Pergamon

Tetrahedron Vol. 51, No. 31, pp. 8447-8458, 1995

0040-4020/95 $9.50+0.00

0040-4020(95)00460-2

Synthesis of Macarpine and its Cytotoxicity: Toward a Synthetic Route for 12-Alkoxybenzo[*c*]phenanthridine Alkaloids through Aromatic Nitrosation under Basic Condition

Tsutomu Ishikawa,* Tatsuru Saito and Hisashi Ishii

Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi, Inage, Chiba 263, Japan

Abstract: Macarpine (**1**), a 2, 3, 7, 8, 10, 12-hexaoxygenated benzo[*c*]phenanthridine alkaloid (O_6 base), was effectively synthesized from sesamol methyl ether (**7**) through the newly developed aromatic nitrosation under basic condition. The tests for the cytotoxicity of **1** and its analogous 2, 3, 7, 8, 10-pentaoxygenated (O_5) bases **23**, **24** against HeLa S3 tumor cell showed that **1** exhibited the highest activity among them.

INTRODUCTION

Benzo[*c*]phenanthridine alkaloids have attracted much attention because of their antitumor activities.[1] Macarpine (**1**) is only one naturally occurring 2, 3, 7, 8, 10, 12-hexaoxygenated one (O_6 base) first isolated by Slavik *et al.*[3] Though two research groups[4] reported the synthesis of it, its biological activity has never been examined. In the course of our synthetic studies on benzo[*c*]phenanthridine alkaloids for their structure-activity relationship on antitumor activity, we synthesized **1** through regioselective introduction of a nitrogen function into the *para* position of the hydroxyl group in a naphthol **4** by newly developed basic nitrosation. In this paper we present the full details of the synthesis of **1** and its cytotoxic activity against HeLa S3 tumor cell.

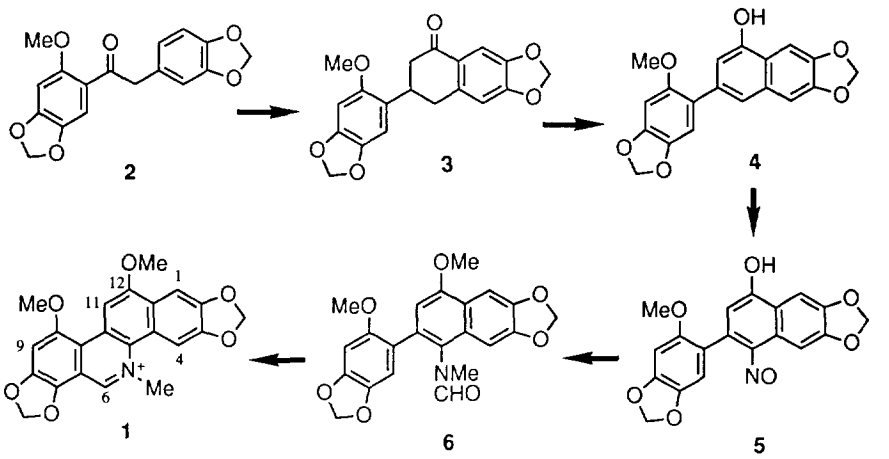
RESULTS AND DISCUSSION

Synthesis of Macarpine (1)

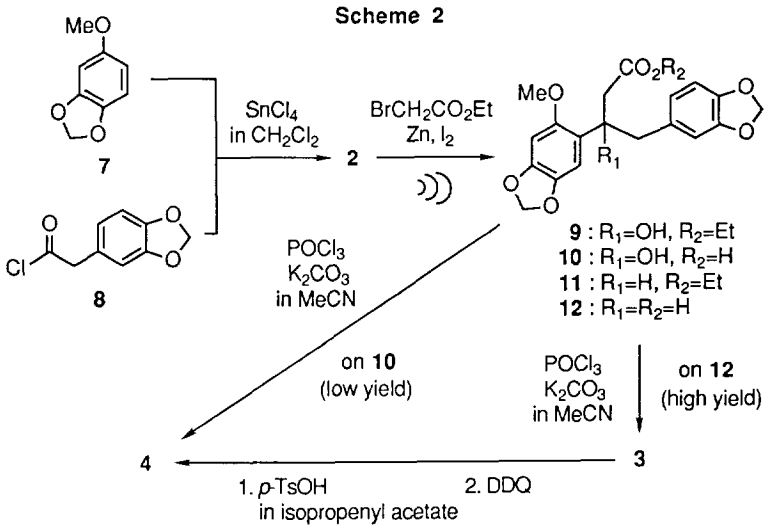
We planned the synthetic strategy for **1** based on nitrosation of a 1-naphthol **4** followed by the Bischler-Napieralski reaction (BNR) of a naphthylformamide **6** as outlined in Scheme 1.

Friedel-Crafts acylation of sesamol methyl ether (**7**) by a phenylacetyl chloride **8** in the presence of stannic chloride afforded a ketone **2** in 88 % yield. 3-Hydroxy-3, 4-diphenylbutyrate **9** was given by the Reformatsky reaction of **2** with ethyl bromoacetate using activated zinc[5] under irradiation of ultrasound.[6] Alkaline hydrolysis of the hydroxyester **9** quantitatively gave a 3-hydroxybutyric acid **10**. However, trial of basic cyclization ($POCl_3$-K_2CO_3 in CH_3CN)[7] to **10** for direct preparation of **4** through dehydration[8] of the tertiary alcoholic function resulted in ineffective cyclization (17 %). Preparation of a naphthol **4** was achieved by a following stepwise operation. Successive treatment of the ester **9** by catalytic hydrogenolysis,[9] alkaline hydrolysis and the basic acylation[7] afforded a 3-aryl-1-tetralone **3** through

Scheme 1



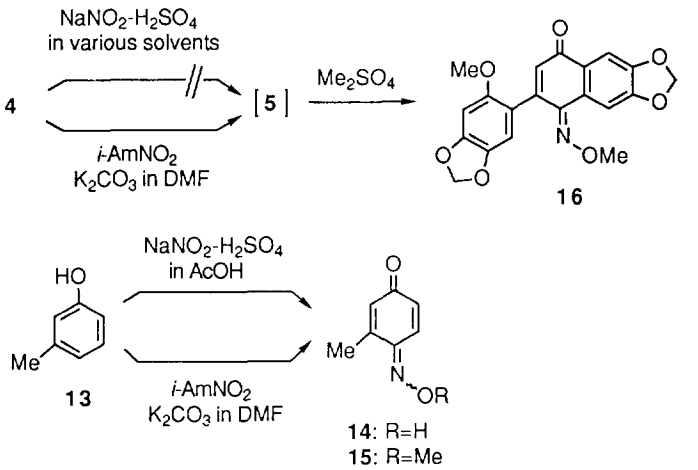
Scheme 2



11 and **12**. The tetralone **3** was converted into **4** by an acetal exchange reaction[10] using isopropenyl acetate followed by dehydrogenation with 2, 3-dichloro-5, 6-dicyano-*p*-benzoquinone (DDQ).[11] (Scheme 2)

Next step is regioselective introduction of a nitrogen function into the *para* position of a phenolic hydroxy group in the naphthol **4**. In the usual synthetic procedure on nitrosation of phenols, nitrous acid is generated *in situ* by the neutralization of nitrite salts with strong (aqueous) mineral acids which also promotes the production of the active nitrosating agents, and the nitrosation occurs at the *para*-position to yield *p*-nitrosophenols, unless the position is blocked.

