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Total Synthesis of Mimosamycin

Hiroshi Fukumi, Hideshi Kurihara, and Hiroshi Mishima

Central Research Laboratories, Sankyo Co., Ltd.[1]

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The synthesis of mimosamycin has been achieved in 6 steps starting from 7-hydroxy-6-methylisoquinoline (**7**). Copper-catalyzed autoxidation of **7**, which was prepared in 80% over-all yield in 5 steps from 3-methoxy-4-methylbenzaldehyde, gave 6-methyl-3,5-dimorpholino-7,8-dioxo-7,8-dihydroisoquinoline (**8a**). Upon treatment with sulfuric acid–methanol followed by diazomethane, the *o*-quinone (**8a**) was converted to 7-methoxy-6-methyl-3-morpholino-5,8-dioxo-5,8-dihydroisoquinoline (**10a**). Reductive acetylation of **10a** with zinc-acetic acid and acetic anhydride gave 5,8-diacetoxy-7-methoxy-6-methyl-3-morpholinoisoquinoline, which was quaternized at the isoquinoline nitrogen with methyl iodide. Treatment of the methiodide (**12a**) with silver oxide gave rise to in one-step 7-methoxy-2,6-dimethyl-3,5,8-trioxo-2,3,5,8-tetrahydroisoquinoline, which was identified with a specimen of natural mimosamycin isolated from the culture filtrate of *Streptomyces lavendulae* No. 314.

Keywords——antibiotic; 3,5,8-isoquinolinetrione; autoxidation; mimosamycin; *Streptomyces lavendulae*; total synthesis

The culture filtrate of *Streptomyces lavendulae* No. 314 contains a number of antibiotics, one of which was designated as mimosamycin.[2] Its structure was determined as 7-methoxy-2,6-dimethyl-3,5,8-trioxo-2,3,5,8-tetrahydroisoquinoline (**1**) by an X-ray crystallographic study.[3] 3,5,8-Trioxo-2,3,5,8-tetrahydroisoquinoline is a new ring system first found in nature.

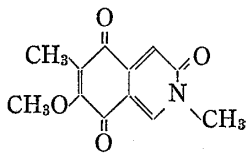

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Chart 1

In connection with efforts towards the total synthesis of **1**, the synthesis of 7-methoxy-2-methyl-3,5,8-trioxo-2,3,5,8-tetrahydroisoquinoline as a model compound was accomplished.[4] Preliminary results concerning mimosamycin have been briefly described.[5] Full experimental details and further work are reported in this paper.

The homologues of 5,8-dioxo-5,8-dihydroisoquinoline have received scant attention, and oxidation of 5,8-diamino-,[6] 5-amino-8-hydroxy-,[7] and 5,8-dihydroxyisoquinolines[8] have been employed in their syntheses. Phenols are known to undergo *ortho*-hydroxylation by aerial oxidation in the presence of cupric ion-amine mixture[9] and this reaction has been employed in the formation of a large number of *ortho*-quinones. Recent extension of the autoxidation to isoquinolinols by Tsizin[10] provided a facile entry to the series of 5,6- and 7,8-dioxoisoquinoline.

1) Location: *Hiro-machi, Shinagawa-ku, Tokyo 140, Japan.*
2) T. Arai, K. Yazawa, Y. Mikami, A. Kubo, and K. Takahashi, *J. Antibiot.* (Tokyo), **29**, 398 (1976); Y. Mikami, K. Yokoyama, A. Omi, and T. Arai, *ibid.*, **29**, 408 (1976).
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9) W. Brockmann and E. Havinga, *Rec. Trav. Chim. Pays-Bas*, **74**, 937 (1955).
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It was apparent that a convenient starting material for the synthesis of **1** was 7-hydroxy-6-methylisoquinoline (**7**). According to the procedure of Jackson's isoquinoline synthesis,[11] **7** was synthesized from 3-methoxy-4-methylbenzaldehyde (**2**) in five steps in 80% over-all yield.

The required 3-methoxy-4-methylbenzaldehyde (**2**) was readily prepared by diazotization of 3-amino-4-methylbenzaldehyde, followed by hydrolysis and methylation. 3-Methoxy-4-methylbenzaldehyde (**2**) was condensed with aminoacetaldehyde dimethylacetal, and the resulting Schiff base (**3**) was catalytically hydrogenated over Adams' catalyst to furnish the benzylaminoacetal (**4**). Treatment of **4** with *p*-toluenesulfonyl chloride in pyridine at room temperature yielded the tosylate (**5**), which was heated under reflux in dioxane containing dilute hydrochloric acid to afford 7-methoxy-6-methylisoquinoline (**6**). Demethylation of **6** with 48% hydrobromic acid afforded 7-hydroxy-6-methylisoquinoline (**7**).

