NITROSATION OF 1-(2-CHLOROETHYL)-3-(2-CHLORO-10*H*-PHENOTHIAZIN-10-YL)PROPYLUREA AND HPLC SEPARATION OF TWO NITROSATED ISOMERS

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Abstract: 1-(2-Chloroethyl)-3-(2-chloro-10*H*-phenothiazin-10-yl)propylurea 1 reacts with sodium nitrite to yield a mixture of isomeric nitrosoureas **2a** and **2b**. The two nitrosoureas **2a** and **2b** could be effectively separated by HPLC and identified.

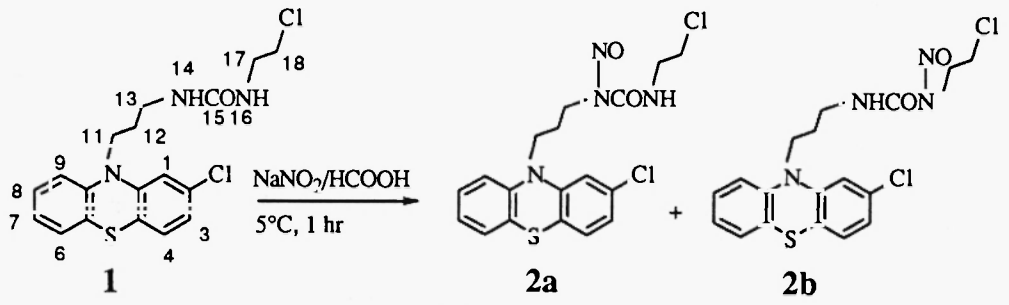
Introduction

Phenothiazines have antitumor activity, especially for brain tumor due to their lipid solubility (1). Nitrosoureas, *i.e.*, 1,3-*bis*-2-chloroethyl-1-nitrosourea with high lipid solubility could also penetrate the blood-brain barrier (BBB) and have been clinically used for intracerebral L1210 mouse leukemia (2) and human meningeal leukemia (3) as the potential in lipophilic tumor cells. The purpose of this paper, it is to synthesize a hybrid compound of phenothiazine and nitrosourea, which could be expected the high penetration into BBB and also high efficacy against some brain tumors.

Results and Discussion

Nitrosation of 1-(2-chloroethyl)-3-(2-chloro-10*H*-phenothiazin-10-yl)propylurea 1 with sodium nitrite in formic acid gave a 74% yield of nitrosated urea (4) (Scheme 1). For the identification of two isomers, first, the ^{1}H-NMR spectrum of the mixture indicated that 3-nitroso **2a** and 1-nitroso **2b** derivatives were formed in a 3:1 ratio. Second, the mixture was separated by HPLC on reversed-phase column (C_{18}) to give two isomers of 3-nitroso **2a** (retention time: 24.71 min) and 1-nitroso **2b** (retention time: 26.74 min) derivatives with the same 3:1 integral ratio of ^{1}H-NMR, respectively. The mixture of **2b** (retention time: 19.62 min) and **2a** (retention time: 28.82 min) by HPLC on normal-phase column (silica-gel) gave almost the same result as that of the reversed-phase column (C_{18}) (Figure 1).

The ^{1}H-NMR spectra **2a** and **2b** were assigned on the bases of ^{1}H-^{1}H correlation spectroscopy (COSY) spectra and comparison of the ^{1}H-NMR spectra with 1. The ^{1}H-^{1}H COSY experiment of 2a and **2b** showed correlations between the following peaks: **2a**: 2.04-2.13 (m)(12-H_2) and 4.04 (m)(13-H_2), 2.04-2.13 (m)(12-H_2) and 4.18 (m)(11-H_2), and 3.73 (t)(17-H_2) and 3.18 (t)(18-H_2); **2b**: 2.31-2.37 (m)(12-H_2) and 3.38 (m)(13-H_2), 2.31-2.37 (m)(12-H_2) and 4.37 (m)(11-H_2), and 4.08 (t)(17-H_2) and 3.44 (t)(18-H_2). The chemical shits for **2a** and **2b** are summarized in Table 2, including those of the starting 1 (6). The spectral identification of isomers is based on similarities to extensive NMR studies of unsymmetrical nitrosourea (5). Thus, the methylene proton signal at δ 4.04 (m, 2H) of compound **2a** is



1 2a 2b

Scheme 1

assigned to 13-H, whose protons resonate at lower field than the 13-H (δ 3.31) of the parent urea 1 by 0.73 ppm. This suggests that the nitroso group in **2a** locates at N-14. Similarly, the 17-H signal at δ 4.08 (t, 2H) in **2b** is deshielded by 0.68 ppm relative to the 17-H (δ 3.40) of **1**, in which the nitroso group locates at N-16. Thus, these deshielding effects in compounds **2a** and **2b** relative to the parent urea **1** are in good agreement with the data for *N*-nitrosoalkylureas reported by the literature (Table 1) (5).