Scheme 3



Thus, **4** was subjected to the conventional acidic nitrosation using sodium nitrite ($NaNO_2$) and conc. sulfuric acid (H_2SO_4). However, several trials for the nitrosation in various solvents (AcOH, dioxane or tetrahydrofuran) led to no isolation of any nitrosated products because of formation of a complex mixture. So, we reexamined the nitrosation reaction using *m*-cresol (**13**) as a model substrate. Treatment of **13** with $NaNO_2$ in acetic acid in the presence of H_2SO_4 afforded a desired nitrosated product **14**[12] in 66 % yield, which could be quantitatively methylated to give a methyl ether **15**. (Scheme 3) The oxime structures[13] for the nitrosated **14** and the methylated product **15** were confirmed by appearance of the signals at δ 188.4 and 188.7 ppm attributable to a carbonyl carbon in the ^{13}C NMR, respectively. These facts suggested that presence of an acid-sensitive methylenedioxy group in the molecule of **4** may cause to fail the nitrosation under acidic condition.

Alkyl nitrites are successfully used for the nitrosation of enolizable carbonyl compounds under both acidic and basic conditions. So, we focused on isoamyl nitrite (*i*-$AmNO_2$) as a nitrogen source under basic condition. Treatment of **13** with *i*-$AmNO_2$ in dimethylformamide (DMF) in the presence of potassium carbonate (K_2CO_3) (basic nitrosation) at 50 °C for 2.5 h afforded the same product **14** in 71 % yield that was obtained under the acidic condition. However, application of the basic nitrosation to a naphthol **4** resulted in low production of a nitrosated product **5** in spite of the smooth disappearance of the starting material on TLC. The step was overcome by successive reaction of the basic nitrosation and methylation with dimethyl sulfate in one pot operation. The desired methyl ether **16** was satisfactorily given (80 % yield). (Scheme 3)

The appearance of two relatively lower shield-shifted signals due to aromatic protons at δ 7.68 and 8.35 in the ^1H NMR of the methyl ether indicated that the substitution with a nitroso group occurred at the 4 position in **4** (*para*-selectivity). The oxime methyl ether structure for **16** was reasonably assigned by the signal at δ 183.6 due to a carbonyl group in the ^{13}C NMR. The geometry of the methoxy group in the oxime ether function could be deduced to be *Z*-configuration, in which a methoxy group locates at an opposite site to the adjacent aryl substituent, by NOE enhancement between the methoxy group and the lowest shield-shifted aromatic proton assignable to 8-H (δ 8.35). (Figure 1)

Further investigation on the basic nitrosation showed its generality and wide applicability. The scope and limitations of this reaction will be discussed in a separate paper.[14]

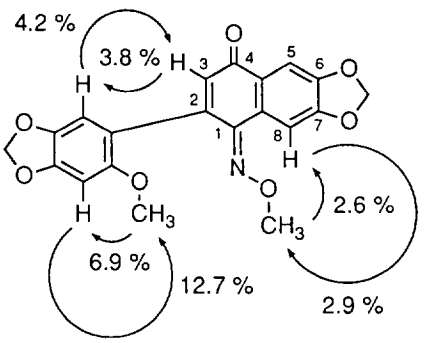


Figure 1. Selected NOE enhancement in the ^{1}H NMR of **16**

Reductions of **16** with sodium borohydride ($NaBH_4$), sodium hydrosulfite ($Na_2S_2O_4$) or Raney Ni gave an iminoquinone **17**, an oxidized product of an expected aminophenol **18**, in low yields as an only isolable product. Monitoring the reaction, in which a limited amount of the reagent was used, by TLC showed generation of two products in the reaction mixture. One of them was corresponded to **17**. The use of large excess of the reagent resulted in complete formation of the alternative product on TLC. However, it disappeared during isolation work and changed into **17**. These facts strongly suggested that an unisolable product would be a fully reduced aminophenol **18**, which could be formed from **16** through **17** under reductive conditions, but that **18** was easily oxidized into **17** by exposure to air. (Scheme 4)

Thus, we tried to directly prepare a formamide from **16** under reductive condition. Catalytic hydrogenation of **16** in dioxane with Pd-C in the presence of formic acid (HCOOH) and formamide ($HCONH_2$) led to the quantitative formation of an expected formamide **19**. Precise examination of the reaction condition indicated that the combination of HCOOH and $HCONH_2$ was necessary for this reductive formylation as shown in Table 1. Methylation of the crude **19** in the presence of a phase transfer catalyst directly afforded a 2-aryl-4-methoxy-1-(*N*-methylformamido)naphthalene **6** in 70 % yield from **16**. Selective methylation (65 %) for the phenol function of **19** giving a methyl ether **20** was also possible under mild condition (Me_2SO_4, K_2CO_3 in DMF). (Scheme 4)

Finally, we attempted cyclization of an *N*-methylformamide **6** by BNR using phosphorus oxychloride ($POCl_3$) as a dehydrating agent. Though a desired cyclized product was not produced when the BNR was