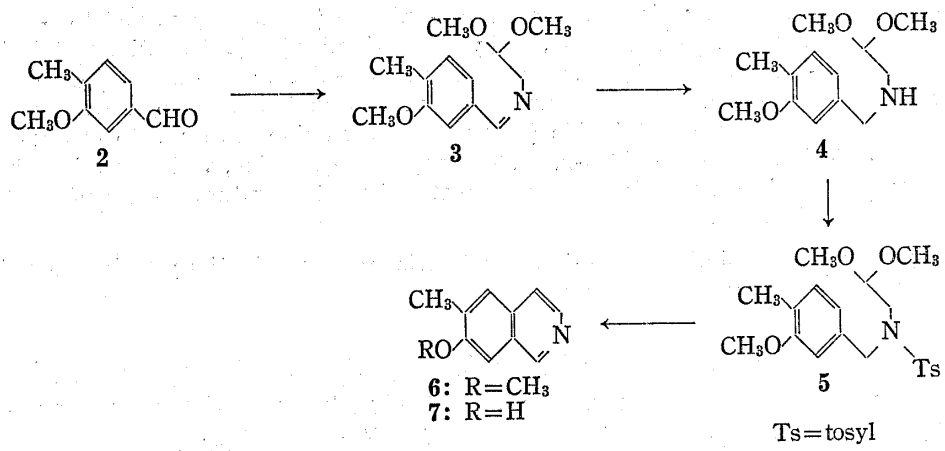

Chart 2

By application of Tsizin's procedure, copper-catalyzed autoxidation of **7** in the presence of morpholine and piperidine gave 6-methyl-3,5-dimorpholino-7,8-dioxo-7,8-dihydroisoquinoline (**8a**) and 6-methyl-3,5-dipiperidino-7,8-dioxo-7,8-dihydroisoquinoline (**8b**), respectively. The spectral data of **8a** and **8b** allow unambiguous assignment of structure and condensation of **8a** and **8b** with *o*-phenylenediamine gave rise to the corresponding phenazine derivatives.

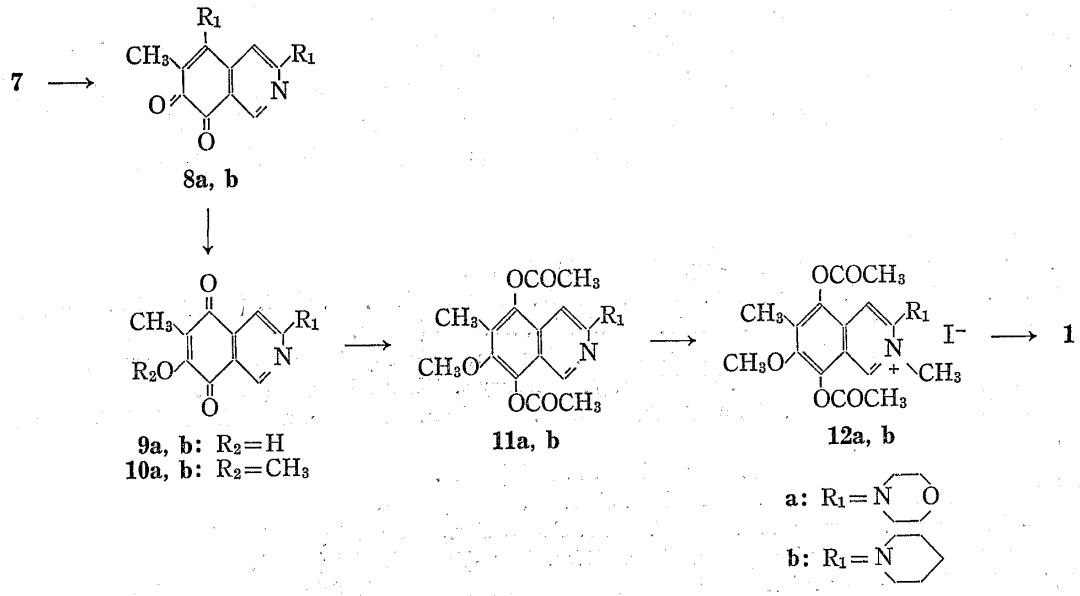

Chart 3

Conversion of **8a** and **8b** to the *p*-quinones (**9a** and **9b**) was effected by warming a methanolic solution of **8a** and **8b** in the presence of catalytic amount of conc. sulfuric acid or sodium hydroxide. Alternatively, methylation of 7-hydroxy-3-morpholino-5,8-dioxo-5,8-dihydroisoquinoline with excess of potassium hydride and methyl iodide afforded a 17% yield of the C-methylated product (**9a**), accompanied by a 32% yield of the O-methylated product.

