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SYNTHESIS OF MIMOSAMYCIN AND 5,8-DIHYDROXY-4,7-DIMETHOXY-2,6-DIMETHYLISOQUINOLINIUM IODIDE

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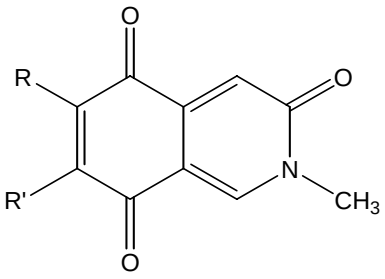
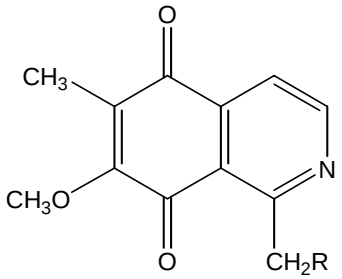
Abstract - The one-pot synthesis of mimosamycin utilizing the Polonovski reaction and a five-step synthesis of 5,8-dihydroxy-4,7-dimethoxy-2,6-dimethylisoquinolinium iodide (**7**) from known compound (**8**) are described.

The antimicrobial activity of mimosamycin (**1**), particularly against mycobacteria, was discovered in the culture filtrate of *Streptomyces lavendulae* in 1976,[1a] and the number of isoquinoline-5,8-dione antibiotics has increased rapidly since then.[1]

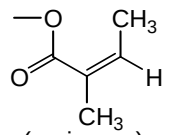


1: R=CH_3,R'=OCH_3
(mimosamycin)

4: R=R'=SCH_3
(perfragilin B)

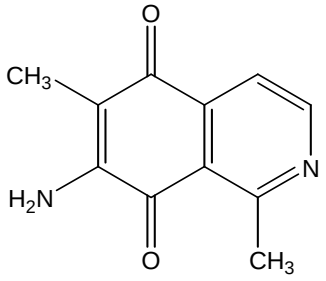


2: R=

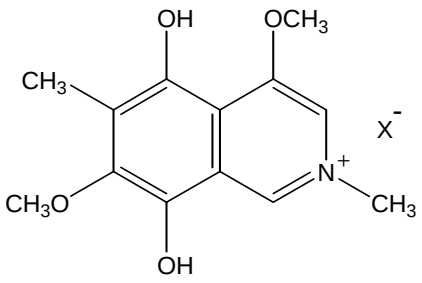


(renierone)

3: R=-$NHCOCOCH_3$
(mimocin)



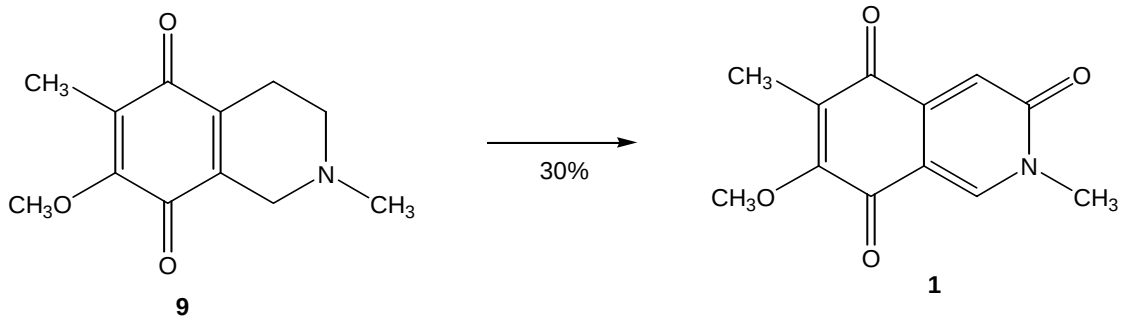
5
(cribrostatin 1)



