

Transnitrosation by *N*-Aryl-*N*-nitrosoareas; NO-Carrying *O*-Nitrosoisourea

Masayuki TANNO,* Shoko SUBYOSHI, and Naoki MIYATA

National Institute of Hygienic Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158, Japan.

Received March 1, 1994; accepted April 18, 1994

Transfer of nitroso groups, so-called transnitrosation, from aromatic *N*-nitroso compounds such as *N*-nitrosoareas, *N*-nitrosamides and *N*-nitrosamines, to aromatic amines or ureas was observed under non-acidic conditions at room temperature. Sterically hindered 3,3-dibenzyl-1-(4-tolyl)-1-nitrosoarea (1a) rapidly nitrosates indoline, *N*-alkylanilines or 3-methyl-1-(4-tolyl)urea to give their *N*-nitroso derivatives. In the case of *N,N*-dimethylanilines, nitrosative demethylation occurs to give *N*-methyl-*N*-nitrosanilines. The transnitrosation is accelerated by electron-releasing groups on the nitroso acceptors, *N*-alkylanilines. The transnitrosation mechanism is considered to be as follows: *N*-nitrosoarea (1) thermally decomposes to nitric oxide and ureidyl radical followed by formation of an *O*-nitrosoisourea intermediate (10), which acts as an NO-carrying agent and nitrosates anilines or ureas.

Keywords nitrosoarea; nitrosoisourea; nitrosamine, nitric oxide; dealkylation; transnitrosation

The migration of a nitroso group from *N*-nitroso compounds to nucleophilic species (secondary amines, alcohols, etc.), so-called transnitrosation, usually occurs under acidic conditions.^{1,2)} This reaction has been studied in connection with the production of carcinogenic nitrosamine in the human stomach.³⁾ Biological nitrosation by the catalytic action of bacteria has been observed even in high pH stomachs of patients with hypochlorhydria⁴⁾ and in the bladders of patients with urinary-tract infections.⁵⁾ Transnitrosation under non-acidic conditions⁶⁾ is little understood. We previously reported that *N*-aryl-*N*-nitrosoareas (1, 2) generate nitric oxide (NO) via radical cleavage of an N–NO bond in a non-acidic solvent at room temperature. Generation of NO was confirmed by trapping as an *N,N'*-ethylenebis(salicylideneiminato)iron–NO₃ complex.⁷⁾ The thermal decompositions of aromatic *N*-nitrosoareas (1, 2) and *N*-nitrosamide (3) proceed by parallel reactions of radical cleavage of an N–NO bond (path A in Chart 1) and diazo ester rearrangement (path B) to give products such as ureas (5, 6), nitro compounds (14), triazenes (15) and diazonium salts, as shown in Chart 1.^{8–11)}

Examples of thermal transnitrosation under non-acidic conditions through the radical N–NO bond cleavage pathway have been demonstrated only in aromatic *N*-nitrosamines,^{6d)} though no detailed study on transnitrosation has been conducted using acyl-type aromatic *N*-nitrosoareas and *N*-nitrosamides. This may be due to the

ease of characteristic diazo ester rearrangement¹²⁾ rather than N–NO bond radical cleavage, in contrast to aromatic *N*-nitrosamines.

3-Benzyl-1-(4-tolyl)-1-nitrosoarea (2a) and 3-methyl-1-(4-tolyl)-1-nitrosoarea (2b) rearranged to the corresponding 3-nitroso isomers (4a, b) by intramolecular 1,3-nitroso shift from the N¹-position toward the N³-position of the ureido group in non-acidic solvents at 33 °C.^{9,10)} Inter-molecular nitrosation of 3-methyl-1-(4-tolyl)urea (6b) with 2a as well as 3-isopropyl-1-(4-tolyl)-1-nitrosoarea (2c) gave 3-methyl-1-(4-tolyl)-3-nitrosoarea (4b) in carbon tetrachloride at 33 °C.^{9,10)}

The present paper describes non-acidic transnitrosation using various *N*-nitroso compounds (1–4, 7, 8), as shown in Fig. 1. The relationship between ease of transnitrosation and structural characteristics of *N*-nitroso compounds is discussed.

Results and Discussion

Transnitrosation with 3,3-Dibenzyl-1-(4-tolyl)-1-nitrosoarea (1a) to *N*-Alkylanilines (9) Compound 1a reacted with *N*-alkylanilines (9) in CCl₄ to produce *N*-alkyl-*N*-nitrosanilines (8) accompanied with 3,3-dibenzyl-1-(4-tolyl)urea (5a). The yields of transnitrosation products (8) were 12–93% (Chart 2, Table I). The time-course of *N*-nitrosaniline (8) formation in the reactions of 1a and *p*-substituted *N*-methylanilines (9a–e) is shown in Fig. 2. The formation of the denitrosated product, 5a increased

