substituted asymmetric N,N-dialkylnitrosamines by high-pressure liquid chromatography (HPLC) and by thin-layer chromatography (TLC). This work was required for the characterization of metabolites encountered in our previous studies.[7]

Materials and Methods

All the N-nitroso compounds were synthesized according to the procedure reported earlier.[8]

Nuclear magnetic resonance (NMR) spectra were taken at 34° in 5% $CDCl_3$ solution 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 Hz: s, singlet; d, doublet; t, triplet; q, quartet.

HPLC measurements were performed on an Oriental Motor 55K25GK-A liquid chromatograph equipped with a 254 nm fixed-wavelength ultraviolet detector made by Nihon Seimitsu Co., Ltd. (Tokyo). Chromatography of the compounds was studied on a column (25 cm × 4.6 mm I.D.) packed with LiChrosorb SI-60 (5 μm) (E. Merck AG). The eluting solvents were as follows: S_1, hexane–ether–CH_2Cl_2 (4 : 3 : 2); S_2, S_1–EtOH

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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8) M. Okada, E. Suzuki, and M. Iiyoshi, *Chem. Pharm. Bull.*, **26**, 3891 (1978).
9) The (*Z*)- and (*E*)-system for specifying double bond stereoisomers unambiguously is employed.

(100: 1), S_3, S_1–EtOH (100: 2); S_4, hexane–CH_2Cl_2–EtOH (80: 20: 2.5). The flow rate was 2.3 ml/min and the column temperature was 25°.

TLC was performed on plates coated with a 0.25 mm layer of silica gel HF_{254} (E. Merck AG) using a mixture of hexane–ether–CH_2Cl_2 (4: 3: 2) as a developing solvent. Spots were detected under ultraviolet (UV) light (254 nm) and using the reagent reported previously.[8]

The compounds were dissolved in EtOAc or CH_2Cl_2 and the freshly prepared solution was subjected to HPLC and TLC.

Results and Discussion

The NMR spectral data for N-alkyl-N-(hydroxyalkyl)nitrosamines are listed in Table I.[10] Because of the partial double bond character of the N-N bond, *syn*- and *anti*-conformers occur in asymmetric N-nitrosamines, and are in dynamic equilibrium[11–16] (Fig. 1).

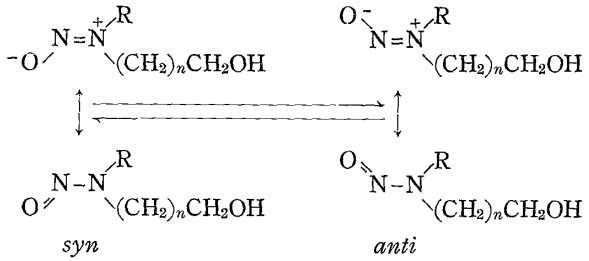

Fig. 1. Polar Resonance Forms of N-Alkyl-N-(ω-hydroxyalkyl)nitrosamines

The designations *syn* and *anti* refer to the position of the nitroso group with respect to the ω-hydroxyalkyl group.

The conformational assignment was made according to Karabatsos and Taller;[12] protons resonate at higher magnetic fields when *syn* than when *anti* to the nitroso oxygen. As shown in Table I, the protons at the carbon atoms (α) adjacent to the amino nitrogen atom resonated at 3.4—4.4 ppm, with two protons *syn* to the nitroso oxygen at 3.4—3.9 ppm and those *anti* at 3.9—4.4 ppm. The two α-protons of *t*-BBN resonated only at 3.4—3.8 ppm, indicating that it exists as the (*E*) (*syn*)-conformer exclusively because of the bulkiness of the *tert*-butyl group as compared with the 4-hydroxybutyl group. The (*E*) (*anti*)-conformer was the preferred one for MHBN owing to the smallness of the methyl group in comparison with the opposite chain. The (*Z*)- and (*E*)-conformer ratio was approximately determined by NMR integration of peak areas, giving values of 24 and 57% for the (*Z*)-conformers of MHBN and EHPN, respectively. The conformer ratio of other compounds could not be determined due to overlapping of signals.