Reductive acetylation of **10a** and **10b**, which were prepared by treatment of **9a** and **9b** with diazomethane, with zinc-acetic acid and acetic anhydride afforded the diacetate of hydroquinones (**11a** and **11b**).

There are two (exocyclic and ring) nitrogens having different environments in **11a** and **11b**. It was hoped that methylation would take place at the ring nitrogen, since this affords a short cut to mimosamycin (**1**). The inspection of the literature[12,13] reveals that methylation of 2-dimethylaminopyridine yields the trimethylammonium isomer which is alkylated at the exocyclic nitrogen, whereas methylation of 3 or 4-dimethylaminopyridine affords the pyridinium salt which is alkylated at the ring nitrogen. The pyridinium salt of 2-dimethylaminopyridine has been formed when its trimethylammonium isomer was heated at high temperature, because the ring alkylated product was more thermally stable.[14] Alkylation of **11a** with methyl iodide in DMF at 100° afforded the quaternary salt (**12a**), as a sole product, in the UV spectrum of which a bathochromic shift of absorption maxima from 290 nm in the parent compound (**11a**) to 358 nm indicated the formation of desired product.[14] The NMR spectrum of **12a** showed that the proton signal of the N-methyl group appeared at δ 4.62 and the proton signal at C-1 appeared at δ 10.18, whereas the corresponding signal in **11a** appeared at δ 8.90.

Treatment of **12a** with silver oxide or **12b** with potassium carbonate gave mimosamycin. Under these conditions, a reaction of three-steps was assumed to proceed *via* hydrolysis of the acetoxyl groups, oxidation of the resultant hydroquinone, and substitution (addition elimination reaction) of the amino (morpholine or piperidine) group with a hydroxyl group. Data on both natural and synthetic mimosamycin agreed in all aspects (IR, UV, NMR, mass spectra and TLC, mixed mp, and antibiotic activities).

Experimental[15]

3-Methoxy-4-methylbenzaldehyde (**2**)——4-Methyl-3-nitrobenzaldehyde (110 g) was added at once to a solution of $SnCl_2 \cdot 2H_2O$ (450 g) in 35% HCl (600 ml). The reaction was highly exothermic. As soon as the inner temperature of the reaction mixture reached at 100°, the mixture was cooled to 0—5° with ice-cooling. The resulting precipitates (stannic complex of 3-amino-4-methylbenzaldehyde) were filtered off and used to the following diazotization reaction without purification. To the suspended mixture of the complex in 35% HCl (600 ml) was added dropwise at 4—5° a solution of $NaNO_2$ (46 g) in H_2O (50 ml) for 50 min. The mixture was stirred at the same temperature for 2 hr, and the resulting pale yellow precipitates were filtered off. A solid of the diazonium salt was added in portions to refluxing water (1.7l) over 30 min and after evolution of nitrogen gas ceased, the mixture was cooled and extracted with CH_2Cl_2. Concentration of CH_2Cl_2 extracts gave a crude product, which was purified by CH_2Cl_2 elution through a short silica gel layer and by recrystallization from benzene. Yield of 3-hydroxy-4-methylbenzaldehyde[16] was

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12) A.E. Chichibabin and R.A. Konowalowa, *Chem. Ber.*, **59**, 2055 (1926).
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15) Melting points were determined using a Büchi capillary melting point apparatus and are uncorrected. Infrared(IR) spectra were taken with a JASCO IRA-2 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian HA-60 spectrometer. Chemical shift values are expressed in ppm relative to internal tetramethylsilane. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br., broad. Ultraviolet (UV) spectra were obtained with a Hitachi 200-20 spectrophotometer. Mass spectra (MS) were recorded on a JEOL JMS-01SG at 75 ev using a direct inlet system. The relative intensity of the ions is indicated in parenthesis as a percent of the base peak.
16) N.V. Sidgwick and E.N. Allott, *J. Chem. Soc.*, **123**, 2819 (1923).

54.5 g (60%). To a solution of 3-hydroxy-4-methylbenzaldehyde (10.6 g) in 2 N NaOH (60 ml) was added dropwise at 40—45° Me_2SO_4 (15.9 g) for 10 min. The mixture was stirred at the same temperature for 1 hr, cooled to 20° and extracted with ether (150 ml). Usual work-up and recrystallization from *n*-hexane gave colorless needles (8.6 g, 88.5%) of **2**, which melted at 45—46° (lit.[17] bp 101—103°/10^{-4} mmHg).