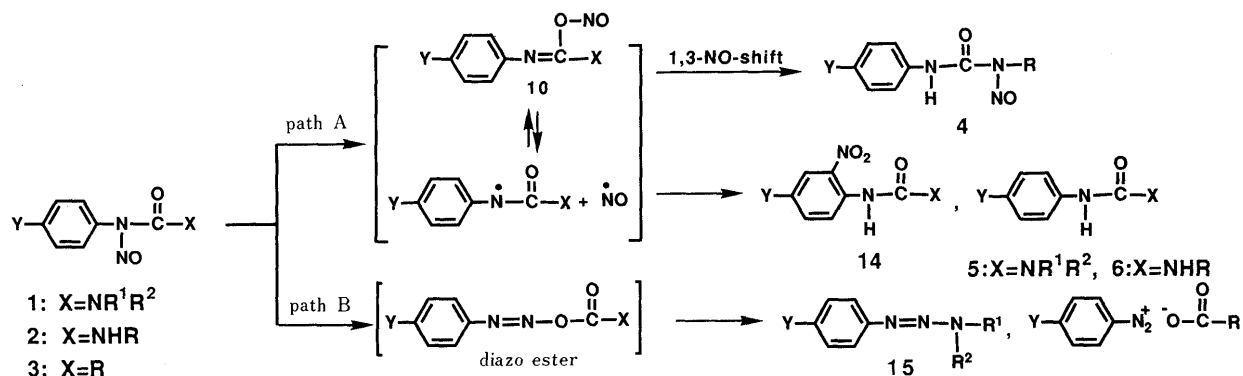


Chart 1

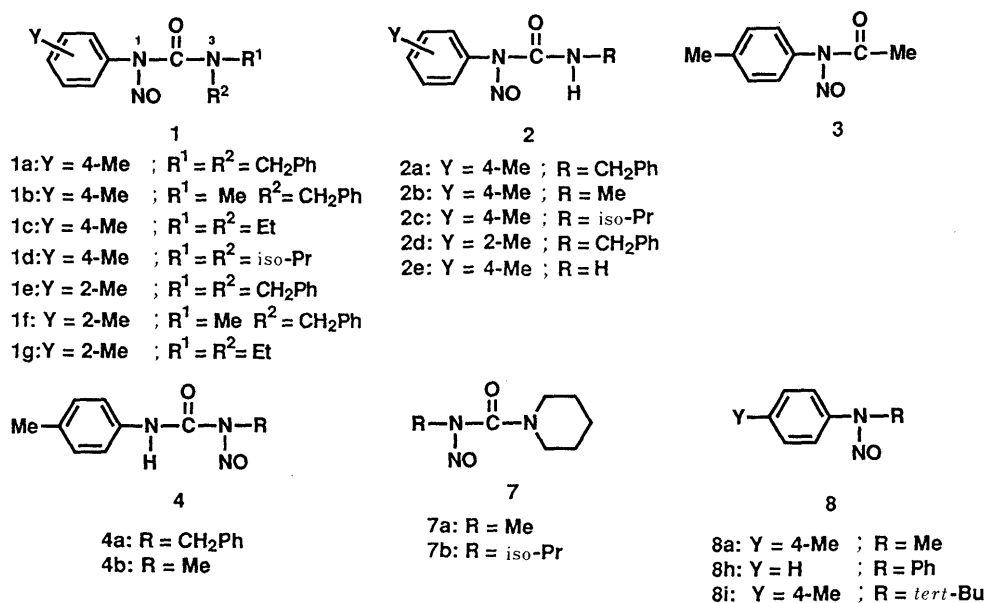


Fig. 1

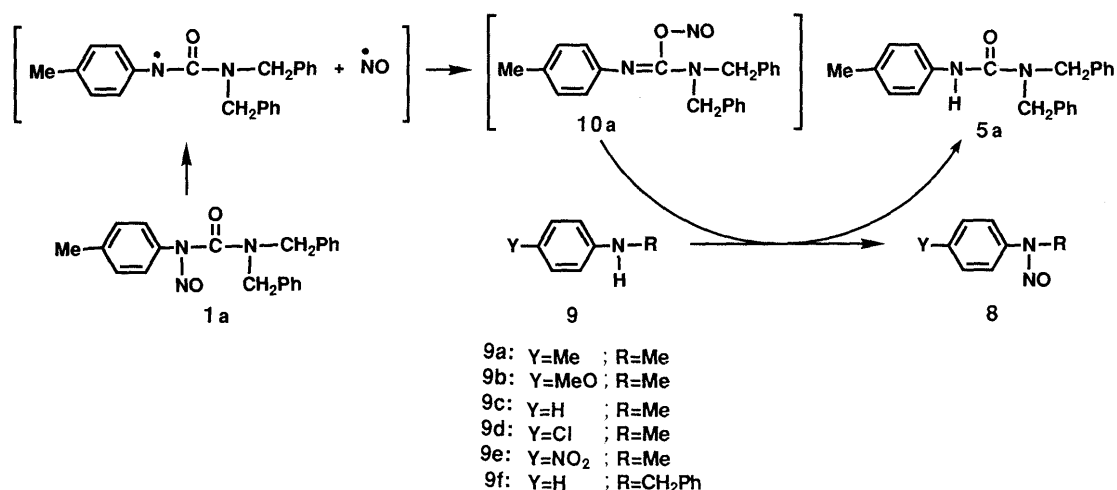


Chart 2

TABLE I. Yields of *N*-Nitrosamines (8) Formed in the Reaction of *N*-Alkylanilines (9) with 3,3-Dibenzyl-1-(4-tolyl)-1-nitrosourea (1a) in CCl₄ at 20 °C^{a)}

Compd.	Y-C ₆ H ₄ NHR		Reaction time (h)	Yield ^{b)} of 8 (%)
	Y	R		
9a	4-Me	Me	5.5	53
9b	4-MeO	Me	3.0	93
9c	H	Me	5.5	42
			1.2 ^{c)}	53
9d	4-Cl	Me	5.5	42
9e	4-NO ₂	Me	5.5	12
			0.8 ^{c)}	67
9g	H	Bn	1.1 ^{c)}	49

a) Concentrations of 1a and 9 were 4.20 × 10⁻³ M. Bn = benzyl. b) Determined by HPLC. c) Reacted at 33 °C.

TABLE II. Rate Constant for the Decomposition of 3,3-Dibenzyl-1-(4-tolyl)-1-nitrosourea (1a) in the Presence of *N*-Methylanilines^{a)} (9) in CCl₄ at 20 °C

Compd.	Y-C ₆ H ₄ NHMe Y	Concentration of 1a × 10 ⁻³ M	Rate constant ^{b)} k × 10 ⁻⁴ s ⁻¹
9a	4-Me	1.0	0.92
9b	4-MeO	1.0	3.18
9c	H	1.0	—
9d	4-Cl	0.25	0.98
		1.0	0.92
		2.0	1.12
		4.0	1.12
9e	4-NO ₂	1.0	0.92
		1.0	0.55 ^{c)}

a) Concentration of *N*-methylanilines (9) was 1.0 × 10⁻³ M. b) Rate constant for the disappearance of 1a. c) Rate constant for the decomposition of 1a in the absence of 9.

with the production of *N*-nitrosanilines (8). In this transnitrosation, electronic effects of *p*-substituents of *N*-methylanilines (9a–e) were observed: electron-releasing

groups at the *p*-position of anilines increased the yield of 8, while electron-attracting groups decreased them, implying that the basicity of aniline nitrogen of 9 influences

the reaction yield.

In the reaction of **1a** and *N*-methyl-4-chloraniline (**9d**), the decomposition rate for **1a** obeyed first-order kinetics (Table II), though formally, it should have followed second-order kinetics. First-order kinetics was also noted in the transnitrosation of **1a** to 3-methyl-1-(4-tolyl)urea (**6b**). The rate constants ranged from $0.92 \times 10^{-4} \text{ s}^{-1}$ to

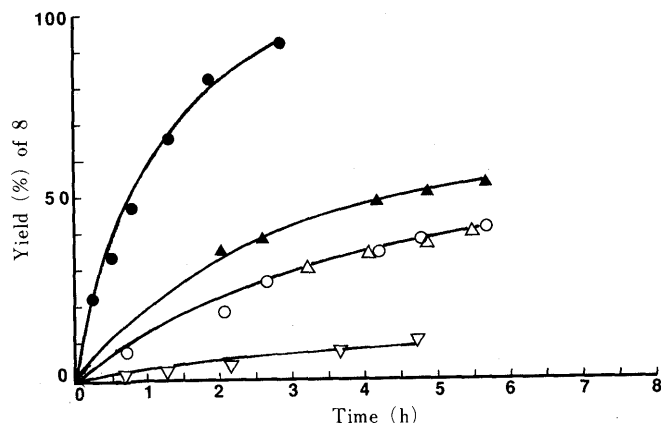


Fig. 2. Formation of *N*-Nitrosamines (**8a–e**) by the Reaction of *p*-Substituted *N*-Methylanilines (**9a–e**) with 3,3-Dibenzyl-1-(4-tolyl)-1-nitroso-urea (**1a**) in CCl_4 at 20°C

●, *p*-OMe; ▲, *p*-Me; △, *p*-H; ○, *p*-Cl; ▽, *p*-NO₂.