The two protons at the carbon atom of the ω-hydroxymethyl group resonated at 3.4—3.9 ppm. Different chemical shifts were observed for the protons of the terminal methyl group of the hydroxyalkyl chain in BHBN-3 and BHPN-2 owing to the anisotropic effect of the N-nitroso group.

10) The following abbreviations are used: MHBN, N-methyl-N-(4-hydroxybutyl)nitrosamine; EHBN, N-ethyl-N-(4-hydroxybutyl)nitrosamine; PHBN, N-propyl-N-(4-hydroxybutyl)nitrosamine; BBN, N-butyl-N-(4-hydroxybutyl)nitrosamine; *t*-BBN, N-*tert*-butyl-N-(4-hydroxybutyl)nitrosamine; AHBN, N-amyl(=pentyl)-N-(4-hydroxybutyl)nitrosamine; EHPN, N-ethyl-N-(3-hydroxypropyl)nitrosamine; PHPN, N-propyl-N-(3-hydroxypropyl)nitrosamine; BHPN, N-butyl-N-(3-hydroxypropyl)nitrosamine; AHPN, N-amyl-N-(3-hydroxypropyl)nitrosamine; EHEN, N-ethyl-N-(2-hydroxyethyl)nitrosamine; BHEN, N-butyl-N-(2-hydroxyethyl)nitrosamine; AHEN, N-amyl-N-(2-hydroxyethyl)nitrosamine; BHBN-2, N-butyl-N-(2-hydroxybutyl)nitrosamine; BHBN-3, N-butyl-N-(3-hydroxybutyl)nitrosamine; BHPN-2, N-butyl-N-(2-hydroxypropyl)nitrosamine; BOBN-2, N-butyl-N-(2-oxobutyl)nitrosamine; BOBN-3, N-butyl-N-(3-oxobutyl)nitrosamine; BOPN, N-butyl-N-(2-oxopropyl)nitrosamine; AOPN, N-amyl-N-(2-oxopropyl)nitrosamine.

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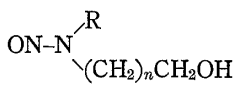
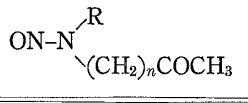
Table I. NMR Spectral Data for N-Alkyl-N-(hydroxyalkyl)nitrosamines



Compound[10]	R	n	Chemical shifts,[a] δppm >N–CH₂– (Z)	(E)	>N–(CH₂)$_n$– (Z)	(E)	–CH₂OH (Z)	(E)	–CH₃ (Z)	(E)
MHBN	CH_3	3			3.64	4.18(t)	3.68(t)		3.77	3.05(s)
EHBN	C_2H_5	3	4.14	3.61(q)	3.4—3.8	4.12(t)	3.68(t)		1.40	1.10(t)
PHBN	C_3H_7	3	4.06	3.4—3.9(t)	3.4—3.9	4.13(t)	3.4—3.9		0.98(t)	
BBN	C_4H_9	3	4.09	3.4—3.8(t)	3.4—3.8	4.12(t)	3.4—3.8		0.98(t)	
t-BBN	*tert*-C_4H_9	3			—	3.4—3.8	3.4—3.8		1.53(s)	
AHBN	C_5H_{11}	3	4.09	3.4—3.8(t)	3.4—3.8	4.12(t)	3.4—3.8		0.92(t)	
EHPN	C_2H_5	2	4.17	3.64(q)	3.52	4.22(t)	3.72(t)		1.42	1.10(t)
PHPN	C_3H_7	2	4.08	3.54(t)	3.63	4.22(t)	3.72(t)		0.98(t)	
BHPN	C_4H_9	2	4.10	3.49(t)	3.60	4.22(t)	3.72(t)		0.98(t)	
AHPN	C_5H_{11}	2	4.08	3.50(t)	3.61	4.21(t)	3.71(t)		0.92(t)	
EHEN	C_2H_5	1	4.22	3.65(q)	3.5—4.4		3.74(s)		1.40	1.10(t)
BHEN	C_4H_9	1	3.5—4.3		3.5—4.3		3.75(s)		0.98(t)	
AHEN	C_5H_{11}	1	3.4—4.3		3.4—4.3		3.72(s)		0.91(t)	
BHBN-2	C_4H_9	–	3.5—4.3		3.5—4.3		3.5—4.3[b]		0.97(t)	
BHBN-3	C_4H_9	–	3.5—4.3		3.5—4.3		3.5—4.3[c]		1.18	1.26(d)[d]
BHPN-2	C_4H_9	–	3.5—4.3		3.5—4.3		3.5—4.3[c]		1.16	1.28(d)[d]