6: X=HCO_2
7: X=I

In 1979, renierone (**2**) was isolated from the major metabolite of *Reniera* sp.[1b] Mimocin (**3**), isolated from a metabolite of *Streptomyces lavendulae*,[1c] contains a pyruvamide side chain in place of the angelate ester side chain of **2**. Perfragilin B (**4**), isolated from the Bryozoan *Biflustra perfragilis*,[1e] contains a methylthio ether group. Cribrostatin 1 (**5**), isolated from the marine sponge *Cribrochalina* sp.[1f] contains an amino group. In 1988, 5,8-dihydroxy-4,7-dimethoxy-2,6-dimethylisoquinolinium formate (**6**) was isolated from the culture broth of *Myxococcus Xanthus*[2] and the structure confirmed by comparison of its spectroscopic properties with those of 5-hydroxy-2-methylisoquinolinium methylsulfate. We have been interested in isoquinolinium formate (**6**) including the 5,8-dihydroxyisoquinoline skeleton, because the catalytic hydrogenation of 8-acetoxy-1-cyano-5-hydroxy-7-methoxy-6-methylisoquinoline caused the intramolecular transfer of the acyl group from the oxygen to the nitrogen atom, followed by air oxidation to produce the corresponding isoquinoline-5,8-dione.[3]

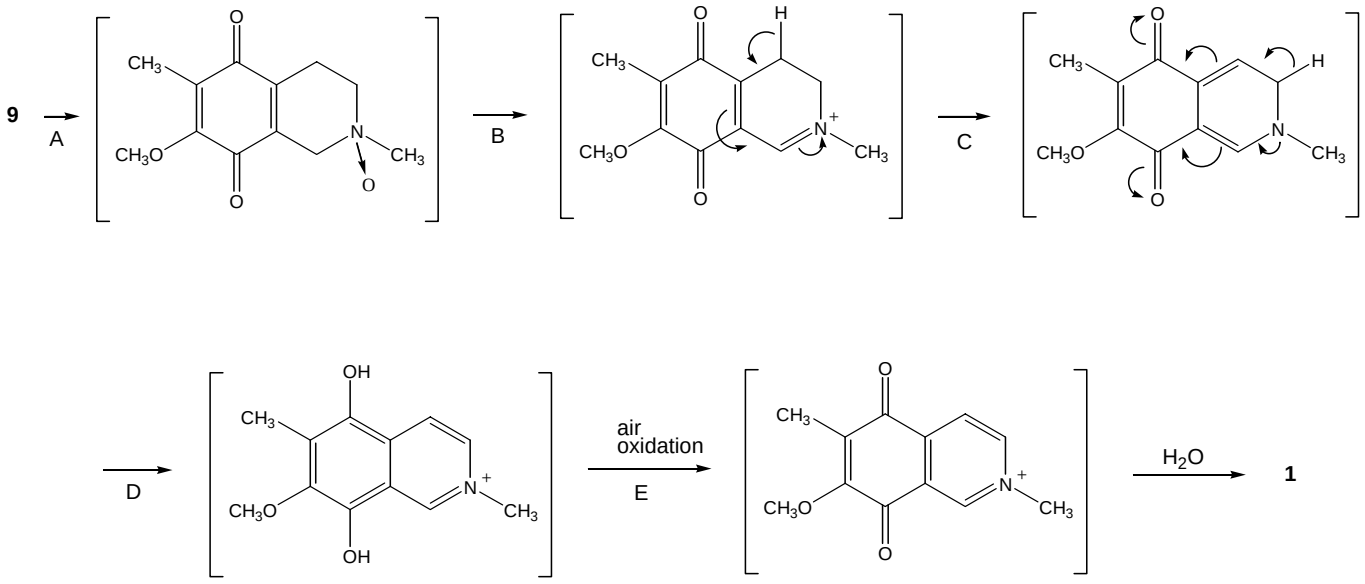
Four synthetic studies of mimosamycin have been conducted.[4] Recently, we reported the oxidative degradation of saframycin S to mimosamycin and mimocin.[5] Here, we describe the one-pot synthesis of mimosamycin and a five-step synthesis of 5,8-dihydroxy-4,7-dimethoxy-2,6-dimethylisoquinolinium iodide (**7**) from 7-methoxy-6-methyl-8-nitroisoquinoline-*N*-oxide (**8**). For the synthesis of mimosamycin, we used the Polonovski reaction[6] for the preparation of the key intermediate immonium salt. Into a stirred solution of tetrahydroisoquinoline-5,8-dione (**9**)[7] in CH_2Cl_2 was added a solution of *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2 at –40°C. After stirring at this temperature for 0.5 h, acetic anhydride and triethylamine were added and the solution was stirred for an additional 1 h at 0°C. Work-up with ice-cold $NaHCO_3/CH_2Cl_2$ afforded mimosamycin (**1**) in 30% yield (Scheme 1). When pyridine was utilized instead of triethylamine, many spots were obtained on TLC; decreasing the yield of **1** to 4%. Synthetic **1** was identical to an authentic sample in terms of TLC behavior and spectroscopic properties.