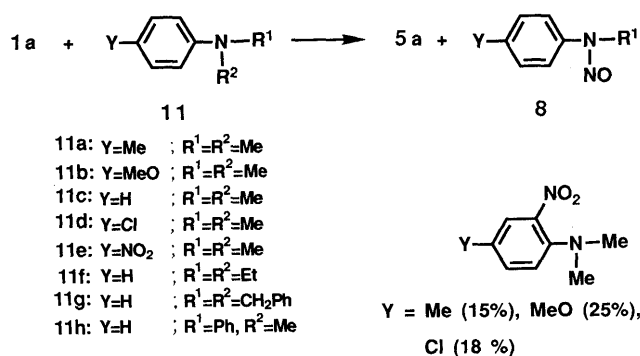
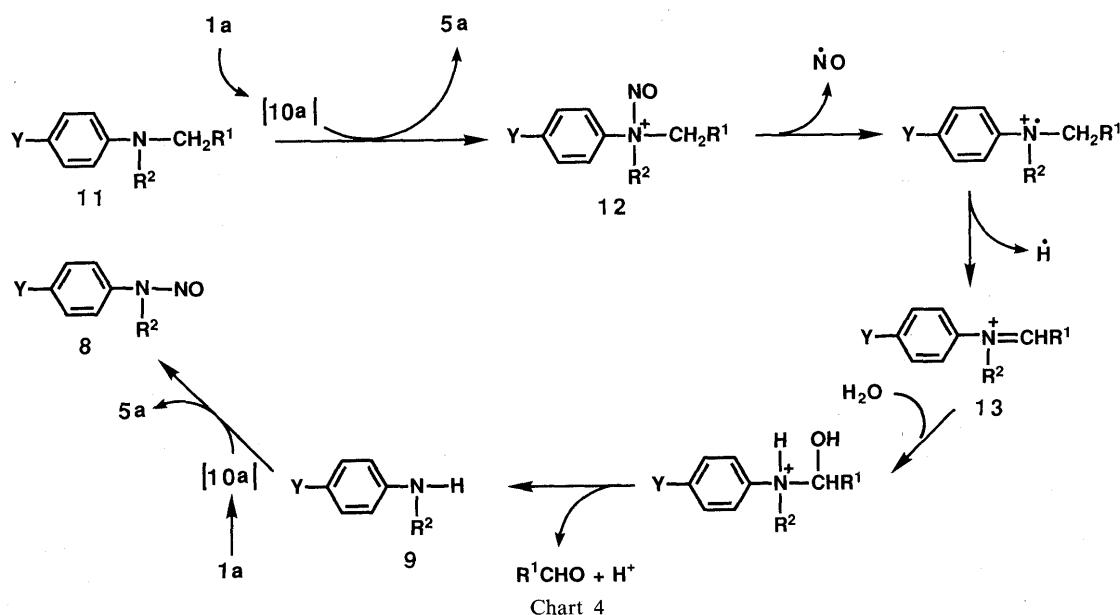


Chart 3



$1.12 \times 10^{-4} \text{ s}^{-1}$. Nevertheless, there was variation in the ratio of **1a** and **9**, and the rate constant was close to the value ($0.55 \times 10^{-4} \text{ s}^{-1}$) in the self-decomposition of *N*-nitroso-urea (**1a**). Thus, an intermediate appears to participate in the reaction. We previously proposed an *O*-nitroso intermediate (**10**)^{7,10} in the self-decomposition of acyl-type aromatic *N*-nitroso compounds (Chart 1). Such an intermediate (**10**) may be a nitrosating agent in transnitrosation. Our proposed non-acidic transnitrosation mechanism is illustrated in Chart 2. The N–NO bond radical cleavage of *N*-nitroso-urea (**1a**) occurs first by thermal decomposition. The resulting NO reacts with ureidyl radical to produce the *O*-nitroso intermediate (**10a**). This intermediate acts as an NO-carrying agent and leads to the urea (**5a**) after nitrosating **9**.

The disappearance rate constant of *N*-methyl-4-methoxyaniline (**9b**) was exceptionally large among *p*-substituted anilines, possibly due to the powerful electron-releasing effect of the MeO group and particularly high

TABLE III. Yields of *N*-Nitrosamines (**8**) Formed in the Reaction of Tertiary Amines (**11**) with 3,3-Dibenzyl-1-(4-tolyl)-1-nitroso-urea (**1a**) in CCl_4 at 20°C ^a

Compd.	Y-C ₆ H ₄ NR ¹ R ²			Reaction time (h)	Yield ^b of 8 (%)
	Y	R ¹	R ²		
11a	4-Me	Me	Me	0.83	54
11b	4-MeO	Me	Me	1.3	25
11c	H	Me	Me	1.3	25
				0.9 ^c	47
				24.0 ^d	47 ^e
11d	4-Cl	Me	Me	2.0	25
11e	4-NO ₂	Me	Me	6.0	—
				6.0 ^c	—
11f	H	Et	Et	0.78 ^c	46
11g	H	Bn	Bn	0.87 ^c	12
11h	H	Ph	Me	0.57 ^c	6

^a Concentrations of **1a** and **11** were $4.20 \times 10^{-3} \text{ M}$. Bn = benzyl. ^b Yield by HPLC. ^c Reacted at room temperature. ^e Isolated yield.

reactivity of **9b** toward the intermediate (**10**). A clear explanation for the abnormal rate constant is not available at present.

Dealkylative Nitrosation of *N,N*-Dialkylanilines (11**) with **1a**** Under similar reaction conditions to those used for *N*-alkylanilines (**9**), **1a** reacted with *N,N*-dimethylanilines (**11a–e**) to give the demethylated nitrosative products, *N*-methyl-*N*-nitrosanilines (**8a–e**) in 6–54% yields (Chart 3, Table III). They were identified by nitrosation of the corresponding *N*-methylanilines (**9**). Formaldehyde was detected in the reaction of **1a** with *N,N*-dimethylaniline (**11c**). The reaction of **1a** with *N,N*-diethylaniline (**11f**) and *N,N*-dibenzylaniline (**11g**) gave *N*-ethyl-*N*-nitrosaniline (**8j**) and *N*-benzyl-*N*-nitrosaniline (**8k**), respectively. In the case of *N*-methyldiphenylamine (**11h**), *N*-nitrosodiphenylamine (**8h**) was obtained by demethylating nitrosation. Nitrosative dearylation was not observed. The formation of *N,N*-dimethyl-2-nitraniline derivatives in 15–25% yields together with the corresponding dealkylated nitrosation products (**8a, b, d**) was observed.

The proposed mechanism for demethylating transnitrosation is shown in Chart 4. After the *O*-nitroso intermediate (**10a**) derived from the N–NO bond radical cleavage of *N*-nitrosoarene (**1a**) reacts with *N,N*-dialkylaniline (**11**) to produce a nitrosammonium ion (**12**), **12** decomposes to give iminium ions (**13**). The addition of water to the ions (**13**) gives aldehyde and *N*-monoalkylaniline (**9**), and the resulting **9** is nitrosated again with **1a** to form *N*-alkyl-*N*-nitrosaniline (**8**). This non-acidic dealkylating nitrosation mechanism is similar to the Leoppky–Tomasik mechanism^{13a)} for dealkylating nitrosation under acidic conditions using sodium nitrite in glacial acetic acid at 85–90 °C, and to the Verardo–Giumanini–Strazzolini mechanism^{13b)} for dealkylating nitrosation by refluxing of *N,N*-dimethylanilines with excess alkyl nitrites under non-acidic conditions. Our non-acidic dealkylation occurred at room temperature. This difference is due to homolytic fission, which proceeds preferentially in aprotic solvents, and *N,N*-dialkylanilines are considered to be predominantly nitrosated with the *O*-nitroso intermediate rather than the nitrosonium cation (NO⁺) intermediate.