a) J=6.0—7.8 Hz.
b) >N–CH₂C<u>H</u>(OH)–.
c) –C<u>H</u>(OH)CH₃.
d) –CH(OH)C<u>H</u>₃.

Table II. NMR Spectral Data for N-Alkyl-N-(oxoalkyl)nitrosamines



Compound[10]	R	n	Chemical shift,[a] δppm >N–CH₂– (Z)	(E)	>N–(CH₂)$_n$– (Z)	(E)	–CH₂COCH₃ (Z)	(E)	–COCH₃ (Z)	(E)	Conformer ratio(%) (Z)	(E)
BOBN-3	C_4H_9	2	4.17	3.54(t)	3.73	4.27(t)	2.70	3.03(t)	2.13	2.21(s)	70	30
BOPN	C_4H_9	1	4.22	3.58(t)	4.23	4.95(s)			2.19	2.27(s)	85	15
AOPN	C_5H_{11}	1	4.20	3.58(t)	4.23	4.95(s)			2.18	2.26(s)	85	15
BOBN-2	C_4H_9	—	4.20	3.60(t)	4.23	4.95(s)	2.51(q)[b]				85	15

a) J=6.5—7.0 Hz.
b) –COC<u>H</u>₂CH₃.

The NMR spectral data for N-alkyl-N-(oxoalkyl)nitrosamines are shown in Table II.[10] The approximate conformer ratios could be determined in these cases by NMR integration of peak areas and are indicated in the table. The (*Z*)-conformer was predominant.

The separation of the conformers of a few substituted asymmetric N-nitrosamines, including EHEN, by HPLC has been reported recently.[17] The conformers of the N-alkyl-N-(hydroxy- and oxo-alkyl)nitrosamines described above were separated by HPLC as shown in Table III. The approximate conformer ratios were determined from the peak heights and are also

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Table III. Separation of (*Z*)- and (*E*)-Conformers of N-Alkyl-N-(hydroxy- and oxo-alkyl)nitrosamines by HPLC

Compound[10]	Solvent system	Retention time (min)		Conformer ratio (%)[a]	
		(*Z*)	(*E*)	(*Z*)	(*E*)
MHBN	S_4	21.2	23	24	76
	S_3	14.0	14.6	24	76
				(24	76)
EHBN	S_4	14.5	15.6	46	54
PHBN	S_4	10.8	11.7	51	49
BBN	S_4	9.1	10.1	51	49
	S_2	8.2	8.7	51	49
t-BBN	S_2		9.4	0	100
AHBN	S_2	7.3	7.9	50	50
EHPN	S_2	13.2	14.7	55	45
				(57	43)
PHPN	S_2	9.2	10.5	59	41
BHPN	S_2	7.3	8.6	59	41
AHPN	S_2	6.6	7.8	58	42
EHEN	S_2	9.8	11.8	56	44
BHEN	S_2	5.8	7.3	58	42
AHEN	S_2	4.9	6.2	60	40
BHBN-3	S_1	7.2	8.8	61	39
BHBN-2	S_1	3.3	3.8	54	46
BHPN-2	S_1	5.3	6.2	56	44
AHPN-2	S_1	4.8	5.8	56	44
BOBN-3	S_1	2.8	3.6	67	33
				(70	30)

a) Values determined by NMR spectroscopy are given in parentheses.

indicated in the table. The ratio did not change after allowing a solution of each compound to stand for 24 hr at room temperature.