2,2-Dimethoxy-N-(3-methoxy-4-methylbenzylidene)ethylamine(3)——Aminoacetaldehyde dimethylacetal (22 g) was added to a solution of **2** (30 g) in benzene (100 ml). The mixture was refluxed in a Dean-Stark apparatus until no further water was appeared. Evaporation of solvent gave Schiff base (51 g) as a colorless oil. Yield was virtually quantitative and this was normally used without purification. Purification for analysis was carried out by distillation. bp 165° (0.5 mmHg). *Anal.* Calcd. for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.85; H, 8.03; N, 5.43. MS *m/e*: 237 (M^+, 21), 207 (11), 163 (13), 136 (9), 76 (100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1648, 1608. NMR ($CDCl_3$) δ ppm: 2.23 (3H, s, CH_3), 3.41 (6H, s, $2CH_3O$), 3.82 (2H, d-d, J=1 and 5.5, CH_2N), 3.88 (3H, s, CH_3O), 4.70 (1H, t, J=5.5, OCHO), 7.17 (2H, s, ArH), 7.37 (1H, s, ArH), 8.25 (1H, d, J=1, CH=N).

2,2-Dimethoxy-N-(3-methoxy-4-methylbenzyl)ethylamine (4)——The benzylideneaminoacetal (3, 46.6 g) was dissolved in MeOH (100 ml) and hydrogenated over platinum oxide (0.2 g) at 1 atm and 20° until the theoretical amount of hydrogen was taken up for about 1 hr. Filtration and removal of solvent yielded quantitatively **4** as an oil, which boiled at 145° in 0.5 mmHg. A portion of this material was redistilled to give an analytical sample. *Anal.* Calcd. for $C_{13}H_{21}NO_3$: C, 65.24; H, 8.85; N, 5.85. Found: C, 65.35; H, 8.87; N, 5.94. MS *m/e*: 239 (M^+, 8), 165 (21), 136 (100), 76 (32). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3320, 1612. NMR ($CDCl_3$) δ ppm: 1.50 (1H, br. s, NH), 2.18 (3H, s, CH_3), 2.70 (2H, d, J=5.5, CH_2N), 3.35 (6H, s, $2CH_3O$), 3.80 (2H, d, J=3, $ArCH_2N$), 3.82 (3H, s, CH_3O), 4.48 (1H, t, J=5.5, OCHO), 6.78 (1H, d, J=8, ArH), 6.83 (1H, s, ArH), 7.07 (1H, d, J=8, ArH).

2,2-Dimethoxy-N-(3-methoxy-4-methylbenzyl)-N-tosylethylamine (5)——*p*-Toluenesulfonyl chloride (36.6 g) was added in portions to a solution of **4** (46 g) in pyridine (150 ml) for 30 min. The solution was stirred for 10 hr at 20°, then poured into ice-water (800 ml) and extracted with ether. The ether extracts were washed with 5% HCl and water. Evaporation of ether afforded the crystal (69.2 g, 91.7%), which was used without purification. Recrystallization from benzene gave an analytical sample as colorless prisms, which melted at 70—72°. *Anal.* Calcd. for $C_{20}H_{27}NO_5S$: C, 61.07; H, 6.87; N, 3.56. Found: C, 61.22; H, 6.75; N, 3.70. MS *m/e*: 393 (M^+, 2), 330 (3), 238 (8), 135(39), 76(100). IR ν_{max}^{KBr} cm^{-1}: 1610. NMR ($CDCl_3$) δ ppm: 2.15 (3H, s, CH_3), 2.40 (3H, s, CH_3), 3.22 (2H, d, J=5.5, CH_2N), 3.25 (6H, s, $2CH_3O$), 3.70 (3H, s, CH_3O), 4.38 (1H, t, J=5.5, OCHO), 4.47 (2H, s, $ArCH_2N$), 6.63 (1H, d, J=1, ArH), 6.70 (1H, d-d, J=1 and 8, ArH), 7.05 (1H, d, J=8, ArH), 7.30 (2H, d, J=8.5, ArH), 7.78 (2H, d, J=8.5, ArH).

7-Methoxy-6-methylisoquinoline (6)——To a solution of **5** (69.2 g) in dioxane (800 ml) was added 6 N HCl (100 ml). The solution was refluxed for 5 hr, then poured into water (1600 ml) and extracted with ether, and the aqueous layer was made alkaline with 10% NH_4OH and reextracted with ether. The ether extracts were washed with water and dried. After removal of the solvent 7-methoxy-6-methylisoquinoline (**6**) was obtained as a solid, which was recrystallized from ethanol to give colorless prisms (25.3 g, 83.1%) melting at 104—105°. *Anal.* Calcd. for $C_{11}H_{11}NO$: C, 76.27; H, 6.40; N, 8.09. Found: C, 75.93; H, 6.32; N, 8.12 MS *m/e*: 173 (M^+, 85), 130 (100). IR ν_{max}^{KBr} cm^{-1}: 1628. NMR ($CDCl_3$) δ ppm: 2.37 (3H, d, J=1, CH_3), 3.93 (3H, s, OCH_3), 7.13 (1H, s, H_8), 7.48 (1H, d, J=9, H_4), 7.53 (1H, s, H_5), 8.45 (1H, d, J=9, H_3), 9.15 (1H, s, H_1).