Scheme 4

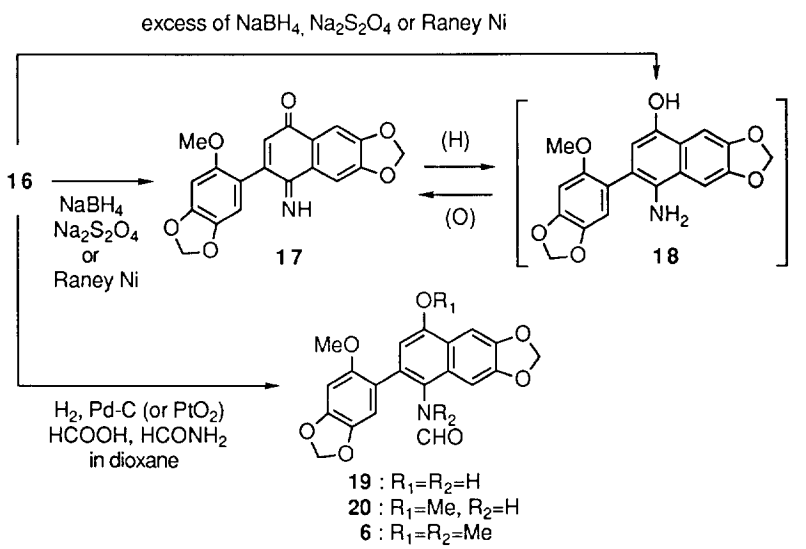
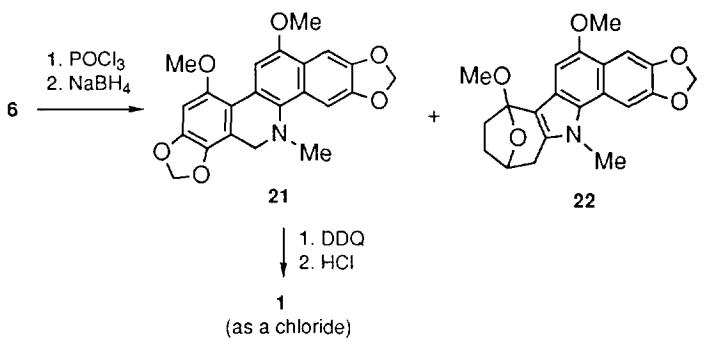


Table 1. Reductive Formylation of the Quinone Monooxime Methyl Ether 16 under the Condition of Catalytic Hydrogenation

run	conditions			result
	catalyst	reagent/solvent	time (d)	
1	10 % Pd-C	HCO_2H-$HCONH_2$ in dioxane	2	**19** : quant.
2	10 % Pd-C	i) HCO_2H	1	complete reduction but not formylation
		ii) $HCONH_2$	3	**19** : quant.
3	10 % Pd-C	HCO_2H-$HCONH_2$	6	**19** : quant.
4	PtO_2	i) $HCONH_2$ in dioxane	1	complete reduction but not formylation
		ii) HCO_2H	7	**19** : quant.

Scheme 5



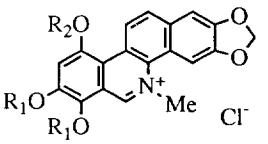
carried out using solvents such as acetonitrile (MeCN), xylene or chloroform, successful cyclization was observed in the reaction without solvent. The reduction of a crude BNR product with $NaBH_4$ afforded an intended dihydromacarpine (**21**)[4] in 30 % yield from **6**, together with an unexpected benz[*g*]indole **22**[15] with a unique ring system (24 %). Dehydrogenation of **21** with DDQ followed by treatment with 10 % hydrochloric acid quantitatively provided macarpine (**1**) chloride, which was directly identified with an authentic sample.[3] (Scheme 5)

Macarpine (**1**) was, thus, synthesized in 12 % overall yield through 12 steps from sesamol methyl ether (**7**). Though the last cyclization step by the BNR of the formamide **6** was entirely unsatisfactory, this synthetic method could be served as a new route for 12-alkoxybenzo[*c*]phenanthridine bases[16] because of simple operation of each step.

Cytotoxic Activities of Macarpine (1) Chloride and the Analogous O_5 Base Chlorides 23, 24

Table 2. Cytotoxic Activities of Macarpine (1) Chloride and the Analogous O_5 Base Chlorides 23, 24 on HeLa S3 Cell[a]

run	substrate	IC_{50} (mg/mL)
1	**1**	0.192
2	**23**	0.316
3	**24**	1.58



23 : $2R_1=CH_2$, $R_2=i$-Pr
24 : $R_1=R_2$=Me

[a] Substrates tested were dissolved in dimethyl sulfoxide and then the solution was diluted with saline. The methylene blue cytotoxity of IC_{50} values was performed as reported previously.[19]

Cytotoxic activitiy of macarpine (**1**) chloride was tested on HeLa S3 tumor cell. Its acitivity was compared to those of the structurally related 2, 3, 7, 8, 10-pentaoxygenated (O_5) base chlorides, 10-isopropoxysanguinarine (**23**)[17] and chelilutine (**24**)[18] chlorides. (Table 2) Interestingly, **1** showed the strongest activity among them. On the other hand the least one was observed in **24**. In other words additional introduction of a methoxy group into the 12 position of a benzo[*c*]phenanthridine skeleton may cause to enhance the activity, while replacement of a methylenedioxy function at the 7, 8 positions by two methoxy groups led to reduction of the activity, in spite of limited examples.

Previously we[1] reported antineoplastic activities of some benzo[*c*]phenanthridine bases including **24** against Sarcoma 180 in mice. In the report we had suggested that either enhanced or unchanged activity was observed by replacement of a methylenedioxy function at the 7, 8 positions by two methoxy groups. The discrepancy of these results may depend on the difference of experimental system used.

EXPERIMENTAL

General. All melting points were measured on a micro melting-point hot stage (Yanagimoto) and are uncorrected. IR spectra were recorded for (nujol) on JASCO IR-700 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution with JEOL JNM GSX-500α spectrometers, unless otherwise stated, with tetramethylsilane as internal reference. EI-MS (MS) and high resolution MS (HR-MS) were recorded on a Hitachi M-60, and high resolution first-atom bombardment MS (HR-FABMS) on a JEOL JMX-HX 110A spectrometers, respectively, with direct inlet system. For column and flash chromatography, silica gel 60 (70-230 mesh ASTM; Merck) and silica gel 60 (230-400 mesh ASTM; Merck) were used, while for TLC and preparative TLC (PLC) and silica gel GF_{254} (Merck) were used. In general the extraction was washed with brine, dried over magnesium sulfate, then filtered, and the filtrate was evaporated to dryness under reduced pressure, unless otherwise stated. Raney® Ni was purchased from Aldrich Chemical Company Inc.

