Nitration of 5,6,7,8-Tetrahydro-5,8-methanoisoquinoline *N*-Oxide with Other Aromatic Substitutions. Isolation and Mutagenicity of an α-Nitropyridine *N*-Oxide

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Treatment of 5,6,7,8-tetrahydro-5,8-methanoisoquinoline *N*-oxide (**2**) with fuming nitric acid afforded 3-nitro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline *N*-oxide (**3**), an example of formation of an α-nitropyridine *N*-oxide derivative by nitration of *N*-oxides. Further reaction of **3** resulted in deoxygenation giving 3-nitro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline (**4**). No aromatic nitration was observed by similar treatment of 5,6,7,8-tetrahydro-5,8-methanoisoquinoline (**1**) or 5,6,7,8-tetrahydroisoquinoline *N*-oxide (**11**). Some other aromatic substitutions with **1** and **2** were caried out to obtain mainly the 3-substituted derivatives. Significant mutagenicity of **3** is briefly reported.

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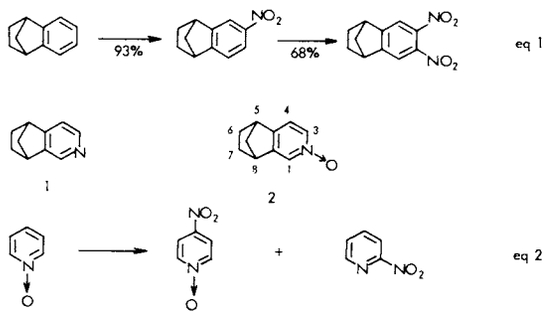
Our previous study [1] on the relative reactivities of some benzocyclenes in aromatic nitration showed that the benzonorbornene system is most reactive at the aromatic positions β to the fused norbornene ring and also has a larger steric hindrance at the α positions, compared to other systems such as indane and tetralin derivatives. Thus, preferred introduction of the nitro group into the β-positions of benzonorbornene was observed by the β-isomer ratio of 93% in mononitration and by the β,β'-isomer ratio of 68% in dinitration of the β-mononitro derivative (eq 1). We recently synthesized a pyridine analogue of benzonorbornene, 5,6,7,8-tetrahydro-5,8-methanoisoquinoline and its *N*-oxide (**1**, **2**) [2]. Nitration of pyridine *N*-oxide yields mainly 4-nitropyridine *N*-oxide with a small quantity of deoxygenated 2-nitropyridine (eq 2) [3]. However, the nitration usually fails when the 4-position of pyridine *N*-oxide is occupied by a moderately electron-supplying substituent such as alkyl [3b]. This leads to two possibilities. In view of the conformation analogous to benzonorbornene, the *N*-oxide **2** would exhibit a high reactivity in nitration at the 3-position (α to the ring nitrogen atom). On the other hand, because the γ and one of β positions to the ring nitrogen are occupied by the norbornene ring group and the other β position is sterically very hindered, nitration of **2** may not proceed. Furthermore, if any nitro-substituted *N*-oxide is obtained from **2**, a test of mutagenicity would be of interest, because a related compound, 4-nitroquinoline *N*-oxide, is known as a very strong mutagen [4] and 4-nitropyridine *N*-oxide a moderate mutagen [5].

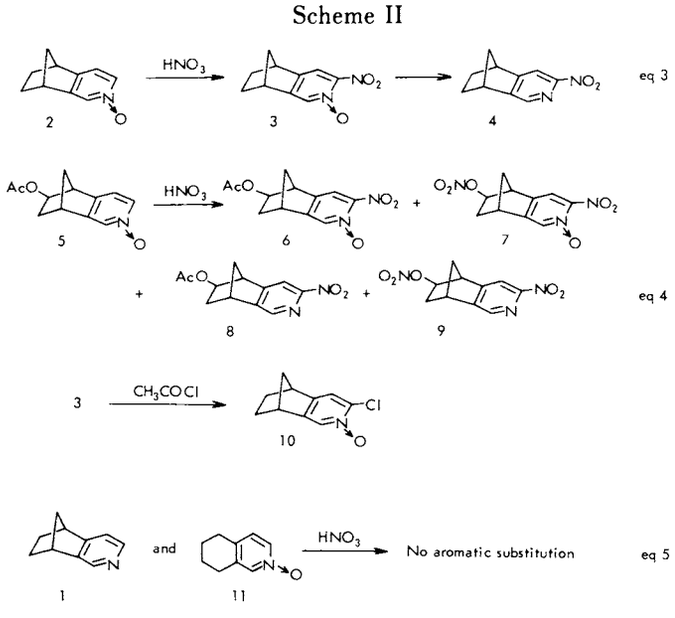
Scheme I



Results and Discussion.