7-Hydroxy-6-methylisoquinoline (7)——A solution of **6** (19.3 g) in 48% HBr (160 ml) was refluxed for 4 hr. The solution was then cooled and the resulting precipitate was filtered off. The crude product was dissolved in hot water (300 ml) and neutralization with 10% NH_4OH gave a crystalline precipitate, which was filtered off and washed with water. Yield was 16.7 g (94%). Recrystallization from MeOH gave colorless prisms, which melted at 184—186°. *Anal.* Calcd. for $C_{10}H_9NO$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.43; H, 5.50; N, 8.94. MS *m/e*: 159 (M^+, 100), 130 (31). NMR (d_6-DMSO) δ ppm: 2.35 (3H, s, CH_3), 7.33 (1H, s, H_8), 7.63 (1H, d, J=6, H_4), 7.70 (1H, s, H_5), 8.33 (1H, d, J=6, H_3), 9.05 (1H, s, H_1), 10.28 (1H, br. s, OH).

6-Methyl-3,5-dimorpholino-7,8-dioxo-7,8-dihydroisoquinoline (8a)——A mixture of **7** (5.25 g) and morpholine (8.6 g) in MeOH (30 ml) was stirred under an atomosphere of oxygen in the presence of cupric acetate (0.3 g) for 4 hr. The reaction was slightly exothermic. Reddish black crystals, precipitated from the initially homogenous reaction mixture, were filtered and wahsed with MeOH. Recrystallization from MeOH gave an analytical sample as a reddish black prism, which melted at 223—225° with decomposition. Yield was 6.7 g (59%). *Anal.* Calcd. for $C_{18}H_{21}N_3O_4$: C, 62.96; H, 6.16; N, 12.24. Found: C, 63.01; H, 6.12; N, 12.14. MS *m/e*: 343 (M^+, 10), 315 (100), 284 (70), 270 (35), 258 (61). IR ν_{max}^{KBr} cm^{-1}: 1665, 1638, 1622. UV λ_{max}^{EtOH} nm(ε): 427 (15400), 317 (96300), 245 (21500). NMR ($CDCl_3$) δ ppm: 2.10 (3H, s, CH_3), 3.2—3.4 (4H, m, $2CH_2N$), 3.7—4.0 (4H, m, $2CH_2O$), 3.83 (8H, s, $2NCH_2CH_2O$), 6.92 (1H, s, H_4), 8.78 (1H, s, H_1).

A mixture of **8a** (0.343 g) and *o*-phenylenediamine (0.108 g) in EtOH (20 ml) was refluxed with AcOH (1 ml) for 30 min. Concentration of the solvent gave orange needles (0.37 g, 89%) of 6-methyl-3,5-dimor-

17) K. Tsuda, S. Ikuma, M. Kawamura, R. Tachikawa, Y. Baba, and T. Miyadera, *Chem. Pharm. Bull.* (Tokyo), **10**, 856 (1962).

pholinopyrido[3,4-*a*]phenazine. Recrystallization from MeOH gave orange needles melting at 305° (dec.). *Anal.* Calcd. for $C_{24}H_{25}N_5O_2$: C, 69.38; H, 6.07; N, 16.86. Found: C, 69.51; H, 6.10; N, 16.96. IR ν_{max}^{KBr} cm^{-1}: 1605. MS *m/e*: 415 (M^+, 100), 384 (43), 370 (31), 358 (30). NMR ($CDCl_3$) δ ppm: 2.88 (3H, s, CH_3), 3.1—4.2 (16H, m), 7.43 (1H, s, H_4), 7.5—8.4 (4H, m, ArH), 10.17 (1H, s, H_1).