Table 1: ^{1}H-NMR spectral data for compounds **1**, **2a** and **2b** (δ in $CDCl_3$, 500 MHz)[a)]

Numbering	Compound 1[b)]	2a	2b
1		7.30 (d, 1H, *J*=1.8)	7.40 (d, 1H, *J*=1.5)
3		7.23 (dd, 1H, *J*=1.8, 8.2)	7.21 (dd, 1H, *J*=1.8, 8.2)
4	7.21-7.23 (m, 2H)	7.84 (d, 1H, *J*=8.2)	7.83 (d, 1H, *J*=8.2)
6	7.12 (d, 1H, *J*=8.2)	7.92 (dd, 1H, *J*=1.5, 7.9)	7.90 (dd, 1H, *J*=1.5, 7.6)
7	6.88-7.20 (m, 4H)	7.29 (ddd, 1H, *J*=1.5, 7.9, 8.5)	7.27 (ddd, 1H, *J*=1.5, 7.6, 8.5)
8		7.65 (ddd, 1H, *J*=1.5, 7.9, 8.5)	7.59 (ddd, 1H, *J*=1.5, 7.6, 8.5)
9		7.34 (d, 1H, *J*=8.5)	7.44 (d, 1H, *J*=8.5)
11	3.96 (br s, 2H)	4.18 (m, 2H)	4.37 (m, 2H)
12	1.96-2.05 (m, 2H)	2.04-2.13 (m, 2H)	2.31-2.37 (m, 2H)
13	3.31 (t, 2H, *J*=5.8)	4.04 (m, 2H)	3.38 (m, 2H)
17	3.40 (t, 2H, *J*=5.8)	3.73 (t, 2H, *J*=5.5)	4.08 (t, 2H, *J*=6.7)
18	3.50 (t, 2H, *J*=5.8)	3.81 (t, 2H, *J*=5.5)	3.44 (t, 2H, *J*=6.7)
NH	4.82 (br, 1H)	7.37-7.40 (br, 1H)	7.38-7.42 (br, 1H)
NH	5.11 (br, 1H)		

a) Chemical shifts (δ) are expressed in ppm, and coupling constants (*J* values) are in Hz in parenthesis; abbreviations are:s, singlet; d, doublet; t, triplet; m, multiplet; and br, broad.
b) Aromatic protons of 1 can not assign due to their broadening.

Experimental

Melting points were determined using a Yanagimoto melting apparatus No. 2308 and are uncorrected. Microanalyses were carried out in the microanalytical laboratory of Josai University. Elemental analyses were in agreement with the proposed structures within ± 0.41% of theoretical values. ^{1}H-NMR spectra were

performed on a JEOL JNM-GSX 500 (500 MHz) spectrometer using TMS as internal standard (δ=0). IR spectra were recorded on a JASCO IR810.

The HPLC apparatus consisted of a JASCO Model 880-PU pump (Japan Spectroscopic, Tokyo, Japan), a JASCO Model 860-CO column oven, a JASCO Model 870 UV detector, a Rheodyne Model 7125 injector equipped with a 20 μL loop (Rheodyne, Berkeley, CA, USA) and a Chromatopac CR-6A digital integrator (Shimadzu, Kyoto, Japan).

Chemicals The following chemicals and reagents were obtained from the indicated companies. Formic acid (Wako Chemicals. 066-00466, Osaka). Sodium nitrite (Wako Chemicals. 195-02562, Osaka). 2-Chloroethyl isocyanate (Tokyo Kasei, B1451, Tokyo). 1-(2-Chloroethyl)-3-(2-chloro-10*H*-phenothiazin-10-yl)propylurea **1** were prepared as described previously (6). TLC (Thin layer chromatography) was performed on a Merck Kieselgel 60 F_{254} (Merck. 5549, USA).

Preparation of 1-(2-chloroethyl)-3-(2-chloro-10*H*-phenothiazin-10-yl)propyl-3-nitrosourea 2a and 1-(2-chloroethyl)-3-(2-chloro-10*H*-phenothiazin-10-yl)propyl-1-nitrosourea 2b

To a solution of 0.396 g (1 mmol) of 1-(2-chloroethyl)-3-(2-chloro-10*H*-phenothiazin-10-yl)propylurea **1** in 3.88 mL of formic acid at 5°C, 265 mg (3.8 mmol) of sodium nitrite was portionwise added for 1 hour with stirring at 5°C. After adding, the reaction mixture was continued for 30 minutes with stirring at 5°C. The reaction mixture was neutralized to pH 4-6 by 60 mL of 10% sodium carbonate at 5°C. The neutralized reaction mixture was extracted with 30 mL of dichloromethane. The extracted organic layer was washed 15 mL of brine and dried over $MgSO_4$ overnight. The dried organic layer was concentrated to give a dark brown oil, which was dissolved in small amount of ether and concentrated to give brown mixtured crystals (mp about 95°C, 0.4 g, yield 74%) of two isomers. TLC (AcOEt): A mixture of two isomers **2a** and **2b**, R_f=0.53; 1-(2-chloroethyl)-3-(2-chloro-10*H*-phenothiazin-10-yl)propylurea **1**, R_f=0.67 (Scheme 1).

