Studies on Methoxylation in the 7*H*-Naphtho[1,2,3-*i*,*j*][2,7]naphthyridin-7-one System

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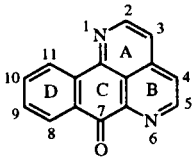
Received March 31, 1997

Studies on methoxylation in the 7H-naphtho[1,2,3-*i*,*j*][2,7]naphthyridin-7-one (sampangine) system represented by 3-bromo- and 4-bromosampangine as well as sampangine itself are described. We have found that regioselectivity of nucleophilic substitution in the sampangine system can be directed by reaction conditions. Under kinetic control (lower temperatures) substitution at C-4 is the predominant reaction, regardless of whether 3-bromo or 4-bromosampangine were used. At higher temperatures, when the reaction is thermodynamically controlled, substitution of the bromine atom at C-3 predominates. This is the first reported example of nucleophilic substitution in ring A of the 7*H*-naphtho[1,2,3-*i*,*j*][2,7]naphthyridin-7-one system.

J. Heterocyclic Chem., **34**, 1233 (1997).

A natural alkaloid, sampangine, **1** (7*H*-naphtho[1,2,3-*i*,*j*][2,7]naphthyridin-7-one), the prototype of a novel group of compounds with antifungal activity, was isolated from *Cananga odorata* in 1986 [1].

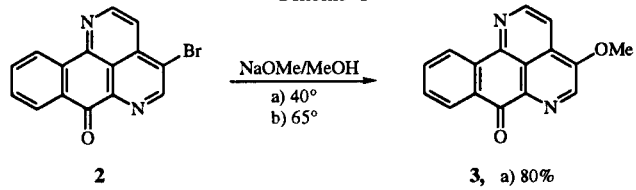


1, sampangine
7*H*-naphtho[1,2,3-*i*,*j*][2,7]naphthyridin-7-one

The biological activity was not known however until 1990, when the first natural sampangine derivative, 3-methoxysampangine **6**, was isolated in our laboratories [2]. 3-Methoxysampangine was found to be very active *in vitro* against several AIDS-related opportunistic pathogens such as *Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus* and *Mycobacterium intracellulare* [3]. Several other synthetic sampangine derivatives were obtained, and showed antifungal activity *in vitro* comparable with that of sampangine [3]. The natural derivative, 3-methoxysampangine was isolated from a West African tree, *Cleistopholis patens* in 0.0001% yield. In order to obtain a larger supply of this compound for biological studies, several synthetic efforts were undertaken. The total synthesis of this compound that we first reported suffers from a low yield (less than 1%) and is very tedious and troublesome [3]. We now report a new method of synthesis of 3-methoxysampangine as a result of our studies on nucleophilic substitution reactions in the sampangine system.

We studied the methoxylation of sampangine and its ring A and ring B bromo derivatives (3- and 4-bromosampangine) at various temperatures. The reaction of 4-bromosampangine **2** with sodium methoxide in metanol was straightforward and provided the 4-methoxyderivative **3** as the only product, regardless the temperature of the reaction. The yield of methoxylation of 4-bromosampangine is high, and comparable at different temperatures (80% at 40° and 84% in refluxed methanol) [3,4] (Scheme 1).