6-Methyl-3,5-dipiperidino-7,8-dioxo-7,8-dihydroisoquinoline (8b)——The autoxidation of **7** with oxygen was carried out according to the procedure described for **8a**, except fot the use of piperidine instead of morpholine. From 6.36 g of **7** was obtained 9.9 g (73%)of **8b**. Recrystallization from MeOH gave red needles of **8b** melting at 223—225° (dec.). *Anal.* Calcd. for $C_{20}H_{25}N_3O_2$: C, 70.77; H, 7.43; N, 12.38. Found: C, 71.06; H, 7.51; N, 12.61. MS *m/e*: 339 (M^+, 7), 311 (100), 282 (36), 268 (16), 256(27), 228 (37). UV λ_{max}^{EtOH} nm(ε): 435 (18300), 324 (9840), 249 (22600). IR ν_{max}^{KBr} cm^{-1}: 1666, 1638, 1620, 1592. NMR ($CDCl_3$) δ ppm: 1.5—2.0 (12H, br. s, $6CH_2$), 2.05 (3H, s, CH_3), 3.1—3.5 (4H, m, $2CH_2N$), 3.6—4.0 (4H, m, $2CH_2N$), 6.90 (1H, s, H_4), 8.98 (1H, s, H_1).

A mixture of **8b** (0.10 g) and *o*-phenylenediamine (0.05 g) in EtOH (10 ml) was refluxed with AcOH (0.5 ml) for 30 min. Concentration of the solvent gave orange needles (0.09 g, 74%) of 6-methyl-3,5-dipiperidinopyrido[3,4-*a*]phenazine. Recrystallization from EtOH gave orange needles melting at 220—222°. *Anal.* Calcd. for $C_{26}H_{29}N_5$: C, 75.88; H, 7.10; N, 17.02. Found: C, 75.65; H, 6.83; N, 16.74. IR ν_{max}^{KBr} cm^{-1}: 1598. MS *m/e*: 411 (M^+, 100), 382 (26), 355 (29), 328 (38). NMR ($CDCl_3$) δ ppm: 1.6—2.0 (12H, m, $6CH_2$), 2.83 (3H, s, CH_3), 3.2—3.5 (4H, m, $2CH_2N$), 3.7—4.0 (4H, m, $2CH_2N$), 7.45 (1H, s, H_4), 7.6—8.4 (4H, m, ArH), 10.16 (1H, s, H_1).

7-Hydroxy-6-methyl-3-morpholino-5,8-dioxo-5,8-dihydroisoquinoline (9a)——i) A solution of **8a** (6.7 g) in MeOH (100 ml) was refluxed with conc. H_2SO_4 (4 ml) for 1 hr. The solution was poured into ice-water and the resulting precipitate was filtered off and dried. Yield of **9a** was 5.04 g (94%). Recrystallization from $CHCl_3$ gave an analytical sample of **9a** as red needles melting at 250—252°. *Anal.* Calcd. for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.15; N, 10.21. Found: C, 61.54; H, 5.05; N, 10.32. MS *m/e*: 272 (M^+, 100), 243 (94), 229 (28), 217 (87), 189 (98). IR ν_{max}^{KBr} cm^{-1}: 3340, 1652, 1640, 1584, 1235. UV λ_{max}^{EtOH} nm(ε); 250 (22700), 325 (14800). NMR (d_6-DMSO) δ ppm: 1.90 (3H, s, CH_3), 3.5—3.95 (8H, m, $2NCH_2CH_2O$), 7.18 (1H, s, H_4), 8.78 (1H, s, H_1).

ii) A solution of **8a** (6.4 g) in 50% aq. EtOH (60 ml) containing NaOH (2.98 g) was heated at 80° for 30 min. The mixture was cooled, diluted with water (60 ml), and acidified with 10% HCl. The resulting precipitate was filtered off and dried to give **9a** (3.38 g, 66%).

iii) To a solution of 7-hydroxy-3-morpholino-5,8-dioxo-5,8-dihydroisoquinoline (0.25 g) in DMF (10 ml) was added KH (0.2 g of 22.7% in oil) under argon. After stirring until foaming subsided, methyl iodide (1 ml) was added dropwise to the mixture. The mixture was then heated at 70° for 3 hr, cooled, poured into ice water, neutralized with 10% HCl, and extracted with CH_2Cl_2. The residue obtained from CH_2Cl_2 extracts was chromatographed on silica gel using 10% MeOH–$CHCl_3$ as an eluent. Final purification of three components, starting material (73 mg, 29%), **9a** (45 mg, 17%), and 7-methoxy-3-morpholino-5,8-dioxo-5,8-dihydroisoquinoline[4] (85 mg, 32%), was achieved by purification through a preparative TLC using 10% MeOH in $CHCl_3$.