Treatment of **2** with fuming nitric acid at room temperature for 24 hours followed by the usual workup gave a mixture of three compounds. Purification was carried out by preparative layer chromatography with 100% acetone solvent to obtain 3-nitro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline *N*-oxide (**3**) in 21% yield and its deoxygenated **4** in 13% yield with recovery of **2** in 25% yield. No other nitro derivative was obtained. Further treatment of **3** with

Scheme II



fuming nitric acid resulted in formation of the deoxygenated **4** (eq 3). The positions of the introduced nitro groups in **3** and **4** were assigned by mainly observing the singlet signals due to the C_1- and C_4- aromatic protons in ^{1}H nmr. To secure crystals satisfactory for X-ray structure analyses of the nitro derivatives, the same nitration was carried out with 5,6,7,8-tetrahydro-5,8-methanoisoquinolin-6-(*exo*)-ol acetate *N*-oxide (**5**). Treatment of the reaction mixture with preparative layer chromatography gave four compounds; the 3-nitroacetate **6**, the 3-nitronitrate **7**, the deoxygenated 3-nitroacetate **8**, and the deoxygenated 3-nitronitrate **9** (eq 4). X-ray analyses of **6** and **8** confirmed the ^{1}H nmr structure assignments for the nitro derivatives. As well demonstrated for the nitro groups γ to the ring nitrogen atom in 4-nitropyridine *N*-oxide derivatives [3], high reactivity of the 3-nitro substituent (α to the ring nitrogen) in **3** for nucleophilic aromatic substitution was exemplified by the reaction with acetyl chloride [7], which led **3** to 3-chloro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline *N*-oxide (**10**). For identification, a sample of **10** was prepared by *N*-oxidation of 3-chloro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline (**12**) with *m*-chloroperbenzoic acid.

The yield of **3** is unusual for nitration of *N*-oxides. To our knowledge, this is the first report describing the isolation of an α-nitro-substituted pyridine *N*-oxide in nitration. Further, we observed that similar treatments of related compounds, **1** and the methano bridge-absent 5,6,7,8-tetrahydroisoquinoline *N*-oxide (**11**) [8], did not cause any aromatic nitration (eq 5). Since the γ position to the ring nitrogen in **11** is occupied by an alkyl, the negative nitration will strengthen the statement in Introduction and literature [3b]. The high reactivity of **2** in nitration will be illustrated by assuming 7,8-carbon bond participation in a cationic transition state, as pictured in Scheme III, eq 6. Participation of this kind was suggested by us in the solvolysis of 1-(5-benzonorbornenyl)ethyl chloride (eq 7) [1].

Some nucleophilic aromatic substitutions known in heterocyclic *N*-oxide chemistry [3a,b] showed high regioselectivities of the 3-position. The reaction of **2** with phosphorus oxychloride exclusively afforded the 3-chloro derivative **12**. Transformation of **2** into the *N*-methoxy quaternary salt with methyl iodide followed by treatment with methylmagnesium iodide provided only the 3-methyl derivative **13** without detectable amounts of the 1-methyl derivative. Treatment of the *N*-methoxy quaternary salt with potassium cyanide gave the 3-cyano derivative **14** with a