Scheme 1

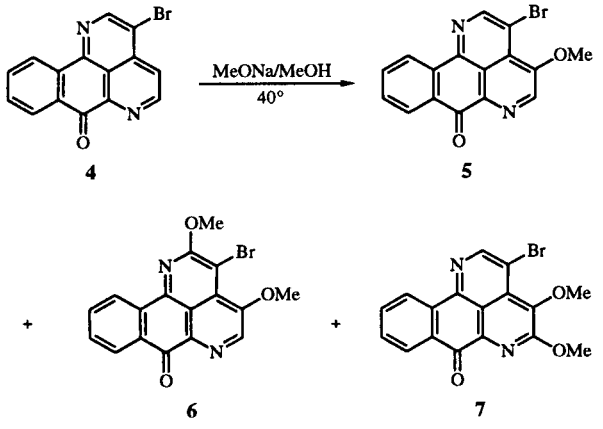


2 **3**, a) 80% b) 84%

When 3-bromosampangine **4** [4], was subjected to reaction with sodium methoxide in methanol, the reaction was found to be temperature-dependent. At 0° and at room temperature the reaction was completely non-selective, producing a complex mixture of products. At 40°, substitution of the hydrogen atom at C-4, instead of bromine at C-3, predominantly takes place [4]. The major product formed is 3-bromo-4-methoxysampangine **5**, accompanied by two minor dimethoxybromo-derivatives **6** and **7** (Scheme 2). This rather unusual pathway of nucleophilic substitution of hydrogen atom at C-4, with a retention of

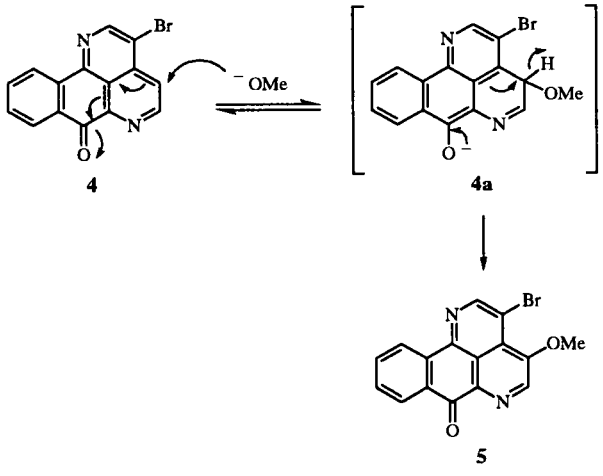
bromine at 3-position is the illustration of an exceptionally high susceptibility of carbon 4 to nucleophilic attack.

Scheme 2



The resonance structure **4a**, with a negative charge on the oxygen atom, is the major contributing intermediate for substitution at the position 4 (Scheme 3). Such cases of

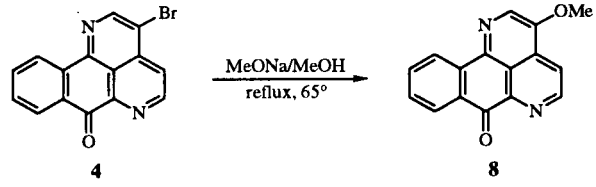
Scheme 3



preferential substitution of hydrogen atom in heterocyclic systems (S_NH) in the presence of halogen (bromine or chlorine) are rather rare [5], but as in our case they are due to the high π-deficiency of a particular carbon atom in a heterocyclic ring. Nucleophilic substitution of a hydrogen atom in azines reported in the literature [6] usually requires the presence of an outer oxidant, which promotes the removal of hydrogen ion and aromatizes an intermediate σ-adduct. In our case air plays this role. This was proved by the reaction of **4** with sodium methoxide in methanol with the exclusion of air (in an argon atmosphere). In these circumstances the formation of a burgundy-red colored Meisenheimer complex **4a** was observed. We were able to isolate the intermediate salt **4a**, characterize it by nmr spectra, and convert it to **5**, by air oxidation in methanol. The nmr spectra of σ-adduct of **4a** obtained shows the proton at the newly formed tetrahedral carbon C-4 resonating at a higher field ($\Delta\delta$ = 4 ppm) relative to that of the parent compound (3-bromosampangine). Resonance signal of proton at sp^3-carbon C-4 shows as a doublet (δ = 4.13 ppm, J = 14 Hz), due to the coupling with aromatic proton at C-5. Although the formation of σ-adducts is well documented in the literature of aromatic and heteroaromatic compounds [7], the isolation of the Meisenheimer complex **4a**, without stabilizing functional groups is worthy to note. Our observations have also made clear that the nucleophilic substitution of hydrogen by methoxyl anion in sampangine system proceeds through the addition-elimination [S_N(AE)] mechanism.