i 85%MCPBA, CH_2Cl_2, -40°C, 5 h ii $(CH_3CO)_2O$, TEA, 0°C, 1 h

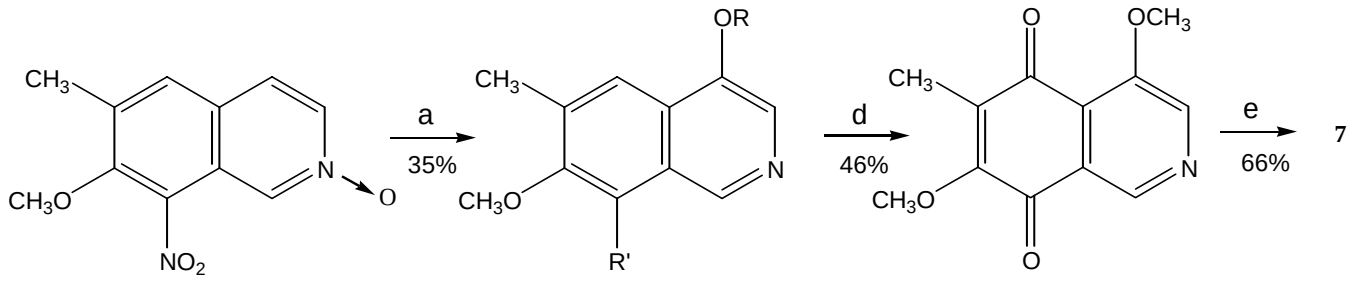
Scheme 1

The proposed mechanism is shown in Scheme 2. The immonium salt was formed in step B and air oxidation of the 5,8-dihydroxy compound to the 5,8-dione occurred during step E.



Scheme 2

The preparation of 5,8-dihydroxy-4,7-dimethoxy-2,6-dimethylisoquinolinium iodide (**7**) was conducted as shown in Scheme 3. Introduction of a hydroxyl group into the C-4 position of isoquinoline (**8**) was achieved using the procedure of Ochiai and Ikehara.[8]



a) i TsCl, $CHCl_3$, reflux, 2 h ii KOH, EtOH, reflux, 2 h b) CH_2N_2, MeOH-ether, rt, 0.5 h c) 10%Pd-C, MeOH, rt, 4 h
d) Fremy's salt, KH_2PO_4, acetone,35℃,0.5 h e) i 10%Pd-C, MeOH, rt, 1 h ii MeI, rt, 1 h

Scheme 3

Treatment of 7-methoxy-6-methyl-8-nitroisoquinoline-*N*-oxide(**8**)[9] with tosyl chloride in $CHCl_3$ under reflux for 2 h, followed by potassium hydroxide in aqueous EtOH for 2 h, gave the 4-hydroxyisoquinoline (**10**) in 35% yield. Treatment of **10** with diazomethane in ether for 0.5 h afforded 4-methoxyisoquinoline (**11**) in 59% yield. Catalytic hydrogenation of **11** over 10% Pd-C

in MeOH afforded 8-aminoisoquinoline (**12**) in 65% yield. The oxidation of **12** with potassium nitrosodisulfonate (Fremy's salt)[10] furnished the isoquinoline-5,8-dione (**13**) in 46% yield. Finally, catalytic hydrogenation of **13** over 10% Pd-C in MeOH followed by methyl iodide treatment at room temperature for 1 h afforded 5,8-dihydroxy-4,7-dimethoxy-2,6-dimethylisoquinolinium iodide (**7**) in 66% yield.