Scheme III

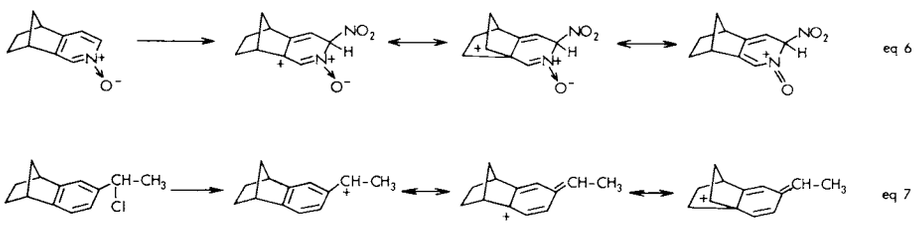


Table I

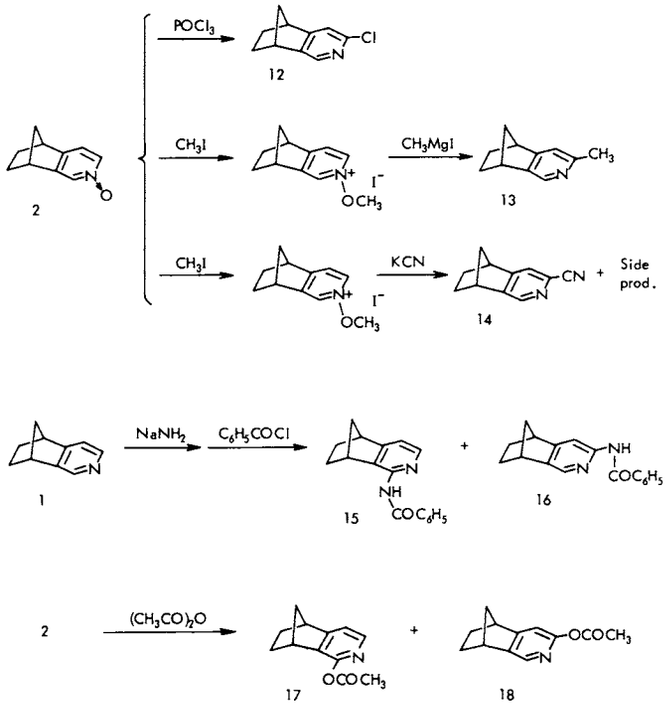
Reverse Mutation of 3-Nitro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline *N*-Oxide (**3**) and 4-Nitroquinoline *N*-Oxide (4NQO) on Salmonella Mutagenicity Test

Materials μg/plate		His$^+$ or Try$^+$ revertant colonies/plate					
		Base-pair substitution type			Frame-shift type		
		TA 100	TA 1535	WP2uvr A	TA 98	TA 1538	TA 1537
Spontaneous		153	15	24	36	21	9
3	0.12	167	16	24	35	18	8
	0.37	246	20	25	39	16	7
	1.11	388	30	25	62	14	7
	3.33	732	75	33	147	15	7
	10.0	1998	181	62	407	15	8
4NQO	0.12	960	22	29	112	96	9
	0.37	2192	44	79	292	219	15
	1.11	3448	160	794	610	472	103
	3.33	2436	0 [a]	1300	421 [a]	89 [a]	6 [a]
	10.0	6 [a]	0 [a]	314 [a]	5 [a]	5 [a]	0 [a]
Positive Control [b]		MMS	NaN_3	ENNG	AF2	4NQO	9AA
(μg/plate)		200	0.5	2	0.1	0.25	80
		755	379	562	472	136	250

[a] Background lawn disappearance or decreased. [b] MMS: methyl methanesulfonate, NaN_3: sodium azide, ENNG: *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine, AF2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, 9AA: 9-aminoacridine.

minor amount of by-product (see Experimental) [9-11]. Teatment of **1** with sodium amide (Chichibabin reaction) was not regiospecific, leading to the 1-amino and 3-amino derivatives (**15** and **16**) in a 1:1 ratio. However, this result should be compared to our observation that Chichibabin reaction of 5,6,7,8-tetrahydroisoquinoline [12] gave only the 1-amino derivative and to the known facts that the reactions with 3-substituted pyridines cause predominantly the amino introduction into the sterically more crowded 2-position [13]. The rearrangement reaction of a *N*-oxide by treatment with acetic anhydride was also carried out with **2** to afford the 1-acetoxy and 3-acetoxy derivatives (**17** and **18**), though in poor yield. Structures of the products were all assigned by ^{1}H nmr. The major factor for the high regioselectivity toward the 3-position in nucleophilic aromatic substitutions is considered to be steric hindrance of the 1-position due to the norbornene ring, as we previously suggested [1].

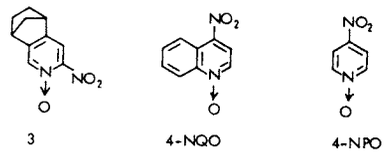
Scheme IV



Mutagenic Activity of the Nitro *N*-Oxide **3**.