The formation of a nitro compound appears to support NO radical release from **12**.¹⁰⁾

Transnitrosation with *N*-Nitrosoarenes (1**) to 3-Methyl-1-(4-tolyl)urea (**6b**)** Compound **6b** was used as a nitroso group acceptor having a less basic amido nitrogen instead

of anilines. When three *N*-nitrosoarenes, 3,3-dibenzyl- (**1a**), 3-benzyl-3-methyl- (**1b**), and 3,3-diethyl-1-(4-tolyl)-1-nitrosoarene (**1c**), were used as transnitrosating agents, **6b** was converted to 3-methyl-1-(4-tolyl)-1-nitrosoarene (**2b**) and its 3-nitroso isomer (**4b**) (Chart 5) in the yields given in Table IV.

Formation curves of transnitrosated products (**2b, 4b**) are compared in Fig. 3a and 3b. Compound **1**, having bulky substituents, rapidly gave **2b** and **4b**. The first-order rate constants decreased in the order of bulkiness of the N³-alkyl groups; **1a** (dibenzyl) > **1b** (benzyl,methyl) > **1c** (diethyl). It is of particular interest that 1-nitrosoarene (**2b**) was produced predominantly compared with the 3-nitroso isomer (**4b**). The basicity of the N¹-nitrogen of the urea (**6b**) adjacent to the tolyl group must surely have been less than that of the N³-nitrogen bonded to the methyl group. The formation of **2b** started earlier in the reaction than that of **4b**. Time-courses of formation of the products obtained by reaction of **1c** with **6b** indicated a 1,3-shift of the nitroso group after 6 h.

Transnitrosation with *N*-Nitroso Compounds to Indoline (16**)** When *N*-methylanilines (**9**) or indoline (**16**) of high basicity were used as nitroso acceptors in the transnitrosation of **1c**, the self-decomposition product, 3,3-diethyl-1-(4-tolyl)triazene (**15c**), was not observed. *N*-Methyl-*N*-nitrosaniline (**8c**) or *N*-nitrosoindoline (**17**) was produced

TABLE IV. Rate Constant for the Decomposition of 3,3-Dialkyl-1-(4-tolyl)-1-nitrosoarenes (**1a–c**) and Yields of 3-Methyl-1-(4-tolyl)-1-nitrosoarene (**2b**) and Its 3-Nitroso Isomer (**4b**) in the Reaction of **1a–c** with 3-Methyl-1-(4-tolyl)urea (**6b**)^{a)} in CCl₄ at 33 °C

Compd.	Y-C ₆ H ₄ N(NO)CONR ¹ R ²			Rate constant ^{b)} (<i>k</i> × 10 ^{−4} s ^{−1})	Reaction time (h)	Yield of products (%) ^{c)}	
	Y	R ¹	R ²			2b	4b
1a	4-Me	Bn	Bn	3.49	2.0	17.0	7.4
				2.94 ^{d)}	2.0	—	—
1b	4-Me	Bn	Me	1.35	2.0	10.4	2.0
					4.0	17.5	3.7
1c	4-Me	Et	Et	1.12 ^{d)}	2.0	—	—
				0.65	2.0	2.5	0.0
					4.0	4.2	0.0
					6.0	8.0	16.0
				0.60 ^{d)}	2.0	—	—

a) Initial concentrations of *N*-nitrosoarenes (**1a–c**) and 3-methyl-1-(4-tolyl)urea (**6b**), 3.4 × 10^{−3} M. b) Rate constant of the decomposition for the *N*-nitrosoarenes (**1a–c**). c) Determined by HPLC. d) Blank, self-decomposition of *N*-nitrosoarene (**1**).

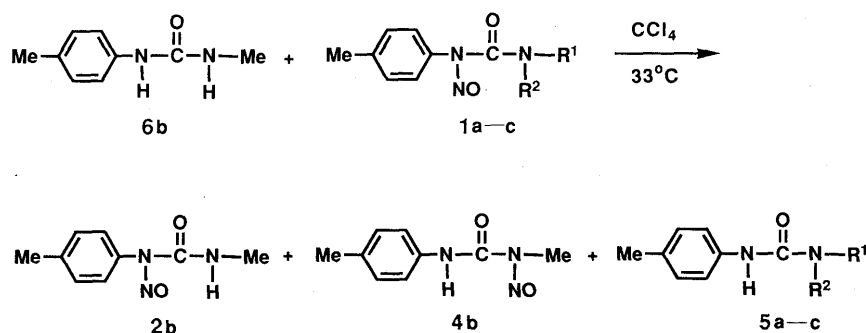


Chart 5

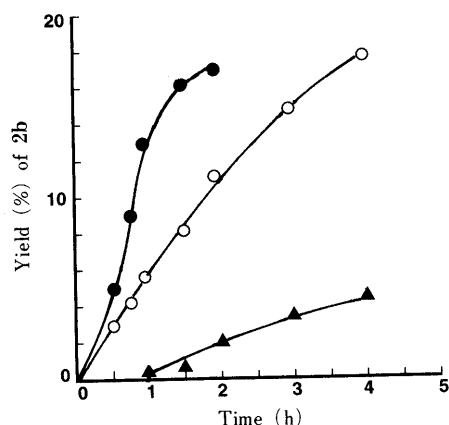


Fig. 3a. Formation of 3-Methyl-1-(4-tolyl)-1-nitrosoourea (**2b**) by the Reaction of 3,3-Dialkyl-1-(4-tolyl)-1-nitrosoourea (**1a–c**) with 3-Methyl-1-(4-tolyl)-1-nitrosoourea (**6b**) in CCl_4 at 33°C

●, **1a**; ○, **1b**; ▲, **1c**.

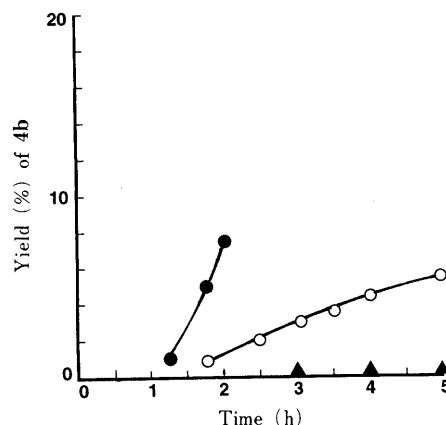


Fig. 3b. Formation of 3-Methyl-1-(4-tolyl)-3-nitrosoourea (**4b**) by the Reaction of 3,3-Dialkyl-1-(4-tolyl)-1-nitrosoourea (**1a–c**) with 3-Methyl-1-(4-tolyl)urea (**6b**) in CCl_4 at 33°C

●, **1a**; ○, **1b**; ▲, **1c**.