2-Methoxy-4,5-methylenedioxyphenyl 3,4-Methylenedioxybenzyl Ketone (2). Oxalyl chloride (26 mL, 0.311 mol) was slowly added to 3,4-methylenedioxyphenylacetic acid (20 g, 0.111 mol) under ice-cooling and the whole was stirred at rt for 2 h. After evaporation of the excess oxalyl chloride under reduced pressure the residual yellow viscous liquid **8** was dissolved in dry

dichloromethane (300 mL). To the solution was added a solution of sesamol methyl ether (**7**) (17 g, 0.111 mol) in dry dichloromethane (300 mL) followed by stannic chloride (52 mL, 0.444 mol) at -15 °C. The mixture was stirred at the same temperature for 2 h, poured into water and extracted with dichloromethane. Work-up gave **2** (31 g, 88 %) as colorless needles (from methanol), mp 103-105.5 °C; IR ν_{max} 1660 cm^{-1}; ^1H NMR δ 3.87 (s, 3H, OMe), 4.18 (s, 2H, $COCH_2Ar$), 5.90, 5.96 (each s, 2H, OCH_2O), 6.52 (s, 1H, 3-H), 6.70 (m, 3H, ArH), 7.27 (s, 1H, 6-H); Anal. Calcd for $C_{17}H_{14}O_6$: C, 64.97; H, 4.49. Found: C, 64.76; H, 4.54.

Ethyl 3-Hydroxy-3-(2-methoxy-4,5-methylenedioxyphenyl)-4-(3,4-methylenedioxyphenyl)butyrate (9). A mixture of the ketone **2** (10 g, 31.8 mmol), ethyl bromoacetate (4.2 mL, 37.9 mmol), zinc[5] (7.46 g, 114 mgatom) and iodine (1.55 g, 6.12 mmol) in dry dioxane (64 mL) was irradiated with ultrasound[6] at rt for 2.5 h, poured into water and extracted with dichloromethane. The organic solution was washed with 1% aqueous potassium iodide solution. Work-up gave **9** (12 g, 91 %) as colorless prisms (from ethanol), mp 110-112 °C; IR ν_{max} 3500, 1710 cm^{-1}; ^1H NMR δ 1.07 (t, *J*=7 Hz, 3H, CH_2CH_3), 2.63 (d, *J*=15 Hz, 1H, 2-Ha), 3.08 (s, 2H, 4-H_2), 3.37 (d, *J*=15 Hz, 1H, 2-Hb), 3.82 (s, 3H, OMe), 3.97 (q, *J*=7 Hz, 2H, OCH_2CH_3), 4.38 (s, 1H, OH), 5.87 (s, 4H, OCH_2Ox2), 6.50 (s, 1H, 3'-H), 6.55-6.64 (m, 3H, ArH), 7.02 (s, 1H, 6'-H); Anal. Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found: C, 62.81; H, 5.46.

3-Hydroxy-3-(2-methoxy-4,5-methylenedioxyphenyl)-4-(3,4-methylenedioxyphenyl)butyric Acid (10). A mixture of the hydroxy ester **9** (1.0 g, 2.48 mmol), 17 % aqueous potassium hydroxide solution (5 mL) and ethanol (10 mL) was refluxed for 0.5 h and washed with ether. After acidification with 10 % hydrochloric acid the mixture was extracted with dichloromethane. Work-up gave a hydroxy acid **10** (1.0 g, quant.) as a colorless amorphous mass; IR ν_{max} 3480, 1750 cm^{-1}; ^1H NMR δ 2.75, 3.25 (each d, *J*=15 Hz, 1H, 2-H_2), 3.06 (s, 2H, 4-H_2), 3.79 (s, 3H, OMe), 5.87 (s, 4H, OCH_2Ox2), 6.38-6.83 (m, 5H, ArH); MS *m/z*: 374 (M^+, 0.8 %); Anal. Calcd for $C_{19}H_{18}O_8$: C, 60.96; H, 4.85. Found: C, 61.07; H, 4.80.

Intramolecular Basic Acylation of the Hydroxy Acid 10. To the stirred solution of **10** (0.10 g, 0.268 mmol) in MeCN (1.0 mL) in the presence of K_2CO_3 (0.81 g, 0.59 mmol) was added $POCl_3$ (0.13 mL, 1.34 mmol) under ice-cooling. The mixture was stirred at 50 °C for 5 h, diluted with water and extracted with chloroform. After work-up purification of the crude product by flash chromatography (with benzene : ethyl acetate=50 : 1) gave the same naphthol **4** (0.015 g, 17 %) described later.

Ethyl 3-(2-Methoxy-4,5-methylenedioxyphenyl)-4-(3,4-methylenedioxyphenyl)butyrate (11). A mixture of the hydroxy ester **9** (1.0 g, 2.48 mmol), the 1% palladium solution[20] (4 mL) and Norit (0.36 g) in acetic acid (33 mL) was hydrogenated at rt and at atmospheric pressure untill absorption of hydrogen ceased. The catalyst was removed by filtration and the filtrate was evaporated. After work-up purification of the crude product by column chromatography (with chloroform) afforded an ester **11** (0.98 g, quant.) as a pale yellow oil; IR ν_{max} ($CHCl_3$) 1720 cm^{-1};^1H NMR δ 1.14 (t, *J*=7.1 Hz, 3H, CH_2CH_3), 2.58 (d, *J*=7.5 Hz, 2H, 2-H_2), 2.74, 2.81 (each dd, *J*=13.5, 7.5 Hz, 1H, 4-H_2), 3.68 (m, 1H, 3-H), 3.71 (s, 3H, OMe), 4.01 (q, *J*=7.1 Hz, 2H, OCH_2CH_3), 5.87, 5.88 (each d, *J*=1.5 Hz, 2H, OCH_2O), 5.89 (s, 2H, OCH_2O), 6.48 (s, 1H, 3'-H), 6.52 (dd, *J*=7.8, 1.7 Hz, 1H, 6"-H), 6.58 (s, 1H, 6'-H), 6.60 (d, *J*=1.7 Hz, 1H, 2"-H), 6.66 (d, *J*=7.8 Hz, 1H, 5"-H); MS *m/z*: 386 (M^+, 9 %).

3-(2-Methoxy-4,5-methylenedioxyphenyl)-4-(3,4-methylenedioxyphenyl)butyric Acid (12). The ester **11** (11 g, 27.4 mmol) was hydrolyzed with 17 % aqueous potassium hydroxide solution (55 mL) and ethanol (110 mL) as above. After work-up purification of the crude product by column chromatography (with chloroform) afforded an acid **12** (8.7 g, 89 %) as colorless needles (from dichloromethane-hexane), mp 125-127 °C; IR ν_{max} 1700 cm^{-1}; ^1H NMR δ 2.61 (d, J=7.6 Hz, 2H, 2-H_2), 2.74, 2.82 (each dd, J=13.5, 7.6 Hz, 1H, 4-H_2), 3.65 (m, 1H, 3-H), 3.70 (s, 3H, OMe), 5.88 (m, 4H, OCH_2Ox2), 6.48 (s, 1H, 3'-H), 6.52 (dd, J=7.8, 1.7 Hz, 1H, 6"-H), 6.57 (s, 1H, 6'-H), 6.59 (d, J=1.7 Hz, 1H, 2"-H), 6.65 (d, J=7.8 Hz, 1H, 5"-H); MS m/z: 358 (M^+, 34 %); Anal. Calcd for $C_{19}H_{18}O_7$: C, 63.68; H, 5.06. Found: C, 63.46; H, 5.00.