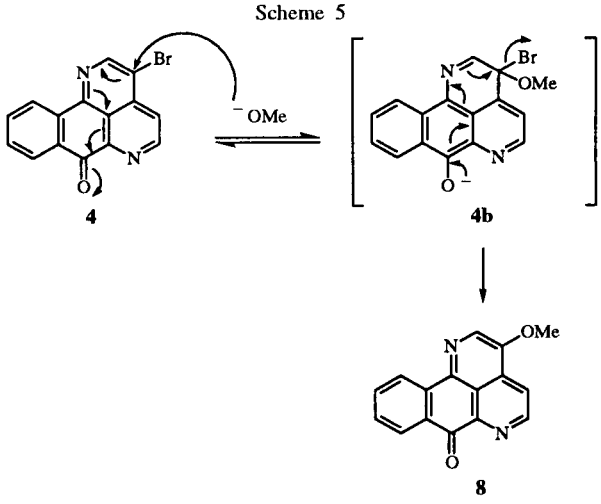
At the reflux temperature of methanol (65°), the direction of nucleophilic attack is changed, and the product of bromine substitution *i.e.* 3-methoxysampangine **8** is formed in 44% yield (Scheme 4).

Scheme 4



The reaction proceeds now through the formation of a thermodynamically preferred σ-adduct **4b** (Scheme 5). It is now evident that 3-bromo-4-methoxysampangine **5** is kinetically, and 3-methoxysampangine **8** thermodynamically controlled products of the reaction of 3-bromosampangine **4** with sodium methoxide in methanol. The formation of these products, **5** and **8**, were observed in both reactions (Schemes 2 and 4) although in different ratios.

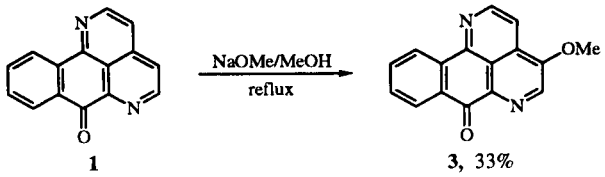
Scheme 5



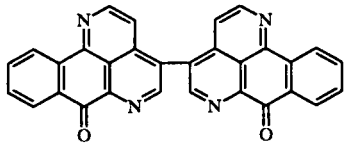
A similar phenomenon regarding site selectivity in relation to temperature was observed in the amination of quinoline or naphthyridines with potassium amide in liquid ammonia (Chichibabin reaction) [8,9].

The fact that the 7*H*-naphtho[1,2,3-*i*,*j*][2,7]naphthyridin-7-one heterocyclic ring system is highly susceptible to nucleophilic substitution at the position C-4 prompted us to evaluate the methoxylation reaction for unsubstituted sampangine. At 0°, room temperature, and even at 40° the reaction was nonselective, producing a complex mixture of products. At 65° however, we were able to obtain two products: 4-methoxysampangine (33%) and surprinsigly enough a dimer of sampangine, 4,4'-bisampangine (15%) (Scheme 6).

Scheme 6

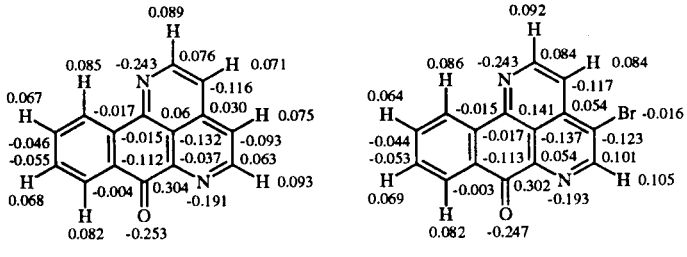


1 3, 33%



9, 15%

The regioselectivity of nucleophilic substitution in some heterocyclic systems such as pyridine, quinoline and naphthyridines can be predicted by quantum chemical calculations [10], FMO calculations [11,12], or by MNDO/PMO theory [13]. Atomic charges calculated by the semiempirical molecular orbital method [14] for sampangine 1,4-bromosampangine **2**, and 3-bromosampangine **4** [Figure 1], provide support for the direction of nucleophilic attack at position C-4.