TABLE V. Yields of *N*-Nitrosoindoline (**17**) Formed in the Reaction of Indoline (**16**) with *N*-Nitroso Compound (**1–3, 7, 8**) in CCl_4 at 33°C ^{a)}

Compd.	X-N(NO)-Y		Reaction time (h)	Yield ^{b)} of 17 (%)
	X	Y		
1a	4-Tol	CON(Bn) ₂	0.5 10.0	88 87 ^{c)}
1b	4-Tol	CONMeBn	1.2	75
1c	4-Tol	CON(iso-Pr) ₂	1.0	62
1d	4-Tol	CON(Et) ₂	1.2	47
1e	2-Tol	CON(Bn) ₂	1.0	73
1f	2-Tol	CONMeBn	1.7	80
1g	2-Tol	CON(Et) ₂	2.2	90
2a	4-Tol	CONHBn	8.0	59
2d	2-Tol	CONHBn	24.0	7
2e	4-Tol	CONH ₂	3.0	26 ^{d)}
3	4-Tol	COMe	0.8	43
7a	Me	CONC ₅ H ₁₀	24.0	0
7b	iso-Pr	CONC ₅ H ₁₀	24.0	0
8a	4-Tol	Me	24.0	16
8h	Ph	Ph	24.0	53
8i	4-Tol	<i>tert</i> -Bu	24.0	9

a) Concentrations of *N*-nitroso compounds and indoline were 4.20×10^{-3} M. Bn = benzyl, Tol = tolyl. b) Determined by HPLC. c) Reacted at 0°C . d) Reacted in CHCl_3 .

in fairly good yield. Many aromatic *N*-nitroso compounds (**1–3, 8**) reacted with indoline (**16**) to give *N*-nitrosoindoline (**17**) in carbon tetrachloride at 33°C . The results of transnitrosation are summarized in Table V. The yield of **17** per unit time was excellent when trisubstituted *N*-aryl-*N*-nitrosooureas (**1a–g**) were used. With 3,3-dibenzyl-1-(4-tolyl)-1-nitrosoourea (**1a**), a particularly high yield of 88% was obtained in 0.5 h. Among disubstituted *N*-aryl-*N*-nitrosooureas (**2a–d**), the 4-tolyl derivative (**2a**) gave **17** in moderate yield, but the 2-tolyl isomer (**2d**) formed **17** in only 7% yield after 24 h. With aliphatic *N*-nitrosooureas (**7**), no *N*-nitrosoindoline (**17**) was produced.

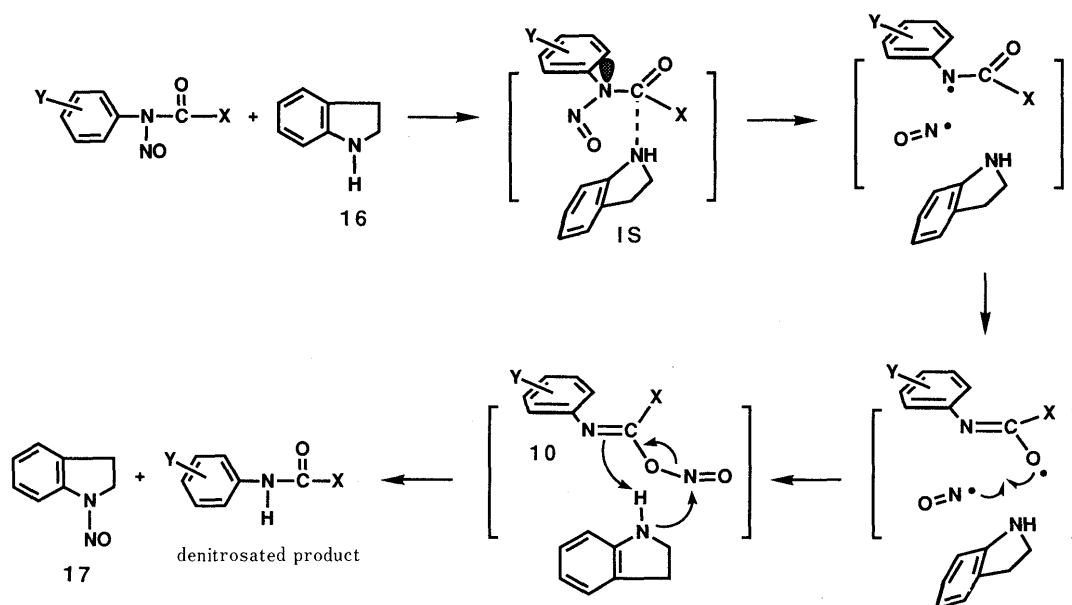
N-Nitroso-4-tolylacetamide (**3**) produced *N*-nitrosoindoline (**17**) in 43% yield. This is anomalous, since the amido carbonyl carbon in the aromatic *N*-nitrosamide (**3**) is positively polarized compared with that of ureido

carbonyl in aromatic *N*-nitrosooureas (**1, 2**).¹¹⁾ Compound **3** should thus favor diazo ester rearrangement (path B in Chart 1) rather than the N–NO bond cleavage (path A). This can be explained as due to interaction between the *N*-nitroso compound and indoline. We reported that aromatic *N*-nitroso compounds can adopt two conformers.¹¹⁾ One, with a co-planar phenyl–N–NO part, may undergo NO radical fission (path A) and the other, in a twist form, may undergo diazo ester rearrangement (path B). Indoline nitrogen is attracted to the polarized carbonyl carbon of *N*-nitrosamide in a twist conformer (Chart 6). Approach of the nitroso group to the carbonyl and formation of the diazo ester intermediate are inhibited. Consequently, *O*-nitroso intermediate formation by N–NO bond radical cleavage is accelerated.

Aromatic *N*-nitrosamines (**8**) are of particular interest, for they can not form *O*-nitroso compounds corresponding to those produced from *N*-nitrosooureas and *N*-nitrosamides. The transnitrosation of *N*-substituted *N*-nitrosanilines occurred in the order of phenyl > methyl > *tert*-butyl as *N*-substituents. *N*-Nitrosodiphenylamine (**8h**) produced the NO radical in organic solvents^{6c,d)} and nitrosated piperidine or diethylamine *via* the nitroxide radical intermediate, $\text{Ph}_2\text{N-N}(\text{O})\cdot$ obtained by reaction of **8h** and secondary amines.^{6d)} A similar intermediate may be expected in the present reaction.

The ease of radical cleavage of the N–NO bond would appear to be reflected by the C form in the resonance hybrid, Ar-N-N=O (A) $\leftrightarrow$ $\text{Ar-N}^+=\text{N-O}^-$ (B) $\leftrightarrow$ $\text{Ar}^-=\text{N}^+-\text{N=O}$ (C) (Ar = aromatic ring). A contribution of the resonance hybrid C has not been observed in aliphatic nitrosamines. Perhaps the N–NO bond radical fission of aromatic *N*-nitroso compounds occurs through one-electron transfer with a single bond contribution at the N–NO bond in a transition state.

Since the π electronic system of the aromatic ring and lone pair of nitrogen bonded to nitroso group are conjugated, N–NO bonds in aromatic *N*-nitrosamines easily undergo radical cleavage compared with aliphatic



N-nitroso compounds. We previously discussed conjugation based on a ^{13}C -NMR experiment in which the *ortho*-carbons of phenyl in *N*-methyl-*N*-nitroso-4-toluidine (**8a**) resonated at higher field than those of *N*-*tert*-butyl-*N*-nitroso-4-toluidine (**8i**).¹¹ This may have been due to differences in the degree of twisting about phenyl- $\text{N}(\text{NO})$ bonds in the nitrosamines **8**. Conjugation in compound **8i** having a bulky *tert*-butyl group is weakened compared with that in other nitrosamines (**8a, h**)¹¹ having a methyl or phenyl group owing to twisting of the $\text{Ph}-\text{N}(\text{NO})$ bond for steric reasons. The $\text{N}-\text{NO}$ bond of **8i** is thus thermally stable.