3,4-Dihydro-3-(2-methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxynaphthalen-1(2*H*)-one (3). To a mixture of an acid **12** (3.1 g, 8.6 mmol) and K_2CO_3 (2.65 g, 19.2 mmol) in MeCN (25 mL) was slowly added $POCl_3$ (4.0 mL, 42.9 mmol). The mixture was stirred at 60 °C for 2.5 h, poured into ice-water, basified with 10 % aqueous sodium hydroxide solution and extracted with choloroform. Work-up gave a tetralone **3** (2.7 g, 92 %) as colorless needles (from chloroform-methanol), mp 210-212 °C; IR ν_{max} 1665 cm^{-1}; ^1H NMR δ 2.72 (m, 2H, 2-H_2), 3.00 (d, J=7 Hz, 2H, 4-H_2), 3.73 (m, 1H, 3-H), 3.76 (s, 3H, OMe), 5.90, 6.00 (each s, 2H, OCH_2O), 6.54 (s, 1H, 3'-H), 6.66 (s, 1H, 6'-H), 6.70 (s, 1H, 5-H), 7.49 (s, 1H, 8-H); Anal. Calcd for $C_{19}H_{16}O_6$: C, 67.05; H, 4.74. Found: C, 66.95; H, 4.46.

3-(2-Methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxy-1-naphthol (4). A solution of a tetralone **3** (1.0 g, 2.94 mmol) in isopropenyl acetate (15 mL) containing *p*-TsOH H_2O (0.085 g, 0.448 mmol) was refluxed for 7 h under argon and then DDQ (0.801 g, 3.53 mmol) was added to the mixture. The mixture was stirred at rt for 40 min, diluted with dichloromethane and then washed with 5 % aqueous sodium hydrogen carbonate solution. After evaporation of the solvent 5 % aqueous sodium hydroxide solution (50 mL) was added to the residue. The mixture was stirred at 85 °C for 15 min, poured into water, acidified with 10 % hydrochloric acid and extracted with dichloromethane. After work-up purification of the crude product by column chromatography (with ether : hexane=1 : 1) afforded a naphthol **4** (0.94 g, 95 %) as colorless needles (from dichloromethane-hexane), mp 179-180 °C; IR ν_{max} 3275 cm^{-1}; ^1H NMR δ 3.73 (s, 3H, OMe), 5.97, 6.04 (each s, 2H, OCH_2O), 6.63 (s, 1H, 3'-H), 6.877 (s, 1H, 6'-H), 6.880 (s, 1H, 2-H), 7.09 (s, 1H, 5-H), 7.31 (s, 1H, 4-H), 7.49 (s, 1H, 8-H); MS m/z: 338 (M^+, 28 %); Anal. Calcd for $C_{19}H_{14}O_6$: C, 67.45; H, 4.17. Found: C, 67.39; H, 4.05.

Nitrosation of *m*-Cresol (13). **(i) Under Acidic Condition:** A solution of $NaNO_2$ (0.65 g, 9.34 mmol) in a limited volume of water (*ca.* 1.4 mL) was added to a stirred mixture of *m*-cresol (**13**) (1.0 g, 9.25 mmol) in acetic acid (2.8 mL) and conc. H_2SO_4 (0.37 mL) under ice-cooling during 1 h. The whole was stirred at the same temperature for 10 min, poured into water and extracted with dichloromethane. After work-up purification of the crude product by column chromatography (with dichloromethane) followed by recrystallization from ethyl acetate-hexane gave a nitrosocresol **14** (0.834 g, 66 %) as reddich brown needles, mp 166-168 °C (lit.[12] 165 °C).
(ii) Under Basic Condition: A mixture of *m*-cresol (**13**) (0.20 g, 1.85 mmol), *i*-$AmNO_2$ (0.3 mL, 2.23 mmol) and K_2CO_3 (0.38 g, 2.75 mmol) in DMF (2.0 mL) was stirred at rt for 1 h and then at 50 °C for 1 h. After acidification with 10 % hydrochloric acid the mixture was extracted with ethyl acetate. Work-up gave the same nitrosocresol **14** (0.18 g, 71 %) obtained under the acidic condition.

2-Methyl-1,4-benzoquinone 1-Monooxime Methyl Ether (15). A mixture of a nitrosocresol **14** (0.10 g, 0.74 mmol), K_2CO_3 (0.24 g, 1.75 mmol) and Me_2SO_4 (0.08 mL, 0.84 mmol) in DMF (3.0 mL) was stirred at 40 °C for 40 min. After decomposition of the excess Me_2SO_4 with 5 %

ammonium hydroxide solution, work-up gave a quinone oxime ether **15** (0.11 g, 100 %) as pale yellow needles (from hexane), mp 69-70.5 °C; ^{1}H NMR δ 2.20 (s, 3H, ArMe), 4.17 (s, 3H, OMe), 6.29 (d, *J*=2 Hz, 1H, 3-H), 6.38 (dd, *J*=10, 2 Hz, 1H, 5-H), 7.61 (d, *J*=10 Hz, 1H, 6-H); ^{13}C NMR δ 17.4 (CH_3), 64.0 (OCH_3), 124.9 (CH), 128.8 (CH), 131.3 (CH), 146.5 (C), 149.2 (C), 187.5 (CO); HR-MS *m/z* 151.0620 (Calcd for $C_8H_9NO_2$: 151.0632).