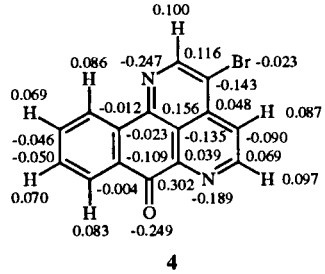


Figure1. Calculated atomic charges of sampangine **1**, 4-bromosampangine **2**, and 3-bromosampangine **4**.

In sampangine **1**, and 3-bromosampangine **4**, the partial charges indicate that the C-4 position will be more prone to nucleophilic attack due to much lower charge density than the C-3 position. In 4-bromosampangine **2**, the situation is different; the charge densities at C-3 and C-4 are very close in value. In that case the presence of the Br leaving group at C-4 makes it the preferred position of nucleophilic attack.

The reported method herein, of the synthesis of 3-methoxysampangine **8**, provides a practical and feasible way of manufacturing this antifungal natural product, which otherwise would be difficult to be obtained [2,3].

EXPERIMENTAL

Sampangine, 3-bromosampangine, and 4-bromosampangine were prepared by the published methods [4,15]. Other reagents and solvents were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received. Melting points (uncorrected) were determined in open capillary tubes with a Thomas-Hoover capillary melting point apparatus. Infrared (ir) spectra were recorded on a Perkin-Elmer 281B spectrophotometer. The nmr spectra were obtained on a Varian VXR-300 FT spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C nmr. Chemical shifts are reported as ppm downfield relative to tetramethylsilane. Two-dimensional nmr spectra were obtained using standard Varian pulse sequences for COSY and HETCOR. The long-range HETCOR experiments were optimized for $^3J_{C-H}$ = 10Hz and $^3J_{C-H}$ = 5Hz. High resolution mass spectra were obtained at the Mass Spectrometry Laboratory, Department of Chemistry, Univeristy of Kansas, Lawrence, Kansas. Elemental analyses were obtained from Chemical Analysis Group of Oneida Research Services Inc. in Whitesboro, New York and from Elemental Microanalysis of Organic Compounds Laboratory of Atlantic Microlab Inc. in Norcross, Georgia.

Analytical thin-layer chromatography (tlc) was performed on Merck silica gel F-254 glass plates. Visualization was achieved with shortwave ultraviolet light and/or Dragendorff reagent spray. Column chromatography was performed on Merck 230-400 mesh silica gel.

Methoxylation of 4-Bromosampangine (2), (a) at Lower Temperature (40°) [4].

The solution of 4-bromosampangine (**6**) (0.311 g, 1 mmole) and sodium methoxide (0.54 g, 10 mmoles, freshly prepared from 0.23 g of sodium metal) in dry methanol (30 ml) was heated at 40° for 48 hours. The methanol was then evaporated and the residue dissolved in chloroform (200 ml). The chloroform solution was washed with water (5 x 50 ml), dried over anhydrous potassium carbonate and concentrated. After trituration of the residue with ethyl acetate the pure 4-methoxysampangine (**3**) was obtained (0.210 g, 80%). An analytical sample was obtained by crystallization from chloroform, mp 279-280° dec; ir (potassium bromide): ν 1670, 1595, 1570, 1500, 1405,

1375, 1320 cm^{-1}. ^{1}H nmr (deuteriochloroform): δ 4.25 (s, 3H, OCH_3), 7.69 (ddd, 1H, J = 7.9, 7.9, 1.2 Hz, H-9), 7.82 (ddd, 1H, J = 7.9, 7.9, 1.2 Hz, H-10), 8.00 (d, 1H, J = 5.8 Hz, H-3), 8.49 (dd, 1H, J = 7.9, 1.2 Hz, H-8), 8.66 (s, 1H, H-5), 8.85 (dd, 1H, J = 7.9, 1.2 Hz, H-11), 8.89 (d, 1H, J = 5.8 Hz, H-2); ^{13}C nmr (deuteriochloroform): δ 56.9, 114.3, 120.0, 125.3, 128.4, 128.9, 130.3, 131.2, 132.8, 134.2, 135.6, 141.0, 146.6, 150.4, 152.7, 181.1 ppm; *Anal.* (exact mass, hreims) Calcd. for $C_{16}H_{10}N_2O_2$ m/e 262.0742. Found 262.0740.