The $\text{N}-\text{NO}$ bonds of trisubstituted *N*-aryl-*N*-nitrosoureas (**1a–g**) are likely to be disrupted by an additional effect, besides conjugation, *i.e.*, the steric hindrance of the N^3 substituents and ureido carbonyl oxygen directed towards the nitroso oxygen.¹¹ Consequently, the 2-tolyl derivatives (**1e, f**) having non-conjugated $\text{N}-\text{NO}$ bonds due to twisting caused by steric hindrance between *ortho* methyl and nitroso groups is less transnitrosated than the 4-tolyl derivatives (**1b, c**) having planar forms.¹⁴ For disubstituted *N*-aryl-*N*-nitrosoureas (**2**), transnitrosation is similar to the case of *N*-aryl-*N*-nitrosanilines (**8**) since the effects of steric hindrance due to the N^3 -alkyl group and electrostatic repulsion between nitroso oxygen and carbonyl oxygen are small.

Conclusion

1) The important factors for $\text{N}-\text{NO}$ bond radical cleavage are planar conformation due to resonance and steric effects about the nitroso group in aromatic *N*-nitroso compounds. The ease of transnitrosation reflects the ease of liberation of the NO radical. Because of these effects, aromatic trisubstituted *N*-nitrosoureas (**1**) are easily transnitrosated under mild conditions compared with aromatic disubstituted *N*-nitrosoureas (**2**) and aromatic *N*-nitrosamines (**8**). Aliphatic *N*-nitroso compounds that lack a resonance effect are stable under the present

experimental conditions.

2) The basicity of the nitroso acceptor is important. An appropriate decrease in basicity is ideal for non-acidic transnitrosation, since the radical reaction may proceed efficiently under aprotic conditions.

3) In the transnitrosation of acyl-type aromatic *N*-nitroso compounds, the *O*-nitroso intermediate is formed after $\text{N}-\text{NO}$ bond radical cleavage and acts as a nitrosating agent. That is to say, *O*-nitrosoisourea is the NO -carrying intermediate in the transnitrosation of aromatic *N*-nitrosoureas and *N*-nitrosamides.

NO has been shown to be essential for physiological responses such as vasorelaxation, neurotransmission, and inhibition of tumor cells by cytotoxic macrophages.¹⁵ We examined the cytotoxic effects toward tumor cells of these transnitrosating compounds (**1–4, 8**) which generate the NO radical. These results will be reported later.

Experimental

All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO A-102 spectrometer. Proton and carbon nuclear magnetic resonance (^1H and ^{13}C -NMR) spectra were taken on a Varian Gemini-300 (300 Mz) NMR spectrometer with tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. High-performance liquid chromatography (HPLC) was done on a JASCO 880-PU chromatograph with an ultraviolet (UV) detector (JASCO 875-UV) operating at 254 nm, using a TSK-Gel LS 310K column (4×300 mm i.d., Toyo Soda) with *n*-hexane and ethyl acetate (80:20 or 87:13, v/v) as a mobile phase. Peak areas were determined with a Hitachi D-2500 Chromato-Integrator. The internal standard employed was 4-methyl-2-nitroacetanilide.^{8,9}

Materials *N*-Methylanilines (**9b, e**), *N,N*-dimethylanilines (**11c, e, f**), and indoline (**16**) were purchased from Wako Pure Chemical Industries, Ltd. Aromatic *N*-nitrosoureas (**1, 2**)^{7–10} and aliphatic *N*-nitrosoureas (**4, 7**)^{9–11} were prepared by nitrosation of the corresponding ureas (**5, 6**) according to the method described in our previous paper. A new aromatic *N*-nitrosourea (**2e**) was prepared by the procedures cited.¹⁶ White-yellow crystals, mp $85\text{--}86^\circ\text{C}$ (dec.). Yield, 30%. *Anal.* Calcd for $\text{C}_8\text{H}_9\text{N}_3\text{O}_2$: C, 53.62; H, 5.06; N, 23.45. Found: C, 53.63; H, 5.03; N, 23.45. IR (CHCl_3): 3520, 3410 (NH), 1730 (CO) cm^{-1} . ^{13}C -NMR [CDCl_3 -

CD₃OD (1:1), -5 — -10°C] δ : 128.9 (C-1), 127.6 (C-2,6), 130.2 (C-3,5), 140.6 (C-4), 155.5 (CO), 21.4 (Me).

Aromatic *N*-nitrosamides (**3**)^{12a} and *N*-nitrosamines (**8**)^{11,13} were prepared by the procedures cited.

Preparations of *N*-Methyl- (9a, b, d) and *N,N*-Dimethylanilines (11a, b, d)¹⁷ Typical Examples (9a and 11a): A solution of Me₂SO₄ (6.6 g, 0.052 mol) in CHCl₃ (100 ml) was added to a solution of 4-toluidine (5.3 g, 0.044 mol) in CHCl₃ (100 ml), while the temperature was kept below 5°C . The reaction mixture was stirred for 3 h, allowed to stand overnight at room temperature, and then evaporated to dryness under reduced pressure. The residue was extracted with CHCl₃, and the solvent was evaporated off. The residue was chromatographed on a column of silica gel with *n*-hexane-ether (9:1). The first fraction gave *N,N*-dimethyl-4-toluidine (**11a**) in 6% yield as an oily product [lit.^{17b} bp₇₆₀ 204—206 $^{\circ}\text{C}$], and the next fraction gave *N*-methyl-4-toluidine (**9a**) in 15% yield as an oily product [lit.^{17c} bp₇₁₅ 207—209 $^{\circ}\text{C}$]. **9a**: ¹³C-NMR (CDCl₃) δ : 147.7 (C-1), 113.0 (C-2, 6), 130.1 (C-3, 5), 126.6 (C-4), 31.0 (N-Me), 20.4 (Me). **11a**: ¹³C-NMR (CDCl₃) δ : 149.4 (C-1), 113.6 (C-2, 6), 130.0 (C-3, 5), 126.4 (C-4), 41.1 (N-Me), 20.3 (Me).

Other *N*-alkylanilines (**9b, d**) and *N,N*-dialkylanilines (**11b, d**) were similarly prepared. *N*-Methyl-4-methoxyaniline (**9b**): oily product [lit.^{17d} bp₁₉ 135—136 $^{\circ}\text{C}$]. Yield, 66.5%. ¹³C-NMR (CDCl₃) δ : 144.2 (C-1), 115.3 (C-2, 6), 114.1 (C-3, 5), 152.6 (C-4), 31.7 (N-Me), 56.0 (Me). *N*-Methyl-4-chloraniline (**9d**): oily product [lit.^{17e} bp₇₆₄ 239—240 $^{\circ}\text{C}$]. Yield, 15.5%. ¹³C-NMR (CDCl₃) δ : 140.8 (C-1), 120.0 (C-2, 6), 129.4 (C-3, 5), 121.9 (C-4), 30.8 (N-Me). *N,N*-Dimethyl-4-methoxyaniline (**11b**): mp 48.0—48.5 $^{\circ}\text{C}$ [lit.^{17b} mp 37—38.5 $^{\circ}\text{C}$]. Yield, 14.5%. ¹³C-NMR (CDCl₃) δ : 42.0 (N-Me), 56.0 (Me). *N,N*-Dimethyl-4-chloraniline (**11d**): mp 31.5—32.5 $^{\circ}\text{C}$ [lit.^{17b} mp 32—32.5 $^{\circ}\text{C}$]. Yield, 4.0%. ¹³C-NMR (CDCl₃) δ : 148.5 (C-1), 113.8 (C-2, 6), 129.4 (C-3, 5), 121.9 (C-4), 30.8 (N-Me).