(*Z*)-2-(2-Methoxy-4,5-methylenedioxyphenyl)-1-methoxyimino-6,7-methylenedioxynaphthalen-4(2*H*)-one (16). A mixture of a naphthol **4** (1.5 g, 4.43 mmol), *i*-$AmNO_2$ (1.0 mL, 7.44 mmol) and K_2CO_3 (6.13 g, 44.4 mmol) in DMF (15 mL) was stirred at 0 °C for 1 h. After addition of further *i*-$AmNO_2$ (0.5 mL, 3.72 mmol) the mixture was stirred at rt for 0.5 h and recooled. To the mixture was added Me_2SO_4 (0.51 mL, 5.38 mmol) at 0 °C. The whole was stirred at the same temperature for 0.5 h. After decomposition of the excess Me_2SO_4 with 5 % ammonium hydroxide solution work-up gave a quinone oxime ether **16** (1.35 g, 80 %) as reddish brown prisms (from dichloromethane-hexane), mp 237-240.5 °C; IR ν_{max} 1663 cm^{-1}; ^{1}H NMR δ 3.71, 4.05 (each s, 3H, OMe), 5.98, 6.11 (each s, 2H, OCH_2O), 6.52 (s, 1H, 2-H), 6.57 (s, 1H, 3'-H), 6.73 (s, 1H, 6'-H), 7.68 (s, 1H, 5-H), 8.35 (s, 1H, 8-H); ^{13}C NMR δ 56.8 (CH_3), 64.3 (OCH_3), 95.1 (CH), 101.5 (CH_2), 102.1 (CH_2), 106.0 (CH), 110.1 (CHx2), 119.3 (C), 124.8 (C), 128.6 (C), 129.3 (CH), 140.9 (C), 145.5 (C), 148.6 (C), 149.2 (C), 150.2 (C), 151.2 (C), 152.5 (C), 183.6 (CO); Anal. Calcd for $C_{20}H_{15}NO_7$: C, 62.99; H, 3.97; N, 3.67. Found: C, 62.95; H, 3.70; N, 3.55.

2-(2-Methoxy-4,5-methylenedioxyphenyl)-1-imino-6,7-methylenedioxynaphthalen-4(2*H*)-one (17). **(i) Reduction of a Quinone Oxime Ether 16 with $NaBH_4$:** A mixture of a quinone oxime ether **16** (0.05 g, 0.131 mmol) and $NaBH_4$ (0.008 g, 0.21 mmol) in DMF (3.5 mL) and methanol (0.7 mL) was stirred at rt. After 18 h and additional 43 h $NaBH_4$ [0.012 g and 0.034 g (total 0.054 g, 1.42 mmol)] was added to the reaction mixture, respectively. The mixture was stirred at 40 °C for 5 h, poured into ice-water, acidified with 10 % hydrochloric acid and extracted with dichloromethane. After work-up purification of the crude product by column chromatography (with dichloromethane) afforded a quinone imine **17** (0.015 g, 33 %) as yellow prisms (from dichloromethane-methanol), mp 254-260 °C; IR ν_{max} (KBr) 3462 cm^{-1}; ^{1}H NMR δ 3.70 (s, 3H, OMe), 6.02, 6.12 (each s, 2H, OCH_2O), 6.55 (s, 1H, 3-H), 6.64 (s, 1H, 3'-H), 6.66 (s, 1H, 6'-H), 7.53 (s, 1H, 8-H), 7.88 (s, 1H, 5-H), 10.19 (br s, 1H, NH); MS *m/z*; 351 (M^+, 12 %); Anal. Calcd for $C_{19}H_{13}NO_6$: C, 64.96; H, 3.73; N, 3.99. Found: C, 64.60; H, 3.50; N, 3.84.
(ii) Reduction of a Quinone Oxime Ether 16 with $Na_2S_2O_4$: A mixture of a quinone oxime ether **16** (0.053 g, 0.139 mmol) and sodium hydrosulfite (80 %, 0.130 g, 0.599 mmol) in DMF (1 mL) and 10 % aqueous sodium hydroxide solution (0.5 mL) was heated at 50-60 °C for 2 h under stirring, poured into ice-water, acidified with 10 % hydrochloric acid and extracted with ethyl acetate. After work-up purification of the crude product by PLC (with chloroform) gave a quinone imine **17** (0.012 g, 25 %).
(iii) Reduction of a Quinone Oxime Ether 16 with Raney® Ni: To a solution of a quinone oxime ether **16** (0.104 g, 0.273 mmol) in dioxane (1 mL) was added a suspension[21] of Raney® Ni (*ca* 0.075 g) in dioxane (1 mL). The whole was hydrogenated at rt and at atmospheric pressure until absorption of hydrogen ceased. After work-up purification of the crude product by column chromatography (with dichloromethane) afforded a quinone imine **17** (0.029 g, 30 %).
(iv) Reduction of the Crude Nitrosated Product 5 Prepared from a Naphthol 4 with $Na_2S_2O_4$: A mixture of a naphthol **4** (0.501 g, 1.48 mmol), K_2CO_3 (2.07 g, 14.9 mmol) and *i*-$AmNO_2$ (0.25 mL, 1.86 mmol) in DMF (8 mL) was stirred at 0 °C for 0.5 h under argon. After addition of further *i*-$AmNO_2$ (0.1 mL, 0.744 mmol) the mixture was stirred at rt for 1 h and worked-up. The crude product was suspended to ethanol (1 mL) and 10 % aqueous sodium hydroxide solution (1.5 mL) and then $Na_2S_2O_4$ (0.26 g, 1.20 mmol) was added to the mixture under ice-cooling. The reaction

mixture was stirred at rt for 1.5 h, poured into ice-water, acidified with 10 % hydrochloric acid and extracted with chloroform. After work-up purification of the crude product by column chromatography (with dichloromethane) afforded a quinone imine **17** (0.05 g, 24 %).

4-Formamido-3-(2-methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxy-1-naphthol (19). **(i) With 10 % Pd-C (Table 1, run 1):** A solution of a quinone oxime ether **16** (1.69 g, 4.44 mmol) in dioxane (30 mL) was hydrogenated at rt and at atmospheric pressure in the presence of 10 % Pd-C (0.847 g). After disappearance of the starting **16** on TLC (4 h) HCOOH (4.5 mL) and $HCONH_2$ (13.5 mL) were added to the mixture. The mixture was stirred at rt for 4 days under hydrogen. After removal of the catalyst the filtrate was evaporated to dryness under reduced pressurre and the residue was dissolved in ethyl acetate. The organic solution was washed with water, dried and evaporated to give a phenol formamide **19** as a dark yellow solid (2.04 g, quant.), which was used without further purification; IR ν_{max} ($CHCl_3$) 3600, 3376, 1686 cm^{-1}; ^1H NMR[22] δ 3.71 (s, 3Hx1/3, OMe), 3.72 (s, 3Hx2/3, OMe), 5.66 (br s, 1H, OH), 5.99 (s, 2Hx2/3, OCH_2O), 6.05 (s, 2Hx1/3, OCH_2O), 6.09 (s, 2H, OCH_2O), 6.57 (s, 1Hx1/3, 3'-H), 6.607 (s, 1Hx2/3, 3'-H), 6.613 (s, 1Hx2/3, 2-H), 6.63 (s, 1Hx1/3, 2-H), 6.69 (s, 1Hx2/3, 6'-H), 6.73 (s, 1Hx1/3, 6'-H), 7.14 (s, 1Hx1/3, 8-H), 7.31 (s, 1Hx2/3, 8-H), 7.42 (s, 1Hx1/3, 5-H), 7.51 (s, 1Hx2/3, 5-H), 7.56 (s, 1Hx1/3, NH), 7.94 (s, 1Hx1/3, CHO), 7.97 (s, 1Hx2/3, NH), 8.28 (s, 1Hx2/3, CHO); MS *m/z*; 381 (M^+, 100 %).
(ii) With PtO_2 (Table 1, run 4): A solution of a quinone oxime ether **16** (0.033 g, 0.088 mmol) in dioxane (2 mL) and $HCONH_2$ (1 mL) was hydrogenated at rt and at atmospheric pressure in the presence of PtO_2 (0.005 g). After 1 day HCOOH (0.3 mL) was added to it and the whole was stirred at rt for 7 days under hydrogen. Work-up gave a phenol formamide **19** (0.069 g, quant.)