In summary, mimosamycin was synthesized using the Polonovski reaction and a mechanism is proposed for the synthesis. 5,8-Dihydroxy-4,7-dimethoxy-2,6-dimethylisoquinolinium iodide (**7**) was synthesized from 7-methoxy-6-methyl-8-nitroisoquinoline-*N*-oxide (**8**) in five steps. Compound (**7**) includes a 5,8-dihydroxyisoquinoline skeleton but is stable in air at room temperature.

EXPERIMENTAL

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H-NMR spectra at 100 MHz and 270 MHz were measured in $CDCl_3$ with tetramethylsilane as an internal standard. Anhydrous sodium sulfate was used for drying organic solvent extracts, and the solvent was removed with a rotary evaporator and finally under high vacuum. Column chromatography (flash chromatography) was performed with silica gel 60 (Merck, 230-400 mesh).

Mimosamycin (1)

Into a stirred solution of tetrahydroisoquinoline-5,8-dione (**9**)[7] (45 mg, 0.2 mmol) in 2 mL of CH_2Cl_2 was added at –40℃ a solution of *m*-chloroperbenzoic acid (MCPBA) (215 mg, 1.06 mmol) in 4 mL of CH_2Cl_2. After stirring at this temperature for 0.5 h acetic anhydride (0.1 mL, 1.06 mmol) and triethylamine (0.7 mL, 5.02 mmol) were added to the solution which is then stirred for additional 1 h at 0℃. The whole was poured into 1% aqueous $NaHCO_3$ solutoin (30 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 1 : 1) to afford mimosamycin (**1**) (14 mg, 30%). IR(KBr) cm^{-1}: 1688, 1644, 1586. ^{1}H-NMR($CDCl_3$) δ : 2.06(3H, s), 3.67(3H, s), 4.17(3H, s), 7.09(1H, s), 8.27(1H, s). ^{13}C-NMR(100 MHz, $CDCl_3$) δ : 9.6, 38.4, 61.3, 111.3, 116.7, 133.1, 138.9, 142.1, 159.5, 162.8, 177.3, 183.5. Ms *m/z* (%) : 233(M^+, 100), 218(36), 205(31), 190(23).

4-Hydroxy-7-methoxy-6-methyl-8-nitroisoquinoline (10)

Tosyl chloride (572 mg, 3 mmol) was added in portions to a solution of 7-methoxy-6-methyl-8-nitroisoquinoline-*N*-oxide (**8**)(234 mg, 1 mmol) in 3 mL of $CHCl_3$ with stirring at 0℃. The solution was boiled under reflux for 2 h and then the solvent was removed under reduced pressure. The residue was dissolved in 20 mL of EtOH and 20 mL of 6% aqueous KOH solution was added. The solution was boiled under reflux for 2 h, then diluted with water, adjusted to pH 5~6 with NH_4Cl and extracted with ethyl acetate (3 x 50 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 1 : 1) to afford 4-hydroxyisoquinoline (**10**) (82 mg, 35%). mp 202~202.5℃ (yellow prisms from

MeOH). *Anal.* Calcd for $C_{11}H_{10}N_2O_4$：C, 56.41；H, 4.30； N, 11.96. Found: C, 56.41；H, 4.29；N, 11.95. IR(KBr) cm^{-1}：3440, 1532, 1358. ^{1}H-NMR($CDCl_3$+ CD_3OD) **δ** : 2.55(3H, s), 3.99(3H, s), 8.04(1H, s), 8.24(1H, s), 8.56(1H, s). Ms *m/z* (%)：234(M^+, 100), 130(32), 103(28).

4,7-Dimethoxy-6-methyl-8-nitroisoquinoline (11)

4-Hydroxyisoquinoline (**10**)(456 mg, 1.95 mmol) in 5 mL of MeOH was added to an ether solution containing excess of CH_2N_2 and the mixture was stirred at rt for 0.5 h. The solvent was evaporated and the residue was chromatographed (eluting with hexane-ethyl acetate 2 : 1) to afford **11** (285 mg, 59%). mp 112~113 ℃ (light yellow needles from $CHCl_3$-hexane). *Anal.* Calcd for $C_{12}H_{12}N_2O_4$：C, 58.06；H, 4.87；N, 11.29. Found: C, 58.03；H, 4.80；N, 11.28. IR(KBr) cm^{-1}: 1530, 1362. ^{1}H-NMR($CDCl_3$) **δ** : 2.41(3H, s), 3.82(3H, s), 3.91(3H, s), 8.11(1H, s), 8.14(1H, s), 8.68(1H, s). Ms *m/z* (%)：248(M^+, 100), 188(9), 172(8), 144(10).