Preparations of *N*-Nitrosanilines (8) and *N*-Nitrosoindoline (17) Typical Example (**8e**): A solution of sodium nitrite (170 mg, 2.5 mmol) in water (10 ml) was added in small portions to a solution of *N*-methyl-4-nitraniline (**9e**) (305 mg, 2.0 mmol) in 20% HCl (15 ml) under ice-water cooling. The reaction mixture was stirred for 30 min below 0°C , then cold water (20 ml) was added and the whole was neutralized with sodium bicarbonate, then extracted with CH₂Cl₂. The CH₂Cl₂ layer was filtered through a silicone-treated filter paper (1 ps phase separators, Whatman Ltd.) and evaporated under reduced pressure. The residue was recrystallized from ether to give *N*-methyl-*N*-nitroso-4-nitraniline (**8e**), mp 98.5—100 $^{\circ}\text{C}$ (dec.) [lit.^{18a} mp 100 $^{\circ}\text{C}$]. Yield, 306 mg (84%). ¹³C-NMR (CDCl₃) δ : 147.6 (C-1), 118.3 (C-2, 6), 125.7 (C-3, 5), 146.5 (C-4), 30.2 (N-Me).

Other nitrosamines (**8a, b, d, g, 17**) were similarly prepared. Compound **8b** was purified by silica gel column chromatography with a mixture of *n*-hexane and ether. *N*-Methyl-*N*-nitroso-4-toluidine (**8a**): mp 47.5—49.5 $^{\circ}\text{C}$ [lit.^{18b} mp 52.4 $^{\circ}\text{C}$]. Yield, 96.5%. ¹³C-NMR (CDCl₃) δ : 140.0 (C-1), 119.3 (C-2,6), 129.8 (C-3, 5), 137.2 (C-4), 31.4 (N-Me), 20.8 (Me). *N*-Methyl-*N*-nitroso-4-methoxyaniline (**8b**): oily product [lit.^{18c} mp 48—49 $^{\circ}\text{C}$]. ¹³C-NMR (CDCl₃) δ : 136.2 (C-1), 121.5 (C-2, 6), 115.0 (C-3, 5), 159.5 (C-4), 32.3 (N-Me), 55.7 (N-Me). *N*-Methyl-*N*-nitroso-4-chloraniline (**8d**): mp 48—48.5 $^{\circ}\text{C}$ [lit.^{18d} mp 51 $^{\circ}\text{C}$]. Yield, 80.8%. ¹³C-NMR (CDCl₃) δ : 140.8 (C-1), 120.0 (C-2, 6), 129.4 (C-3, 5), 132.7 (C-4), 30.8 (N-Me). *N*-Benzyl-*N*-nitrosaniline (**8g**): mp 57.5—58.5 $^{\circ}\text{C}$ [lit.^{18e} mp 57—58 $^{\circ}\text{C}$]. Yield, 75.4%. *N*-Nitrosoindoline (**17**): mp 83—84 $^{\circ}\text{C}$ [lit.^{18f} mp 83—84 $^{\circ}\text{C}$]. Yield, 95.5%.

Transnitrosation of *N*-Nitrosoarene (1a) to *N*-Alkylanilines (9) 3,3-Dibenzyl-1-(4-tolyl)-1-nitrosoarene (**1a**) (7.3 mg, 0.02 mmol) was added to a solution of an *N*-alkylaniline (**9**) (0.02 mmol) in CCl₄ (10 ml). The solution was kept at 20°C or 33°C . The yields of the corresponding *N*-nitrosaniline (**8**), denitrosated 3,3-dibenzyl-1-(4-tolyl)urea (**5a**) and 3,3-dibenzyl-1-(2-nitro-4-tolyl)urea (**14a**) were determined by the HPLC method. The results are shown in Table I and Fig. 2.

Formation of *N*-Alkyl-*N*-nitrosanilines (8) from *N,N*-Dimethylanilines (11) by Demethylating Transnitrosation Compound **1a** (7.3 mg, 0.02 mmol) was added to a solution of an *N,N*-dimethylaniline (**11**) (0.02 mmol) in CCl₄ (10 ml). The solution was kept at 20°C or 33°C . The yields of *N*-methyl-*N*-nitrosanilines (**8**) were determined by the HPLC method, and the results are shown in Table III.

When **1a** was allowed to react with **11a—d** in CCl₄ for 4.5 h at 20°C , *N,N*-dimethyl-2-nitranilines were produced in 8.9—16.6% yields, besides the formation of *N*-methyl-*N*-nitrosanilines (**8**) (Table III). These nitro compounds were identified by comparison with authentic samples.^{19a—c}

N,N-Dimethyl-2-nitro-4-toluidine: oily product. Yield, 16.6%. ¹H-NMR (CDCl₃)^{19a,b} δ : 2.30 (3H, s), 2.85 (6H, s), 6.96 (1H, d, $J=8.4\text{ Hz}$), 7.23 (1H, dd, $J=8.1\text{ Hz}$), 7.58 (1H, d, $J=1.44\text{ Hz}$). ¹³C-NMR (CDCl₃) δ : 144.9 (C-1), 140.3 (C-2), 134.7 (C-3), 130.5 (C-4), 126.8 (C-5), 118.9 (C-6), 42.9 (N-Me), 20.1 (Me). *N,N*-Dimethyl-2-nitro-4-methoxyaniline: oily product. Yield, 15.4%. ¹H-NMR^{19a,b} (CDCl₃) δ : 2.80 (3H, s), 3.79 (6H, s), 7.0—7.3 (3H, m). ¹³C-NMR (CDCl₃) δ : 141.5 (C-1), 141.8 (C-2), 110.0 (C-3), 153.4 (C-4), 121.3 (d, C-5, 6), 43.7 (N-Me), 56.1 (MeO). *N,N*-Dimethyl-2-nitro-4-chloraniline: mp 55—56 $^{\circ}\text{C}$ [lit.^{19c} mp 55—56 $^{\circ}\text{C}$]. Yield, 8.9%. ¹H-NMR (CDCl₃) δ : 2.89 (6H, s), 6.97 (1H, d, $J=8.1\text{ Hz}$), 7.35 (1H, dd, $J=9.0\text{ Hz}$), 7.77 (1H, d, $J=2.7\text{ Hz}$). ¹³C-NMR (CDCl₃) δ : 145.4 (C-1), 139.3 (C-2), 133.7 (C-3), 122.8 (C-4), 126.7 (C-5), 119.7 (C-6), 42.6 (N-Me).

Demethylating nitrosation using other *N,N*-dialkylanilines was also undertaken similarly. The results are shown in Table III.