1-Formamido-4-methoxy-2-(2-methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxynaphthalene (20). A solution of a phenolic formamide **19** (0.385 g, 1 mmol), Me_2SO_4 (0.12 mL, 1.28 mmol) and K_2CO_3 (0.426 g, 3.08 mmol) in DMF (7 mL) was stirred at 0 °C for 3.5 h. After work-up purification of the crude product by column chromatography (with dichloromethane) afforded a formamide **20** (0.258 g, 65 %) as light brown needles (from dichloromethane-hexane), mp 261-263 °C; IR ν_{max} ($CHCl_3$) 3378, 1680 cm^{-1}; ^1H NMR[22] δ 3.74 (s, 3H, OMe), 3.96 (s, 3Hx1/3, OMe), 3.97 (s, 3Hx2/3, OMe), 5.99 (br s, 2Hx2/3, OCH_2O), 6.05 (br s, 2Hx1/3, OCH_2O), 6.08 (s, 2H, OCH_2O), 6.62 (s, 1Hx1/3, 3-H), 6.63 (s, 1H, 3'-H), 6.66 (s, 1Hx2/3, 3-H), 6.76 (s, 1Hx2/3, 6'-H), 6.83 (s, 1Hx1/3, 6'-H), 7.15 (s, 1Hx1/3, 8-H), 7.19 (d, *J*=12 Hz, 1Hx2/3, NH), 7.31 (s, 1Hx2/3, 8-H), 7.55 (s, 1Hx1/3, 5-H), 7.56 (br s, 1Hx1/3, NH), 7.58 (s, 1Hx2/3, 5-H), 7.95 (d, *J*=12 Hz, 1Hx2/3, CHO), 8.28 (d, *J*=1.4 Hz, 1Hx1/3, CHO); MS *m/z*; 395 (M^+, 100 %); Anal. Calcd for $C_{21}H_{17}NO_7$: C, 63.80; H, 4.33; N, 3.54. Found: C, 63.55; H, 4.22; N, 3.49.

4-Methoxy-2-(2-methoxy-4,5-methylenedioxyphenyl)-1-(*N*-methylformamido)-6,7-methylenedioxynaphthalene (6). A mixture of a phenolic formamide **19** (0.303 g, 0.795 mmol), Me_2SO_4 (0.78 mL, 8.23 mmol) and benzyl tri-*n*-butylammonium chloride (0.106 g, 0.339 mmol) in chloroform (30 mL) and 2 % aqueous sodium hydroxide solution (15 mL) was stirred at rt for 3 days. After work-up purification of the residue by column chromatography (with dichloromethane) gave an *N*-methyformamide **6** (0.228 g, 70 %) as plae yellow needles (from chloroform-hexane), mp 264-268 °C; IR ν_{max} 1664 cm^{-1}; ^1H NMR δ 2.97 (s, 3H, NMe), 3.67, 3.97 (each s, 3H, OMe), 5.98, 6.06 (each s, 2H, OCH_2O), 6.59 (s, 1H, 3'-H), 6.60 (s, 1H, 3-H), 6.63 (s, 1H, 6'-H), 6.99 (s, 1H, 8-H), 7.60 (s, 1H, 5-H), 8.07 (s, 1H, CHO); ^{13}C NMR δ 33.2 (NCH_3), 55.7 (OCH_3), 56.0 (OCH_3), 95.5 (CH), 99.2 (CH), 99.6 (CH), 101.4 (CH_2x2), 105.9 (CH), 110.1 (CH), 119.7 (C), 122.1 (C), 128.8 (C), 129.4 (C), 134.6 (C), 141.1 (C), 147.6 (C), 148.1 (C), 149.5 (C), 151.3 (C), 154.4 (C), 164.8

(CO); HR-FABMS *m/z*: 410.1239 (Calcd for $C_{22}H_{20}NO_7$: 410.1240); Anal. Calcd for $C_{22}H_{19}NO_7$: C, 64.54; H, 4.68; N, 3.42. Found: C, 64.56; H, 4.47; N, 3.37.

BNR of 4-Methoxy-2-(2-methoxy-4,5-methylenedioxyphenyl)-1-(*N*-methylformamido)-6,7-methylenedioxynaphthalene (6) A mixture of an *N*-methyformamide **6** (0.030g, 0.07 mmol) and $POCl_3$ (0.5 mL, 5.36 mmol) was heated at 70 °C for 4 h. After work-up the crude product was dissolved in methanol (3 mL) and treated with $NaBH_4$ (0.050 g, 1.32 mmol) at rt for 2 h. After work-up the residue was purified by PLC (with ethyl acetate : hexane=1 : 3) to give dihydromacarpine (**21**) (0.009 g, 30 %) as a less polar component and a benz[*g*]indole **22**[15] (0.007 g, 24 %) as a more polar component, respectively: Dihydromacarpine (**21**); pale yellow needles (from ethyl acetate-hexane), mp 179-183 °C (lit. 178-179 °C[4a]; 177-178 °C[4c]); ^1H NMR δ 2.53 (s, 3H, NMe), 3.88, 4.00 (each s, 3H, OMe), 4.09 (s, 2H, 6-H_2), 6.01, 6.04 (each s, 2H, OCH_2O), 6.61 (s, 1H, 9-H), 7.53 (s, 1H, 1-H), 7.67 (s, 1H, 4-H), 7.82 (s, 1H, 11-H).