HPLC separation of two nitrosated isomers 2a and 2b

Two nitrosated isomers were separated by the following HPLC.

HPLC conditions for two nitrosated isomers **2a** and **2b** are shown in Table 2. First, the reversed-phase HPLC on Cosmosil C18 AR column with a mobile phase of MeOH-H_2O (60:40) separated two mixture isomers to give a 3 : 1 of square ratio (74.5 : 25.5) on **2a** (175 mg) and **2b** (59 mg) (Figure 1). In normal-phase HPLC on Zorbax SIL column, also two mixture isomers showed the same ratio (Table 2).

Property of two isomers 2a and 2b

2a: pale yellowish-orange crystals, mp 140-142°C (MeOH-Et_2O); IR (Nujol): 3350, 1700 cm^{-1}. Anal. Calcd for $C_{18}H_{18}Cl_2N_4O_2S \cdot H_2O$: C, 48.76; H, 4.55; N, 12.64. Found: C, 48.93; H, 4.14; N, 12.62.

2b: pale yellow crystals, mp 150-153°C (MeOH-Et_2O); IR (Nujol): 3150-3350, 1725 cm^{-1}. Anal. Calcd for $C_{18}H_{18}Cl_2N_4O_2S \cdot H_2O$: C, 48.76; H, 4.55; N, 12.64. Found: C, 48.85; H, 4.27; N, 12.74.

Figure 1: Chromatogram of isomers **2a** and **2b** (3:1) by HPLC. (A) Column: Cosmosil C18AR; Sample solution: 0.4 mg/mL; retention time (min): **2a** (24.71), **2b** (26.74); for other analytical conditions: see text. (B) Column: Zorbax SIL; Sample solution: 0.4 mg/mL; retention time (min): **2a** (29.82), **2b** (19.62); for other analytical conditions: see text.

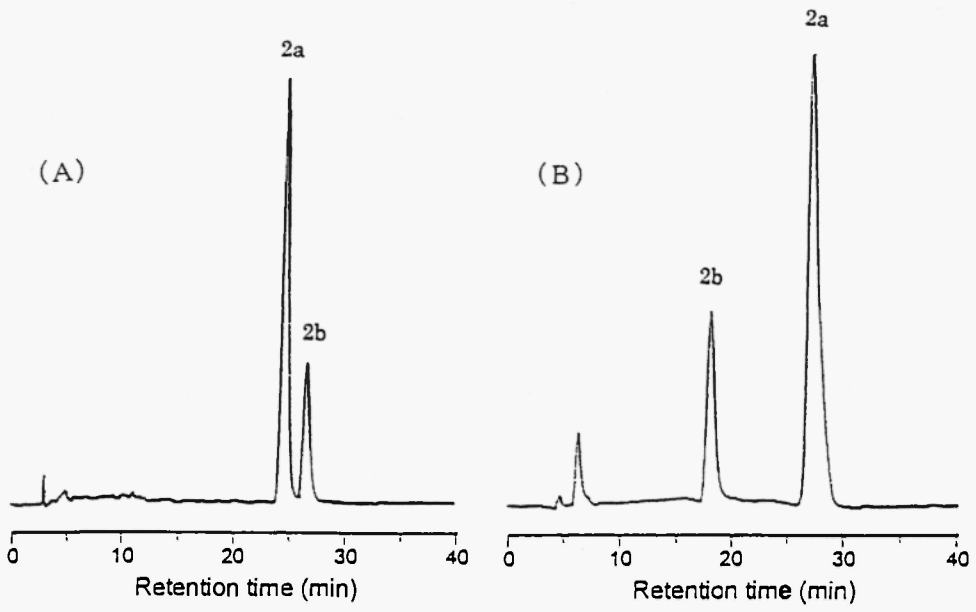


Figure 1: Chromatogram of isomers **2a** and **2b** (3:1) by HPLC. (A) Column: Cosmosil C18AR; Sample solution: 0.4 mg/mL; retention time (min): **2a** (24.71), **2b** (26.74); for other analytical conditions: see text. (B) Column: Zorbax SIL; Sample solution: 0.4 mg/mL; retention time (min): **2a** (28.82), **2b** (19.62); for other analytical conditions: see text.

Table 2: HPLC conditions for isomers **2a** and **2b**

HPLC conditions	Isomers **2a** and **2b**
Column	Cosmosil C18 AR (5 μm, 250 x 4.6 mm i.d., Nacalai Tesque Inc.)
Mobile phase	MeOH-H_2O (60:40)
Flow rate	1 mL/min
Column temperature	40°C
Detector	UV detection for 254 nm
Sample size	5 μL
Column	Zorbax SIL (5 μm, 250 x 4.6 mm i.d., Dupone)
Mobile phase	EtOH-*n*-hexane (10:90)
Flow rate	1 mL/min
Column temperature	40°C
Detector	UV detection for 254 nm
Sample size	5 μL

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