Mutagenicity tests of **3** are presented in the Experimental Section and Table I in comparison with data for 4-nitroquinoline *N*-oxide (4-NQO) and 4-nitropyridine *N*-oxide (4-PNO). While 4-NQO induces both base-pair substitution and frame-shift type of mutation, **3** has only the mutagenic activity of a base-pair substitution type. In a rough comparison with the data of Takahashi *et al.* [5] for 4-NPO, the mutagenic activity of **3** against a strain of the base-pair substitution type is about 10 times more than that of 4-NPO and 30 times less than that of 4-NQO. Of significance is that the α-nitropyridine *N*-oxide derivative shows mutagenic activity like the γ-nitro *N*-oxide system.

Chart I



3 4-NQO 4-NPO

EXPERIMENTAL

Melting points were taken in capillary tubes and are corrected. The ^{1}H nmr spectra were determined with a Varian T-60A.

Nitration of 5,6,7,8-Tetrahydro-5,8-methanoisoquinoline *N*-Oxide (**2**).

A solution of 645 mg of **2** in 4 ml of fuming nitric acid (d = 1.52) was stirred at room temperature for 24 hours. The reaction mixture was poured onto ice, made alkaline with solid sodium carbonate, and extracted with chloroform. The chloroform solution was dried and distilled leaving 450 mg of a product mixture. Preparative layer chromatography with 100% acetone separated three products: 171 mg of 3-nitro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline *N*-oxide (**3**) and 100 mg of 3-nitro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline (**4**) with 162 mg of recovered **2**. Treatment of **3** with fuming nitric acid led to **4**.

3-Nitro *N*-Oxide **3**.

This compound had mp 152.5° dec (from dichloromethane-*n*-hexane); ^{1}H nmr (deuteriochloroform): δ 7.5 (s, 1 H at C_4), and 8.1 (s, 1 H at C_1).

Anal. Calcd. for $C_{10}H_{10}N_2O_3$: C, 58.25; H, 4.89; N, 13.58. Found: C, 58.27; H, 4.80; N, 13.61.

3-Nitro *N*-Oxide **3**.

This compound had mp 152.5° dec (from dichloromethane-*n*-hexane); ^{1}H nmr (deuteriochloroform): δ 1.0-2.2 (m, 6H at C_6, C_7, and C_9), 3.5 (m, 2H, bridgeheads), 7.5 (s, 1 H at C|), and 8.1 (s, 1 H at C☆).

Anal. Calcd. for $C_{10}H_{10}N_2O_3$: C, 58.25; H, 4.89; N, 13.58. Found: C, 58.27; H, 4.80; N, 13.61.

3-Nitro **4**.

This compound had mp 81.5-82.5° (from dichloromethane-*n*-hexane); ^{1}H nmr (deuteriochloroform): δ 1.0-2.3 (m, 6 H at C_6, C_7, and C_9), 3.6 (m, 2 H, bridgeheads), 8.1 (s, 1 H at C_1), and 8.4 (s, 1 H at C_4).

Anal. Calcd. for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.01; H, 5.27; N, 14.77.

Nitration of 5,6,7,8-Tetrahydro-5,8-methanoisoquinoline-6-(*exo*)-ol Acetate *N*-Oxide (**5**).

A solution of 397 mg of **5** in 3 ml of fuming nitric acid (d = 1.52) was stirred overnight at 40°. The same workup as the above yielded 389 mg of a product mixture, which when subjected to preparative layer chromatography with 100% acetone, gave 20 mg of 3-nitro-5,6,7,8-tetrahydro-5,8-methanoisoquinolin-6-(*exo*)-ol acetate *N*-oxide (**6**), 37 mg of 3-nitro-5,6,7,8-tetrahydro-5,8-methanoisoquinolin-6-(*exo*)-ol nitrate *N*-oxide (**7**), 43 mg of 3-nitro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline-6-(*exo*)-ol acetate (**8**), and 73 mg of 3-nitro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline-6-(*exo*)-ol nitrate (**9**). In addition, minor amounts of the starting material (**5**) and 5,6,7,8-tetrahydro-5,8-methanoisoquinoline-6-(*exo*)-ol nitrate *N*-oxide were isolated.

3-Nitro Acetate *N*-Oxide **6**.