Macarpine (1) A mixture of dihydromacarpine (**21**) (0.017g, 0.043 mmol) and DDQ (0.018 g, 0.080 mmol) in benzene (9 mL) and 5 % aqueous sodium hydroxide solution (1 mL) was stirred at rt for 2 h. After evaporation of the benzene the aqueous layer was extracted with ether. The organic solutions were combined, dried and evaporated. The yellow residue was dissolved in a small amount of chloroform, triturated with 5 % hydrochloric acid and recrystallized from methanol-ether to afford reddish brown needles (0.014 g, 91 %), mp 291-295 °C (lit. 283-285 °C[4a]; 275-278 °C[4c]). The synthetic product was identical with an authentic sample of macarpine (**1**); ^1H NMR (DMSO-d_6) δ 4.17, 4.19 (each s, 3H, OMe), 4.82 (s, 3H, NMe), 6.35, 6.54 (each s, 2H, OCH_2O), 7.22 (s, 1H, 9-H), 7.92 (s, 1H, 1-H), 8.15 (s, 1H, 4-H), 8.84 (s, 1H, 11-H), 9.84 (s, 1H, 6-H).

ACKNOWLEDGMENT

We are grateful to Prof. J. Slavik, J. E. of Purkyne University, The Czecho Republic, for a generous supply of natural macarpine. We also thank to Mr. A. Saimoto, Nippon Kayaku Co Ltd., for the biological tests of macarpine and its analogous compounds. This work was partially supported by the Hohansha Foundation.

REFERENCES AND NOTES

(1) Ishii, H.; Ichikawa, Y.-I.; Kawanabe, E.; Ishikawa, M.; Ishikawa, T.; Kuretani, K.; Inomata, M.; Hoshi, A. *Chem. Pharm. Bull.* **1985**, *33*, 4139-4141. Recently it was reported that fagaronine[2a] and nitidine[2b] inhibited DNA topoisomerases I and II.

(2) *a)* Larsen, A. K.; Grondard, L.; Couprice, J.; Desoize, B.; Comoe, L.; Jardillier, J.-C.; Riou, J.-F. *Biochem. Pharm.* **1993**, *46*, 1403-1412; *b)* Fang, S. D.; Wang, L. K.; Hecht, S. M. *J. Org. Chem.* **1993**, *58*, 5025-5027.

(3) Slavik, J.; Slavikova, L. *Coll. Czech. Chem. Commun.* **1955**, *20*, 356-361.

(4) *a)* Takao, N.; Kamigauchi, M.; Sugiura, M.; Ninomiya, I.; Miyata, O.; Naito, T. *Heterocycles* **1981**, *16*, 221-225; *b)* Hanaoka, M.; Cho, W. J.; Yoshida, S.; Fueki, F.; Mukai, C. *Heterocycles* **1989**, *29*, 857-860; *c)* Hanaoka, M.; Cho, W. J.; Yoshida, S.; Fueki, F.; Mukai, C. *Chem. Pharm. Bull.* **1990**, *38*, 3335-3340.

(5) Kardesky, F. A. J.; Ardecky, R. J.; Lakshmikanthan, M. V.; Cava, M. P. *J. Am. Chem. Soc.* **1981**, *103*, 1992-1996.

(6) Hau, B. H.; Boudjouk, P. *J. Org. Chem.* **1982**, *47*, 5030-5032.
(7) Ishii, H.; Chen, I.-S.; Ueki, S.; Masuda, T.; Morita, K.; Ishikawa, T. *J. Chem. Soc., Perkin Trans. I* **1987**, 2415-2420.
(8) Unsuccessful dehydration of the hydroxy ester **9** was also observed.
(9) Hydrogenolysis of **10** with Pd-C ineffectively afforded an intended acid **12** (66 %). Formation of a 1, 2-diphenylpropane derivative (13 %) as an over-reduction product indicated that decarboxylation could compete with hydrogenation of a benzylic cation formed under acidic condition during the reaction.
(10) Wang, S.; Cushman, M. *Synth. Commun.* **1991**, *21*, 989-996.
(11) Trials of the dehydrogenation either using oxygen in place of DDQ or without pre-treatment of isopropenyl acetate resulted in incomplete reaction with slight decomposition or recovery of the starting **3**, respectively. Ineffective formation of the corresponding methyl ether of **4** was observed when ethyl orthoformate was used in the acetal exchange reaction.
(12) Klages, A. *Ber.*, **1899**, *32*, 2564-2569.
(13) Coomes, R. G. "Comprehensive Organic Chemstry" ed by Barton, D.; Ollis, W. D. Vol 2, 1979, Pergamon Press, London, pp.305-325.
(14) Ishikawa, T.; Watanabe, T.; Tanigawa, H.; Saito, T.; Kotake, K.-I.; Ohhashi. Y.; Ishii, H. Unpublished result.
(15) Ishikawa, T.; Saito, T.; Noguchi, S.; Ishii, H.; Ito, S.; Hata, T. *Tetrahedron Lett.* **1995**, *36*, 2795-2798.
(16) 12-Methoxydihydrochelerythrine has appeared in nature as an additional 12-alkoxybenzo[*c*]phenanthridine alkaloid. [Oechslin, S. M.; Konig, G. M.; Oechslin-Merkel, K.; Whight, A. D.; Kinghorn, A. D.; Sticher, O. *J. Nat. Prod.* **1991**, *54*, 519-524]
(17) Preparation of this new base will be reported elswhere.
(18) Ishii, H.; Ishikawa, T.; Watanabe, T.; Ichikawa, Y.-I.; Kawanabe, E. *J. Chem. Soc., Perkin Trans. I* **1984**, 2283-2289.
(19) Wang, J-.D.; Narui, T.; Takatsuki, S.; Hashimoto, T.; Kobayashi, F.; Ekimoto, H.; Abuki, H.; Niijima, K.; Okuyama, T. *Chem. Pharm. Bull.* **1991**, *39*, 2135-2137.
(20) Ishii, H.; Kawanabe, E.; Harada, K.-I.; Deushi, T.; Ueda, E.; Watanabe, T.; Ichikawa, Y.-I.; Sakamoto, M.; Ishida, T.; Takahashi, T.; Nakajima, K.; Ishikawa, T. *Chem. Pharm. Bull.* **1983**, *31*, 3039-3055.
(21) The water in 50 % slurry Raney® Ni in water was exchanged into dioxane by repeated decantations before use.
(22) The ^{1}H NMR showed a complex signal pattern due to the presence of geometrical isomers[18] derived from the double bond nature of the tertiary amide structure in the naphthyl formamide derivatives.

(*Received in Japan* 10 *May* 1995; *accepted* 8 *June* 1995)