8-Amino-4,7-dimethoxy-6-methylisoquinoline (12)

4,7-Dimethoxy-6-methyl-8-nitroisoquinoline (**11**)(280 mg, 1.13 mmol) in MeOH (30 mL) was hydrogenated at 1 atm for 4 h using 10% Pd-C (100 mg) as a catalyst. The catalyst was filtered off, the solvent was removed and the residue was chromatographed (eluting with hexane-ethyl acetate 1 : 1) to afford 8-aminoisoquinoline (**12**) (160 mg, 65%). mp 198~199℃ (light yellow prisms from $CHCl_3$-hexane). *Anal.* Calcd for $C_{12}H_{14}N_2O_2$：C, 66.03；H, 6.47；N, 12.84. Found: C, 65.84；H, 6.52；N, 12.72. IR(KBr) cm^{-1}: 3396. ^{1}H-NMR($CDCl_3$) **δ** ：2.43(3H, s), 3.78(3H, s), 3.98(3H, s), 4.64~4.06(2H, br s), 7.40(1H, s), 7.93(1H, s), 8.84(1H, s). Ms *m/z* (%)：218(M^+, 70), 203(100), 175(32).

4,7-Dimethoxy-6-methyl-5,8-dihydroisoquinoline-5,8-dione (13)

A solution of Fremy's salt (0.3 g, 1.1 mmol) in 1/15 aq. KH_2PO_4 (12.5 mL) was added to 8-amino-4,7-dimethoxy-6-methylisoquinoline (**12**)(36 mg, 0.16 mmol) in acetone (1.2 mL). The mixture was stirred at 35℃ for 0.5 h, diluted with water, made alkaline with sat. $NaHCO_3$ and extracted with $CHCl_3$ (3 x 20 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 4 : 1) to afford *p*-quinone (**13**) (17 mg, 46%). mp 150~151℃ (yellow needles from $CHCl_3$-MeOH). *Anal.* Calcd for $C_{12}H_{11}NO_4$：C, 61.80；H, 4.75；N, 6.01. Found: C, 61.81；H, 4.63；N, 5.99. IR(KBr) cm^{-1}: 1670, 1656. ^{1}H-NMR($CDCl_3$) **δ** : 2.06(3H, s), 4.13(6H, s), 8.74(1H, s), 8.92(1H, s). Ms *m/z* (%): 233(M^+, 100), 218(28), 190(29).

5,8-Dihydroxy-4,7-dimethoxy-2,6-dimethylisoquinolinium iodide (7)

4,7-Dimethoxy-6-methyl-5,8-dihydroisoquinoline-5,8-dione (**13**) (15 mg, 0.064 mmol) in MeOH (3 mL) was hydrogenated at 1 atm for 1 h using 10% Pd-C (8 mg) as a catalyst. The catalyst was filtered off, the solvent was removed and methyl iodide (2.8 g, 20 mmol) was added. The solution was stirred at rt for 1 h, the precipitated crystals were collected and recrystallized from $CHCl_3$-MeOH to give isoquinolinium iodide (**7**)(16 mg, 66%) as yellow needles melting at 122~124℃. HRMS Calcd for $C_{12}H_{13}NO_4$: 235.0845, Found: 235.0843. Ms *m/z* (%): 235(39), 220(28), 192(32), 142(100), 127(59). IR(KBr) cm^{-1}: 3412. ^{1}H-NMR($CDCl_3$+CD_3OD) **δ** : 2.38(3H, s), 3.84(3H, s), 4.21(3H, s), 4.40(3H, s), 7.98(1H, s), 9.30(1H, s). ^{13}C-NMR(67.5 Hz,

$CDCl_3$+CD_3OD) δ : 10.63, 48.83, 59.11, 61.50, 113.88, 115.46, 119.24, 131.45, 141.20, 143.07, 145.83, 148.66, 156.20.

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