This compound had mp 134-135° (from dichloromethane-*n*-hexane); ^{1}H nmr (deuteriochloroform): δ 1.8-2.2 (m, 4 H at C_7 and C_9), 2.1 (s, 3 H, OAc), 3.5 (m, 2 H, bridgeheads), 4.7 (m, 1 H at C_6-*endo*), 7.5 (s, 1 H at C_4),

and 8.1 (s, 1 H at C_1).

Anal. Calcd. for $C_{12}H_{12}N_2O_5$: C, 54.54; H, 4.58; N, 10.60. Found: C, 54.72; H, 4.49; N, 10.89.

The structure was confirmed by X-ray analysis.

3-Nitro Nitrate *N*-Oxide **7**.

This compound had mp 86-87° (from dichloromethane-*n*-hexane); ^{1}H nmr (deuteriochloroform): δ 1.9-2.2 (m, 4 H at C_7 and C_9), 3.6 and 3.7 (m, 2 H, bridgeheads), 5.0 (m, 1 H at C_6-*endo*), 7.6 (s, 1 H at C_4), and 8.1 (s, 1 H at C_1).

Anal. Calcd. for $C_{10}H_9N_3O_6$: C, 44.95; H, 3.40; N, 15.73. Found: C, 44.98; H, 3.45; N, 15.61.

3-Nitro Acetate **8**.

This compound had mp 95-96° (from dichloromethane-*n*-hexane); ^{1}H nmr (deuteriochloroform): δ 1.8-2.2 (m, 4 H at C_7 and C_9), 2.1 (s, 3 H, OAc), 3.6 (m, 2 H, bridgeheads), 4.7 (m, 1 H at C_6-*endo*), 8.1 (s, 1 H at C_1), and 8.4 (s, 1 H at C_4).

Anal. Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found: C, 57.65; H, 4.89; N, 11.44.

The structure was confirmed by X-ray analysis.

3-Nitro Nitrate **9**.

This compound had mp 126-127° (from dichloromethane-*n*-hexane); ^{1}H nmr (deuteriochloroform): δ 1.9-2.3 (m, 4 H at C_7 and C_9), 3.7 and 3.8 (m, 2 H, bridgeheads), 5.0 (m, 1 H at C_6-*endo*), 8.2 (s, 1 H at C_1), and 8.4 (s, 1 H at C_4).

Anal. Calcd. for $C_{10}H_9N_3O_5$: C, 47.81; H, 3.61; N, 16.73. Found: C, 48.06; H, 3.82; N, 16.54.

Reaction of **2** with Acetyl Chloride.

A reported procedure was used [7]. When 1 ml of actyl chloride was added to 42 mg of **2** at room temperature, a vogorous reaction occurred. The mixture was poured on ice, made alkaline with aqueous sodium carbonate, and extracted with chloroform. The chloroform solution was dried and distilled leaving 34 mg of crystals. Recrystallization from dichloromethane-*n*-hexane gave a pure sample of 3-chloro-5,6,7,8-tetrahydro-5,8-methanoisoquinoline *N*-oxide (**10**), mp 150.5-151.5°; ^{1}H nmr (deuteriochloroform): δ 1.0-2.2 (m, 6 H at C_6, C_7, and C_9), 3.4 (m, 2 H, bridgeheads), 7.2 (s, 1 H at C_4), and 8.1 (s, 1 H at C_1).

Anal. Calcd. for $C_{10}H_{10}ClNO$: C, 61.39; H, 5.15; N, 71.6; Cl, 18.12. Found: C, 61.31; H, 4.97; N, 7.22; Cl, 18.05.

Treatment of **12** with *m*-chloroperbenzoic acid in chloroform gave a sample of **10**.

3-Chloro- and 3-Methyl-5,6,7,8-tetrahydro-5,8-methanoisoquinoline (**12** and **13**).

Essentially the same procedures used for the preparations of 3-chloro- and 3-methyl-5,6,7,8-tetrahydro-5,8-methanoisoquinoline-7-(*exo*)-ol derivatives [6] were applied.

This compound had mp 42.5-43°; ^{1}H nmr (deuteriochloroform): δ 1.0-2.2 (m, 6 H at C_6, C_7, and C_9), 3.4 (m, 2 H, bridgeheads), 7.1 (s, 1 H at C_4), and 8.1 (s, 1 H, at C_1).

Anal. Calcd. for $C_{10}H_{10}ClN$: C, 66.85; H, 5.61; N, 7.80; Cl, 19.74. Found: C, 66.88; H, 5.68; N, 7.84; Cl, 19.47.