7-Hydroxy-6-methyl-3-piperidino-5,8-dioxo-5,8-dihydroisoquinoline (9b)——The derivation of **8b** to **9b** was carried out according to the procedure described for i) of **9a**. From 9.9 g of **8b** was obtained 7.9 g (99%) of **9b**. Recrystallization of **9b** from MeOH gave an analytical sample as red needles melting at 230—231° with decomposition. *Anal.* Calcd. for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found; C, 66.29; H, 5.95; N, 10.31. MS *m/e*: 272 (M^+, 100), 244 (48), 230 (19), 218 (19), 190 (28), UV λ_{max}^{EtOH} nm(ε): 482 (5900), 329 (15700), 252 (24700). IR ν_{max}^{KBr} cm^{-1}: 3430, 3250, 1648, 1638, 1592. NMR (d_6-DMSO) δ ppm: 1.5—1.8 (6H, m, $3CH_2$), 1.92 (3H, s, CH_3), 3.6—4.0 (4H, m, $2CH_2N$), 7.10 (1H, s, H_4), 8.73 (1H, s, H_1), 10.9 (1H, br. s, OH).

7-Methoxy-6-methyl-3-morpholino-5,8-dioxo-5,8-dihydroisoquinoline (10a)——To a suspension of **9a** (5.2 g) in $CHCl_3$ (300 ml) was added excess diazomethane ether solution. The solution was kept at room temperature until no starting material was detected. Recrystallization from EtOH gave an analytical sample as orange needles melting at 179—181°. Yield was quantitative. *Anal.* Calcd. for $C_{15}H_{16}N_2O_4$: C, 62.49; H, 5.59; N, 9.72. Found: C, 62.16; H, 5.49; N, 9.93. MS *m/e*: 288 (M^+, 100), 258 (69), 232 (51), 204 (38). UV λ_{max}^{EtOH} nm(ε): 470 (5100), 322 (12900), 252 (25300). IR ν_{max}^{KBr} cm^{-1}: 1655. NMR ($CDCl_3$) δ ppm: 2.03 (3H, s, CH_3), 3.82 (8H, s, $2NCH_2CH_2O$), 4.20 (3H, s, CH_3O), 7.08 (1H, s, H_4), 8.87 (1H, s, H_1).

7-Methoxy-6-methyl-3-piperidino-5,8-dioxo-5,8-dihydroisoquinoline (10b)——The methylation of **9b** with diazomethane was carried out according to the procedure described for **10a**. From 7.9 g of **9b** was obtained 7.2 g (86%) of **10b**. Recrystallization from MeOH gave an analytical sample as red leaflets melting at 153—154°. *Anal.* Calcd. for $C_{16}H_{18}N_2O_3$: C, 67.11; H, 6.34; N, 9.78. Found: C, 67.19; H, 6.26; N, 9.80. MS *m/e*: 286 (M^+, 100), 271 (26), 257 (52), 243 (28), 203 (36). UV λ_{max}^{EtOH} nm(ε): 488 (6500), 325 (12300), 255 (28500). IR ν_{max}^{KBr} cm^{-1}: 1655, 1585. NMR ($CDCl_3$) δ ppm: 1.5—1.9 (6H, m, $3CH_2$), 2.02 (3H, s, CH_3), 2.6—4.0 (4H, m, $2CH_2N$), 4.18 (3H, s, CH_3O), 7.12 (1H, s, H_4), 8.85 (1H, s, H_1).

5,8-Diacetoxy-7-methoxy-6-methyl-3-morpholinoisoquinoline (11a)——A solution of **10a** (4.4 g) in AcOH (9 ml) and Ac_2O (80 ml) was refluxed with Zn powder (4 g) and anhydrous AcONa (2 g) for 30 min. The mixture was cooled, filtered, and concentrated *in vacuo*. The concentrated mixture was poured into water and the resulting precipitate was filtered off to give **11a** (3.23 g, 57%) after drying. Recrystallization from

benzene–*n*-hexane mixture gave an analytical sample melting at 180—182°. *Anal.* Calcd. for $C_{19}H_{22}N_2O_6$: C, 60.95; H, 5.92; N, 7.48. Found: C, 61.30; H, 5.85; N, 7.36. MS *m/e*: 374 (M^+, 100), 332 (43), 291 (88), 290 (62), 276 (66). UV λ_{max}^{EtOH} nm(ε): 290 (14500), 252 (42700), 216 (26400). IR ν_{max}^{KBr} cm^{-1}: 1765, 1758, 1640, 1595. NMR ($CDCl_3$) δ ppm: 2.23 (3H, s, CH_3), 2.45 (6H, s, $2CH_3COO$), 3.4—3.7 (4H, m, $2CH_2N$), 3.90 (3H, s, CH_3), 3.75—4.10 (4H, s, $2CH_2O$), 6.52 (1H, s, H_4), 8.90 (1H, s, H_1).