By comparing the conformer ratios of MHBN, EHPN and BOBN-3, which were determined by both NMR spectroscopy and HPLC, it was judged that the (*Z*)-conformer was eluted faster than the (*E*)-conformer. Good separations were achieved with all the compounds examined except for those with a 2-oxo group (BOBN-2, BOPN, and AOPN), the conformers of which were clearly distinguishable by NMR spectroscopy (Table II). In accordance with the NMR spectral data, *t*-BBN gave a single peak in all the solvent systems tested.

A few instances of successful separations of (*Z*)- and (*E*)-conformers of N-nitrosamines by TLC have appeared in the literature.[18,19] Among the three N-butyl-N-(hydroxybutyl)nitrosamines, BBN, BHBN-3 and BHBN-2, having the hydroxyl group at the ω, ω-1, and ω-2 positions respectively, good separation of the conformers was achieved with BHBN-2 using a mixture of hexane, ether and dichloromethane (4: 3: 2) as a solvent. Partial separation was attained with BHBN-3, but no separation with BBN (Fig. 2a). Similar results were obtained with other N-alkyl-N-(hydroxybutyl)nitrosamines. As for the compounds with an oxo group, good separation of the conformers of BOBN-3 was accomplished, while separation was unsuccessful with BOBN-2 and BOPN (Fig. 2b).

In the course of our studies[7] on the metabolism of N,N-dibutylnitrosamine, BBN and related compounds, the characteristic NMR spectral and chromatographic properties of the N-alkyl-N-(hydroxy- and oxo-alkyl)nitrosamines described above were very useful for the

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19) B. Liberek, J. Augustyniak, J. Ciarkowski, K. Plucińska, and K. Stachowiak, *J. Chromatogr.*, **95**, 223 (1974).

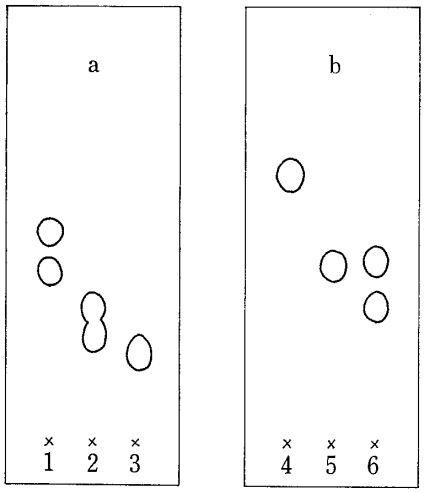

Fig. 2. Thin–Layer Chromatographic Separation of the Conformers

1, BHBN-2; 2, BHBN-3; 3, BBN; 4, BOBN-2; 5, BOPN; 6, BOBN-3.

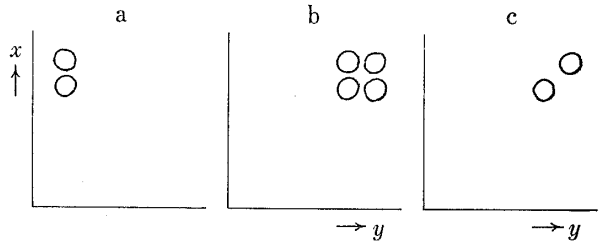

Fig. 3. Two-dimensional Thin–Layer Chromatography for the Detection of Conformers

identification of metabolites and for determining their purity. It was inconvenient, however, that one compound (metabolite) might give two spots on TLC while another compound might afford one spot, so that a question always arose as to whether the two spots represented the conformers of one compound or two compounds, possibly obtained as metabolites. This problem has been settled by using two-dimensional TLC.

As shown schematically in Fig. 3, after developing a compound on the TLC plate in the x-direction the plate was allowed to stand at room temperature in the dark for more than 5 hr. The chromatogram with two spots (Fig. 3a) was developed again in the y-direction with the same solvent. If four spots were obtained (Fig. 3b) on the chromatogram after the second development, the two spots observed after the first development were considered to correspond to the two conformers of the compound examined. If only two spots were detected again on the chromatogram after the second development (Fig. 3c), the material was considered to be a mixture of at least two compounds. This procedure is a very useful and convenient way of detecting conformers, provided that the compound is stable.

Acknowledgement This work was supported in part by Grants-in-Aid for CancerResearch from the Ministry of Education, Science and Culture, Japan.