Compound **13** was obtained as an oil; ^{1}H nmr (deuteriochloroform): δ 1.0-2.1 (m, 6 H at C_6, C_7 and C_9), 2.5 (s, 3 H, CH_3), 3.4 (m, 2 H, bridgeheads), 6.9 (d, 1 H at C_4), and 8.2 (s, 1 H at C_1).

Reaction of the *N*-Methoxy Quaternary Salt of **2** with Potassium Cyanide.

A mixture of 2 g of **2** and 5 ml of methyl iodide was stirred at room temperature for 1.5 hours. The mixture was concentrated under reduced pressure leaving a residue, which was dissolved into 30 ml of 70% aqueous dioxane. To this solution was added 1.62 g of potassium cyanide and the mixture was stirred for 2 hours at room temperature and extracted with chloroform. The chloroform solution was washed with water, dried, and concentrated under reduced pressure leaving 2.27 g of a residue. Chromatography of this residue on a Lobar column B, using a 5:1 mixture solvent of benzene and ethyl acetate, gave 0.31 g of fraction 1 and 0.92 g of fraction 2. Recrystallization of fraction 2 from dichloromethane-*n*-hexane gave 3-cyano-5,6,7,8-tetrahydro-5,8-methanoisoquinoline (**14**) as colorless crystals, mp 83.5-84.5°; ^{1}H nmr (deuteriochloroform): δ 1.0-2.2 (m, 6 H at C_6, C_7, and C_9), 3.5 (m, 2 H, bridgeheads), 7.5 (s, 1 H at C_4), and 8.4 (s, 1 H at C_1).

Anal. Calcd. for $C_{11}H_{10}N_2$: C, 77.62; H, 5.92; N, 16.46. Found: C, 77.84; H, 5.89; N, 16.54.

Fraction 1 had ^{1}H nmr (deuteriochloroform): δ 1.1-2.1 (m, 6 H at C_6, C_7, and C_9), 3.2 and 3.4 (m, 2 H, bridgeheads), 3.9 (s, 3 H, OCH_3), 5.5 (d, 1 H at C_4), 7.2 (d, 1 H at C_3), and 7.9 (s, 1 H at C_1). On the basis of ^{1}H nmr data, the structure for fraction 1 was either 1-cyano-*N*-methoxy-1,2,5,-6,7,8-hexahydro-5,8-methanoisoquinoline or 3-cyano-*N*-methoxy-2,3,5,-6,7,8-hexahydro-5,8-methanoisoquinoline. Lengthening of the reaction time from 1.5 hours to overnight did not affect the ratio of products. Also, further treatment of fraction 1 under the above reaction conditions caused no transformation into **14**. Therefore, the 1-cyano-*n*-methoxy structure is more reasonable than the 3-cyano-*N*-methoxy structure [11].

Reaction of **1** with Sodium Amide.

A solution of 300 mg of **1** and 121 mg of sodium amide in 1 ml of *N,N*-dimethylaniline was warmed at 160° with stirring overnight under nitrogen atmosphere. The mixture was poured into ice water and extracted with ether. The ether solution was washed with water, dried, and distilled. The residue of 289 mg was dissolved in 5 ml of chloroform and treated with 1 g of benzoyl chloride and 5 drops of pyridine. The usual work-up followed by treatment with preparative layer chromatography afforded 75 mg of 1-benzamido-5,6,7,8-tetrahydro-5,8-methanoisoquinoline (**15**) (14% yield) and 64 mg of 3-benzamido-5,6,7,8-tetrahydro-5,8-methanoisoquinoline (**16**) (12% yield).

Compound **15** had mp 109.5-110°; ^{1}H nmr (deuteriochloroform): δ 1.1-2.2 (m, 6 H at C_6, C_7 and C_9), 3.4 and 3.6 (m, 2 H, bridgeheads), 7.0 (d, 1 H at C_4), 7.3-7.5 (m, 3 H, aromatic), 7.8-8.1 (m, 2 H, aromatic), and 8.0 (d, 1 H at C_3).

Anal. Calcd. for $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60. Found: C, 77.54; H, 6.18; N, 10.53.