Isolation of *N*-Methyl-*N*-nitrosanilines (8) after Demethylating Transnitrosation: Compound **1a** (180 mg, 0.5 mmol) was added to *N,N*-dimethylaniline (**11c**) (60 mg, 0.5 mmol) in CCl₄ (10 ml). The solution was allowed to stand overnight at room temperature. The solvent was evaporated off, and the residue was chromatographed on a column of silica gel with a mixture of *n*-hexane and ether (4:1). The first fraction gave 3,3-dibenzyl-1-(4-tolyl)triazene (**15a**) (1 mg). The second fraction gave **11c** (1.65 mg). The third fraction gave *N,N*-dibenzyl-*N*-nitrosamine (3.7 mg). The last fraction gave *N*-methyl-*N*-nitrosaniline (**8c**) (31.8 mg, 47%).

Detection of Formaldehyde Formed in Demethylating Transnitrosation: When the reagent for formaldehyde detection (Tokyo Kasei Ltd.) was added to the reaction mixture of **1a** and **11c** dissolved in CCl₄, the solution changed from yellow to red color as the reaction progressed.

Transnitrosation of *N*-Nitrosoarene (1a—c) to the Urea (6b) A 3,3-dialkyl-1-(4-tolyl)-1-nitrosoarene (**1a—c**) (0.034 mmol) was added to a suspension of 3-methyl-1-(4-tolyl)urea (**6b**) (5.74 mg, 0.034 mmol) in CCl₄ (10 ml) containing an internal standard. The solution was kept at 33°C for 6 h. The time course of the yields of 3-methyl-1-(4-tolyl)-1-nitrosoarene (**2b**) and 3-methyl-1-(4-tolyl)-3-nitrosoarene (**4b**), which were determined by the HPLC method, was plotted.

In the reaction of 3,3-diethyl-1-(4-tolyl)-1-nitrosoarene (**1c**) with **6b**, the denitrosation product 3,3-diethyl-1-(4-tolyl)urea (**5c**), 3,3-diethyl-1-(2-nitro-4-tolyl)urea (**14c**), and 3,3-diethyl-1-(4-tolyl)triazene (**15c**) were also determined by the HPLC method. These products (**2b, 4b, 5c, 14c, 15c**) were identified by comparison with authentic samples obtained by the method described in our previous paper.^{9,10} The yields of products **2b, 4b, 5c, 14c** and **15c** were 8%, 16%, 24%, 47% and 12%, respectively. The results are shown in Table IV and Fig. 3a,b.

Transnitrosation of Various *N*-Nitrosoarene (1—4, 7, 8) to Indoline (16) Typical Example (**1a**): Compound **1a** (7.3 mg, 0.02 mmol) was added to a solution of indoline (**16**) (0.02 mmol) in CCl₄ (10 ml). The solution was kept at 20°C or 33°C . The yield of *N*-nitroindoline (**17**) was determined by the HPLC method. The transnitrosation to indoline using other *N*-nitroso compounds was examined similarly, and the results are shown in Table V.

Kinetics of the Transnitrosation All rate measurements were carried out at 20°C using HPLC, by following the disappearance of the peak of **1a** in CCl₄ containing *N*-alkylanilines (**9a—f**) or containing 3-methyl-1-(4-tolyl)urea (**6b**) in the presence of an internal standard as a function of time using a chart recorder. The results are given in Tables II and IV.

References

- 1) B. C. Challis, M. R. Osborne, *J. Chem. Soc., Perkin Trans. 2*, **1973**, 1526.
- 2) a) S. S. Singer, B. B. Cole, *J. Org. Chem.*, **46**, 3461 (1981); b) R. Bonnett, P. Nicolaidou, *J. Chem. Soc., Perkin Trans. 1*, **1979**, 1969.
- 3) a) S. S. Singer, W. Lijinsky, G. M. Singer, *Tetrahedron Lett.*, **1977**, 1613; b) S. S. Singer, G. M. Singer, B. B. Cole, *J. Org. Chem.*, **45**, 3931 (1980).
- 4) S. A. Leach, M. Thompson, M. Hill, *Carcinogenesis*, **8**, 1907 (1987).
- 5) H. Ohsima, S. Calmels, B. Pignatelli, P. Vincent, H. Bartsch, *IARC Sci. Publ.*, **84**, 384 (1987).
- 6) a) C. L. Bumgardner, K. S. McCallum, J. P. Freeman, *J. Am. Chem. Soc.*, **83**, 4417 (1961); b) D. B. Parihar, S. P. Sharma, *Chem. Ind. (London)*, **28**, 1227 (1966); c) P. Welzel, *Chem. Ber.*, **104**, 808 (1971); d) A. J. Buglass, B. C. Challis, M. R. Osborne, *IARC Sci. Publ.*, **9**, 94 (1974).

- 7) M. Tanno, S. Sueyoshi, S. Kamiya, *Chem. Pharm. Bull.*, **38**, 2644 (1990).
- 8) S. Sueyoshi, M. Tanno, *Chem. Pharm. Bull.*, **33**, 488 (1985).
- 9) M. Tanno, S. Sueyoshi, *Chem. Pharm. Bull.*, **35**, 1360 (1987).
- 10) M. Tanno, S. Sueyoshi, S. Kamiya, *Chem. Pharm. Bull.*, **38**, 49 (1990).
- 11) M. Tanno, S. Sueyoshi, *Chem. Pharm. Bull.*, **35**, 1353 (1987).
- 12) a) D. H. Hey, J. Stuart-Webb, G. H. Williams, *J. Chem. Soc.*, **1952**, 4657; b) P. Miles, H. Suschitzky, *Tetrahedron*, **18**, 1369 (1962).
- 13) a) R. N. Leopky, W. Tomasik, *J. Org. Chem.*, **48**, 2751 (1983); b) G. Verardo, A. G. Giumanini, P. Strazzolini, *Tetrahedron*, **46**, 4303 (1990).
- 14) K. Yamaguchi, G. Matsumura, M. Tanno, S. Sueyoshi, N. Miyata, *Acta. Crystallogr., Sect. C*, **48**, 1051 (1992).
- 15) S. Moncada, R. M. J. Palmer, E. A. Higgs, *Pharmacol. Rev.*, **43**, 109 (1991).
- 16) R. Uehara, *Bull. Chem. Soc. Jpn.*, **33**, 12 (1960).
- 17) a) F. Ullmann, *Justus Liebigs Ann. Chem.*, **327**, 104 (1903); b) D. G. Thomas, J. H. Billman, C. E. Davis, *J. Am. Chem. Soc.*, **68**, 895 (1946); c) *Beilstein*, **12**, 902; d) *Beilstein*, **13**, 442; e) *Beilstein*, **12**, 609.
- 18) a) *Beilstein*, **1671**, 727; b) C. E. Looney, W. D. Phillips, E. L. Reilly, *J. Am. Chem. Soc.*, **79**, 6136 (1957); c) S. F. Nelsen, D. H. Heath, *ibid.*, **91**, 6452 (1969); d) *Beilstein*, **12**, 609; e) J. W. Pensabene, W. Fiddler, C. J. Dooley, R. C. Doerr, A. E. Wasserman, *J. Agr. Food Chem.*, **20**, 274 (1972); f) J. T. D'Agostino, H. H. Jaffé, *J. Am. Chem. Soc.*, **92**, 5160 (1970).
- 19) a) T. Yokoyama, *Aust. J. Chem.*, **27**, 915 (1974); b) *Idem, ibid.*, **29**, 1469 (1976); c) N. Ishikawa, K. Kuroda, *Nippon Kagaku Zasshi*, **88**, 1010 (1967).