Anal. Calcd. for $C_{16}H_{10}N_2O_2$: C, 73.27; H, 3.84; N, 10.68. Found: C, 73.08; H , 3.88; N, 10.68.

(b) At 65° (Reflux of Methanol) [3].

Following the same procedure as above in refluxing methanol for 12 hours, 0.220 g, 84% of 4-methoxysampangine (**3**) was obtained. The mp, ir, and ^{1}H and ^{13}C nmr data are identical with those obtained from experiment (a).

Methoxylation of 3-Bromosampangine (**4**), (a) at 40° (Kinetic Control).

A solution of 3-bromosampangine (**4**) (0.311 g, 1 mmole) and sodium methoxide (0.27 g, 5 mmoles, freshly prepared from 0.115 g of sodium metal) in dry methanol (50 ml) was heated at 40° for 48 hours. The methanol was then evaporated and the residue dissolved in chloroform (200 ml). The chloroform solution was washed with water (5 x 50 ml), dried over anhydrous potassium carbonate and concentrated. The residue was chromatographed on flash silica gel column eluting with the mixture of chloroform-ethyl acetate (94:6 v/v) to give 120 mg (35%) of 3-bromo-4-methoxysampangine **5**, as the major product, mp 248-250° dec; ir (potassium bromide): ν 1665, 1600, 1545, 1490, 1400 cm^{-1}. ^{1}H nmr (deuteriochloroform): δ 4.25 (s, 3H, OCH_3), 7.70 (ddd, 1H, J = 7.8, 7.8, 1.4 Hz, H-9), 7.82 (ddd, 1H, J = 7.8, 7.8, 1.4 Hz, H-10), 8.46 (dd, 1H, J = 7.8, 1.4 Hz, H-8), 8.73 (brs, 1H, H-5), 8.83 (dd, 1H, J = 7.8, 1.4 Hz, H-11), 9.04 (s, 1H, J, H-2); ^{13}C nmr (deuteriochloroform): δ 56.7, 93.6, 113.6, 121.4, 125.6, 128.3, 130.9, 131.4, 132.1, 134.3, 135.3, 141.0, 149.1, 150.8, 152.7, 181.0 ppm; *Anal.* (exact mass, hreims) Calcd. for $C_{16}H_9BrN_2O_2$ + H, m/e 340.9926. Found 340.9931.

Anal. Calcd. for $C_{16}H_9BrN_2O_2 \cdot 1/4H_2O$: C, 55.59, H, 2.77, N, 8.10. Found: C, 55.75, H, 2.85, N, 7.88; and the mixture (90 mg) of 3-bromo-2,4-dimethoxysampangine (**6**) with 3-bromo-4,5-dimethoxysampangine (**7**) in about 3:8 ratio based on the nmr spectrum. All efforts to separate these compounds by column chromatography or crystallization failed.

(b) At 65° (Thermodynamic Control).