Compound **16** had mp 142-143.5° (from dichloromethane-*n*-hexane); ^{1}H-nmr (deuteriochloroform): δ 1.9-2.0 (m, 6 H at C_6, C_7, and C_9), 3.2 and 3.3 (m, 2 H, bridgeheads), 7.4 and 8.0 (m, 5 H, aromatic), 7.6 (s, 1 H at C_4), and 8.3 (s, 1 H, at C_1).

Anal. Calcd. for $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60. Found: C, 77.31; H, 6.10; N, 10.54.

Reaction of 5,6,7,8-Tetrahydroisoquinoline with Sodium Amide.

The same treatment of the tetrahydroisoquinoline [12] to the above at a reaction temperature of 135° afforded 1-benzamido-5,6,7,8-tetrahydroisoquinoline in about 35% yield, mp 157-158° (from dichloromethane-*n*-hexane); ^{1}H nmr (deuteriochloroform): δ 1.7 and 2.65 (m, 8 H at C_5, C_6, C_7, and C_8), 6.85 (d, 1 H at C_4), 7.4 and 7.9 (m, 5 H, aromatic), and 7.9 (d, 1 H at C_3).

Anal. Calcd. for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10. Found: C, 76.25; H, 6.35; N, 11.14.

3-Amino-5,6,7,8-tetahydro-5,8-methanoisoquinoline was prepared by catalytic reduction of **4** over palladium-on-charcoal in methanol, mp 79-80° (from ether); ^{1}H nmr (deuteriochloroform): δ 1.0-2.0 (m, 6 H, at C_6, C_7, and C_9), 3.2 (m, 2 H, bridgeheads), 5.1 (broad s, 2 H, NH_2), 6.4 (s, 1 H at C_4), and 7.7 (s, 1 H at C_1).

Anal. Calcd. for $C_{10}H_{12}N_2$: C, 74.96; H, 7.55; N, 17.49. Found: C, 75.23; H, 7.76; N, 17.51.

Reaction of **2** with Acetic Anhydride.

A solution of **2** in acetic anhydride was refluxed for 5 hours. The mixture was concentrated under reduced pressure leaving a residue, which was treated with preparative layer chromatography using 100% ethyl acetate solvent. The product was shown to be an oily mixture of 1-acetoxy and 3-acetoxy derivatives (**17** and **18**); ^{1}H nmr for **17** (dueteriochloroform): δ 1.0-2.0 (m, 6 H at C_6, C_7, and C_9), 2.3 (s, 3 H, $OCOCH_3$), 3.4 (m, 2 H, bridgeheads), 7.0 (d, 1 H at C_4), and 8.1 (d, 1 H at C_3); ^{1}H nmr for **18**

(deuteriochloroform): δ 1.0-2.0 (m, 6 H at C_6, C_7, and C_9), 2.3 (s, 3 H, $OCOCH_3$), 3.4 (m, 2 H, bridgeheads), 6.8 (s, 1 H at C_4), and 8.05 (s, 1 H at C_1).

Mutagenic Activity of the Nitro *N*-Oxide **3**.

Reverse mutation activity of **3** was measured with the Ames Salmonella mutagenicity system [14]. Six tester strains, *i.e. S. typhimurium* TA 1535, TA 1537, TA 1538, TA 100, TA 98 and *E. coli* WP2uvr A were used. 4-Nitroquinoline *N*-oxide (4-NQO) was used as a reference. The assay results are shown in Table I.

While 4-NQO showed strong or moderate mutagenicity in all tester strains, **3** induced a moderate to weak mutation in TA 100, TA 1535, TA 98 and WP2uvr A, but did not induce any reversion in TA 1538 and TA 1537. The results indicate that 4-NQO induces mutation of both the base-pair substitution type and frame-shift type, but **3** has only a mutagenic activity of the base-pair substitution type. The TA 98 strain is essentially a tester of the frame-shift type, but also has some sensitivity against the base-pair substitution type of mutagens. The weakly positive result of **3** in TA 98 is considered to be due to the latter character.

Takahashi *et al.* [5] have reported that the relative mutagenic activities between 4-nitropyridine *N*-oxide (4-NPO) and 4-NQO are 1 to 379 for TA 100 and 1 to 758 for TA 98. Rough calculation shows that the relative mutagenic activity of **3** to 4-NQO is about 1/30 for both TA 100 and TA 98. If an error range is allowed, the mutagenic activity of **3** is about 10 times that of 4-NPO.

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