5,8-Diacetoxy-7-methoxy-6-methyl-3-piperidinoisoquinoline (11b)——The reductive acetylation of **10b** with zinc-acetic acid and acetic anhydride was carried out according to the procedure described for **11a**. From 5 g of **10b** was obtained 4.0 g (62%) of **11b**. Recrystallization from benzene–*n*-hexane mixture gave an analytical sample melting at 132—134°. *Anal.* Calcd. for $C_{20}H_{24}N_2O_5$: C, 64.50; H, 6.50; N, 7.52. Found: C, 64.64; H, 6.42; N, 7.45. MS *m/e*: 372 (M^+, 100), 330 (71), 289 (62), 288 (100), 274 (98). UV λ_{max}^{EtOH} nm(ε): 294 (17000), 255 (42100), 217 (29100). IR ν_{max}^{KBr} cm^{-1}: 1756, 1638, 1594. NMR ($CDCl_3$) δ ppm: 1.5—2.0 (6H, m, $3CH_2$), 2.22 (3H, s, CH_3), 2.45 (6H, s, $2CH_3COO$), 3.4—3.8 (4H, m, $2CH_2N$), 3.83 (3H, s, CH_3O), 6.51 (1H, s, H_4), 8.87 (1H,s, H_1).

5,8-Diacetoxy-2,6-dimethyl-7-methoxy-3-morpholinoisoquinolinium Iodide (12a)——A solution of **11a** (6.1 g) in methyl iodide (30 ml) and DMF (1 ml) was heated at 90—100° for 40 hr in a sealded tube. After cooling the precipitate was filtered off and washed with ether to give **12a** (7.5 g, $Y=89\%$). Recrystallization from EtOH gave an analytical sample melting at 208° with decomposition. *Anal.* Calcd. for $C_{20}H_{25}IN_2O_6$: C, 46.51; H, 4.84; N, 5.43. Found: C, 46.30; H, 4.75; N, 5.21. MS *m/e*: 275 (43), 260 (36), 190 (33), 142 (100). UV λ_{max}^{EtOH} nm(ε): 358 (1920), 271 (39400), 220 (36300). IR ν_{max}^{KBr} cm^{-1}: 1784, 1762, 1640, 1612. NMR (d_7-DMF) δ ppm: 2.38 (3H, s, CH_3), 2.63 (6H, s, $2CH_3COO$), 3.2—3.6 (4H, m, $2CH_2N$), 3.8—4.0 (4H, m, $2CH_2O$), 4.03 (3H, s, CH_3O), 4.62 (3H, s, CH_3N^+), 8.07 (1H, s, H_4), 10.18 (1H, s, H_1).

5,8-Diacetoxy-7-methoxy-2,6-dimethyl-3-piperidinoisoquinolinium Iodide (12b)——A solution of **11b** (0.48 g) in methyl iodide (10 ml) was heated at 90—100° for 14 hr in a sealed tube. After cooling, the precipitate was filtered and washed with ether to give a quaternary salt (0.53 g, 80%). Recrystallization from MeOH gave an analytical sample as yellow needles melting at 172—174° with decomposition. *Anal.* Calcd. for $C_{21}H_{27}IN_2O_5$: C, 49.03; H, 5.29; N, 5.45. Found: C, 49.13; H, 5.29; N, 5.36. MS *m/e*: 372 (M^+—142, 30), 330 (40), 289 (55), 288 (83), 142 (100). UV λ_{max}^{EtOH} nm (ε): 363 (2400), 298 (6400), 267 (32800), 219 (32500). IR ν_{max}^{KBr} cm^{-1}: 1766, 1640, 1612. NMR (d_7-DMF) δ ppm: 1.5—2.0 (6H, m, $3CH_2$), 2.37 (3H, s, CH_3), 2.63 (6H, s, $2CH_3COO$), 3.1—3.5 (4H, m, $2CH_2N$), 4.03 (3H, s, CH_3O), 4.57 (3H, s, CH_3N^+), 7.95 (1H, s, H_4), 8.47 (1H, s, H_1).

7-Methoxy-2,6-dimethyl-3,5,8-trioxo-2,3,5,8-tetrahydroisoquinoline (1)——i) A solution of **12a** (3.3 g) in MeOH (60 ml) was stirred with silver oxide (4.0 g) at room temperature for 45 min and filtered. The filtrate was stripped off *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$ as an eluent and 0.73 g (49%) of **1** was obtained. Recrystallization from MeOH gave a pure sample of **1** as yellow prisms melting at 219—221°, which was identified with a specimen of mimosamycin by mixed mp, IR, UV, MS, and NMR.

ii) Under an atmosphere of oxygen a solution of **12b** (0.3 g) in MeOH (6 ml) was stirred with K_2CO_3 (0.3 g) at room temperature for 30 min. The filtrate obtained by the filtration of the reaction mixture was concentrate *in vacuo*. The same work-up as in i) gave 0.036 g (29%) of **1**.