A solution of 3-bromosampangine (**4**) (0.311 g, 1 mmole) and sodium methoxide (0.27 g, 5 mmoles, freshly prepared from 0.115 g of sodium metal) in dry methanol (50 ml) was refluxed for 10 hours. The methanol was then evaporated and the residue dissolved in chloroform (200 ml). The chloroform solution was washed with water (5 x 50 ml), dried over anhydrous potassium carbonate and concentrated. The residue was chromatographed on flash silica gel column eluting with the mixture of chloroform-ethyl acetate-ammonia (25%) (90:10:1 v/v) to give 115 mg (44%) of 3-methoxysampangine (**8**). An analytical sample was obtained by crystallization from chloroform, mp 225-227° dec; ir (potassium bromide): ν 1675, 1600, 1570, 1500, 1405, 1380, 1300 cm^{-1}, ^{1}H nmr (deuteriochloroform): δ 4.18 (s, 3H, OCH_3), 7.61 (ddd, 1H, J = 7.8, 7.8, 1.2 Hz, H-9), 7.78 (ddd, 1H, J = 7.8, 7.8, 1.2 Hz, H-10), 8.21 (d, 1H, J = 5.4 Hz, H-4), 8.36 (s, 1H, H-2), 8.43 (dd, 1H, J = 7.8, 1.2 Hz, H-8), 8.65 (dd, 1H, J = 7.8, 1.2 Hz, H-11), 9.13 (d, 1H, J = 5.4 Hz, H-5); ^{13}C nmr (deuteriochloroform): δ 56.6, 118.8, 119.7, 124.6, 126.8, 128.5, 130.2, 131.5, 131.8, 134.6, 135.7, 143.2, 147.2, 148.0, 149.9, 182.0 ppm; *Anal.* (exact mass, hreims) Calcd. for $C_{16}H_{10}N_2O_2$ m/e 262.0742. Found 262.0747.

Anal. Calcd. for $C_{16}H_{10}N_2O_2 \cdot 1/2H_2O$: C, 70.84, H, 4.09, N, 10.33. Found: C, 70.82, H, 3.88, N, 10.68.

Methoxylation of Sampangine (**1**), (a) at Lower Temperature (40°).

A solution of sampangine (**1**) (0.02 g. 0.086 mmole) and sodium methoxide (0.023 g, 0.43 mmole, freshly prepared from 0.0098 g of sodium metal) in dry methanol (10 ml) was heated at 40° for 24 hours. Thin-layer chromatographic analysis of the reaction mixture showed a complex mixture of products. Increasing or decreasing the reaction time did not improve selectivity.

(b) At 65° (Reflux of Methanol).

A solution of sampangine (**1**) (0.232 g, 1 mmole) and sodium methoxide (0.27 g, 5 mmoles, freshly prepared from 0.115 g of sodium metal) in dry methanol (50 ml) was refluxed for 24 hours. The methanol was then evaporated and the residue dissolved in chloroform (200 ml). The chloroform solution was washed with water (5 x 50 ml), dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on flash silica gel column eluting with the mixture of chloroform-*n*-hexane (90:10 v/v) to give 86 mg (33%) of 4-methoxysampangine (**3**), and 69 mg (15%) of 4,4'-bisampangine (**9**), mp >260°; ^{1}H nmr (deuteriochloroform of **9**): δ 7.35 (d, 1H, J = 5.8 Hz, H-3), 7.78 (ddd, 1H, J = 7.8, 7.8, 1.2 Hz, H-9), 7.91 (ddd, 1H, J = 7.8, 7.8, 1.2 Hz, H-10), 8.54 (dd, 1H, J = 7.8, 1.2 Hz, H-8), 8.87 (d, 1H, J = 5.8 Hz, H-2), 8.91 (dd, 1H, J = 7.8, 1.2 Hz, H-11), 9.23 (s, 1H, H-5); *Anal.* (exact mass, hreims) Calcd. for $C_{30}H_{14}N_4O_2$ m/e 462.1117. Found 462.1132.

Acknowledgements.

The authors wish to thank the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS, Grant No. RO1-AI-32485 for financial support, and Mr. Troy Smilie for preparing sampangine.

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[14] Molecules were constructed using standard parametrization of the SYBYL^R molecular modeling package (version 6.1, 1994, Tripos Associate, Inc., St.Louis, MO), mounted on a Silicon Graphics Indigo^2 workstation with IRIX 5.2 operating system, 64 Megabyte RAM and XZ graphics interface (Silicon Graphics, Inc., Mountain View, CA). Atomic charges were calculated by the semiempirical molecular orbital methods of MOPAC 6.0 program package using MNDO parametrization, which is incorporated into the SYBYL package. Energy minimization was done using the Tripos force field with MOPAC charges.

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